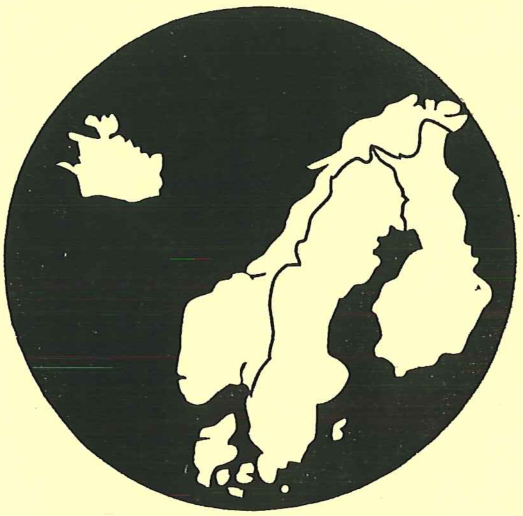
Röbäcksdalen meddelar

Lantbruksväxternas övervintring, teorier och testmetoder

The overwintering of agricultural crops, theories and test methods

Föredrag vid NJF-seminarium nr 221 i Umeå, 10-12 maj 1993

Sven Andersson (red.)



Sveriges lantbruksuniversitet
Institutionen för norrländsk jordbruksvetenskap

Rapport 11:1993

Lantbruksväxternas övervintring, teorier och testmetoder

The overvintering of agricultural crops, theories and test methods

NJF - seminarium nr 221 i Umeå den 10-12 maj 1993

NORDISKA JORDBRUKSFORSKARES FÖRENING

SEKTION II - VÄXTODLING

SEKTION IV - VÄXTSKYDD

ARRANGÖRSKOMMITTÉ:

Bjarni E Gudleifsson
Sven Andersson
Seppo Pulli
Anne-Marte Tronsmo
Ole Bagger

Sveriges lantbruksuniversitet
Institutionen för norrländsk jordbruksvetenskap

Förord

Föreliggande rapport innehåller 22 uppsatser presenterade vid ett NJF-seminarium i Umeå 10-12 maj 1993. Seminariet arrangerades av en arbetsgrupp inom NJF:s sektion II, Växtodling och IV, Växtskydd och hade titeln Lantbruksväxternas övervintring, teorier och testmetoder.

Övervintringen av perenna och vinterannuella växter är av oerhört stor betydelse för nordiskt jordbruk. Seminariet syftade till att förmedla kunskap om de faktorer, såväl biotiska som abiotiska, som påverkar växternas övervintring. Den teoretiska bakgrunden diskuterades och dessutom olika testmetoder för att utvärdera vinterhärdigheten hos växterna. Det senare är viktigt för växtförädlingen och en stor del av deltagarna var också växtförädlare.

Totalt deltog 42 personer i seminariet varav 8 från Finland, 7 från Norge, 4 från Danmark, 18 från Sverige, 2 från Lettland och en från vardera Island, Estland och Litauen.

NJF har medel från Nordiska ministerrådet avsedda att möjliggöra för baltiska forskare att deltaga i NJF:s seminarier och workshops. Med sådana medel finansierades deltagandet från Estland och Lettland medan Kungliga Skogs- och Lantbruksakademien finansierade deltagandet från Litauen. Organisationskommittén tackar för detta.

Ett varmt tack också till ledning och forskare vid Röbäcksdalen samt till lantbrukare Per Andersson, Altjärn, Tavelsjö vilka ställde upp under exkursionsdagen.

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Innehåll Sida

INDUCED RESISTANCE TO FROST AND SNOW MOULD FUNGI, MOLECULAR AND BIOCHEMICAL BASIS.

Anne Marte Tronsmo,
Norwegian Plant Protection Institute, Fellesbygget, N 1432 ÅS, Norway.

INTRODUCTION

Abiotic and biotic environmental factors cause stress on plants. In a biological system stress is defined as: Any environmental factor capable of inducing a potentially injurious strain in living organisms.

Amongst the abiotic stress factors are temperature and water, and among the biotic stress factors are the plant pathogens. Both abiotic and biotic stresses can, under certain conditions, induce resistance to subsequent stresses of the same category. In addition, low temperature stress can induce resistance to fungal pathogens. Which mechanisms are involved in this stress-induced resistance?

As part of the investigation of this topic it is useful to compare effects of abiotic and biotic stresses.

EFFECTS OF ABIOTIC AND BIOTIC STRESSES ON PLANTS.

The effects of stresses can be **injury or induced resistance**. The effect or the strain can often be seen as production of stress metabolites.

The nature of stress injury depends on the cause of injury and the dose (duration, level) of stress. Low doses of stress may induce resistance to an otherwise lethal dose of the same stress.

RESISTANCE TO STRESS

Stress-resistance is a plant's ability to avoid or tolerate the stress or the strain caused by a stress factor. Resistance or susceptibility may depend on the presence or absence of transmissible information coding for mechanisms for stress-resistance (a heritable trait), or it may depend on exposure to a low dose of the stress, which triggers mechanisms for stress-resistance (Kuc 1990, 1992, Levitt 1980).

Cold hardening is induced resistance to freezing stress. Induced resistance to plant diseases is often called immunization or acquire resistance.

STRESS METABOLITES

Stress metabolites are compounds which are synthesized and/or accumulate in the plant in response to stress conditions. We recognize them as indicators of stress. They may have a physiological role in either causing or preventing the injury or may simply be the end product of the strain.

A well known stress metabolite is the amino acid proline, which can prevent freezing injury by regulating the solute water potential of the cell. Plant hormones are also affected by stress. Especially ABA may have a function in development of freezing resistance.

Acquired resistance to diseases involves salicylic acid (SA) as a signal molecule (Klessig et al. 1993, Lawton et al.1993, Metraux et al. 1990). Pathogen infection induces SA, which is translocated throughout the plant and recognized by a specific receptor. The receptor transducts the signal and triggers the expression of systemic acquired resistance (SAR)-genes, and, consequently, SAR-proteins accumulate to high levels.

Another compound, a polypeptide called systemin, is a systemic signal that activates proteinase inhibitor genes in response to wounding (Ryan et al. 1993).

STRESS INDUCED PROTEINS

The stress proteins are a group of stress metabolites believed to have a physiological role. These proteins are selectively synthesized under stress conditions and their assumed function is to reduce the effects of the imposed stress. This function is documented for the so called "heat shock proteins" (HSP). HSP are produced at 8-10°C above the plant's optimal temperature. Plants containing HSP may survive even higher temperatures, which they would not without HSP (Van Loon 1989). Stress proteins induced by plant pathogens are usually called PR-proteins (pathogenesis related proteins). Several genes coding for PR-proteins can be induced by abiotic stress factors such as UV-light, ozone, cold, heat and drought (Didierjean et al. 1993, Schraudner al. 1993).

STRESS INDUCED RESISTANCE.

Can abiotic stress trigger processes in the plants which make them more resistant to pathogen attack?

Abiotic stress may alter activities in plants directed towards resisting a pathogen. This could result in either increased or decreased biotic injury. Exposure of grasses to cold hardening (1°C for 2 wks), increases the freezing resistance of the plants, and also increases resistance to fungal diseases such as snow moulds, leaf spots, rusts and powdery mildews (Tronsmo 1984a, b, c and unpublished).

In barley, resistance to powdery mildew may be induced by virulent and avirulent isolates of powdery mildew as well as by saprophytes (Smedegaard et al. 1992). Are defence mechanisms induced by cold stress the same as those induced by biotic stress ? We have approached this question by studying the gene expression (m-RNA and proteins) in cold hardened barley plants.

GENES:
Expression of genes induced by cold treatment has been analysed by Northern blots, using probes from cDNA libraries prepared from m-RNA isolated from barley leaves 6hrs after inoculation with an incompatible race of powdery mildew (Thordal-Christensen et al. 1992).
RNA from cold-hardened barley plants hybridized to a probe from a 2.0 kb cDNA clone (pBT6-4), a putative sucrose synthetase gene. Induction of this gene may be of importance for mobilizing sucrose in the plants. RNA from cold-hardened plants inoculated with powdery mildew expressed a much stronger signal than RNA from unhardened plants when hybridized to a probe from a 1.4 kb cDNA clone (pBH6-301), a putative peroxidase gene.

PROTEINS:
In the grass species timothy and reed canary-grass we have found enhanced invertase activity in cold hardened plants (Tronsmo et al 1993a). This enzyme is also involved in mobilizing sucrose in the plants.

In cold hardened barley we have found the same acid PR-proteins as found in mildew-inoculated plants (Bryngelsson and Collinge 1991). Some basic PR-proteins are also induced by cold hardening, amongst them a putative chitinase (Tronsmo et al.1993b).

Our results show that in barley, the responses to mildew infection can also be induced or enhanced by the temperature-stress which induces cold hardening. This indicates that several defence responses are unspecific stress responses.

CONCLUSIONS

Lethal and sublethal injuries, caused by stress factors often affect membrane properties and the osmotic balance, and consequently initiate changes in metabolism. The energy balance of the cell will always be affected, often indicated by an increased respiration.

An explanation for stress-induced resistance is not yet documented, but one can speculate that cold stress produces membrane alterations which result in ethylene release. Ethylene may then induce ABA production and the pathway leading to increased freezing resistance (Reeney et al. 1989), and /or affect other plant hormones such as Systemin, or other signal molecules which trigger defence mechanisms against pathogens.

Another explanation may be found in the carbohydrate composition of the plants. After cold hardening the composition of carbohydrates resembles the that found in barley cultivars with so called "adult plant resistance" (Tronsmo et al. 1991, Hwang and Heitefuss 1986). A mobilization of sugars seems to occur. This may be of importance for prevention of injury or injury repair.

LITERATURE CITED

Bryngelsson, T. and Collinge, D.B. 1991. Biochemical and molecular analyses of the response of barley to infection by powdey mildew. p459-480 In: Barley: Genetics, Molecular Biology and Biotechnology (Ed. P.R. Shewry). C.A.B. International, Wallingford.

Didierjean, L., P. Frendo, W. Nasser, J. Marivet, G. Genot, M.Margis-Pinheiro, E. Passelegue, D. Amegninou, C. Martin and G. Burkard 1993: Plant genes induced by chemicals and pollutants. p 276-285 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hwang, B.K., and R. Heitefuss, 1986: Sugar composition and acid invertase activity in spring barley plants in relation to adult-plant resistance to powdery mildew. Phytopathology 76, p365.

Klessig, D.F., J. Malmay, J. Hennig, Z. Chen and P. Sanchez-Casas 1993: Induction, modification, and reception of the salicylic acid signal in plant defence. p185-195 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Kuc, J.1990: Immunization for the control of plant disease. p 355-375 In: Biological control of soil-borne plant pathogens. (Ed.D. Hornby) CAB International.

Kuc, J. and N.A. Strobel 1992: Induced resistance using pathogens and nonpathogens. p 295-303 In: Biological control of plant diseases. Progress and challenges for the future. (Eds. E.C. Tjamos, G.C. Papavizas and R.J. Cook). NATO ASI Series. Plenum Press.

Lawton, K., S. Uknes, L. Friedriech, T. Gaffney, D. Alexander, R. Goodman, J.P. Metraux, H. Kessmann, P. Ahl Goy, M. Gut Rella, E. Ward and J. Ryals1993: The molecular biology of systemic aquired resistance. p 422-432 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Levitt 1980: Responses of plants to environmental stresses.2nd Ed.Vol. 1. Academic Press. 497p

van Loon 1989: Stress proteins in infected plants. In: Plant-Microbe interactions Vol. 3. Eds. Kosuge & Nester. Mc Graw-Hill.

Metraux, J.P., H. Signer, J. Ryals, E. Ward, M. Wyss-Benz, J. Gaudin, K. Schmid, W. Blum, B. Inveradi 1990: Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250: 1004-1006.

Reeney, M.J., L.V. Gusta, S.R. Abrams and A.J. Robertson1989: The effects of abscisic acid, kinetin and gibrellin on freezing tolerance in smooth bromegrass (Bromus inermis) cell suspensions. Can.J.Bot. 67, 3640-3646.

Ryan, C.A., G. Pearce, S. Johnson, B. Mcgurl, M. Orozco-Cardenas and E.E. Farmer 1993: Systemin, a polypeptide signal for proteinase inhibitor gene expression in plants. p196-201 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Schraudner, M., C. Langerbartels, J. Negrel and H. Sandermann 1993: Plant defence reactions induced in tobacco by the air pollutant ozone. p 286-290 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Smedegaard-Petersen, V., D.B. Collinge, H. Thordal-Christensen, J.Brandt, P.L. Gregersen, B.H. Cho, H. Walter-Larsen, H.J. Kristensen and K.Vad. 1992. Induction and molecular analyses of resistance to barley powdery mildew. p 321-326 In: Biological control of plant diseases: Progress and challenge for the future (Eds. E. C. Tjamos, G. Papavizas, R.J. Cook) NATO-ASI Plenum Press, New York.

Thordal-Christensen, H., Brandt, J., Cho, B. H., Gregersen, P., Rasmussen, S.K.,Smedegaard-Petersen, V., and Collinge, D.B., 1992. c-DNA cloning and characterization of two barley peroxidase transcripts induced differentially by the powdery mildew fungus. Physiol. Molec. Plant Pathol. 40, 395-409.

Tronsmo, Anne Marte, 1984a: The effects of hardening, dehardening and freezing on resistance to snow mould fungi in timothy and meadow fescue. In: Proceedings of the 10th general meeting of the European grassland federation. Ås. Eds. Riley and Skjelvåg, 292-296.

Tronsmo, A.M. 1984b: Predisposing effects of low temperature on resistance to winter stress factors in grasses. Acta Agric. Scand. 34: 210-220.

Tronsmo. A. M., 1984c: Resistance to the rust fungus Puccinia poae-nemoralis in Poa pratensis induced by low-temperature hardening. Can. J. Bot. 62, 2891-2892.

Tronsmo, A. M., Kobro, G., Morgenlie, S. og Flengsrud, R.1993a: Carbohydrate content and glycosidase activities following cold hardening in two grassspecies. Physiologia Plantarum. In press.

Tronsmo, A. M., P. Gregersen, L. Hjeljord, T. Sandal, T. Bryngelsson and D. B. Collinge 1993b: Cold induced disease resistance. p369 In: "Mechanisms of Plant Defence Responses." (Eds. B. Fritig and M. Legrand). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Dr.scient. student Marit Sunde
Apelsvoll Research Station, 2858 Kapp, Norway.

EFFECTS OF TEMPERATURE ON FROST HARDENING AND CARBOHYDRATE CONTENT IN TIMOTHY AND RED CLOVER

Introduction

Frostresistant plants get cold acclimated through the energy dependent frost hardening process. The hardening is induced by short days and low temperature in the autumn. Low temperature seems to be more important than short days. Hardening usually occurs when temperature gets lower than 5 - 10 °C, but temperatures below 0 °C are necessary for full hardening. Higher temperatures than 5 - 10 °C are less effective and often cause dehardening of previously hardened plants. Plants may harden, deharden and reharden in response to fluctuating temperatures. Different temperature responses of species and varieties of grasses have been demonstrated for both hardening and dehardening.

The temperature greately influences the accumulation and degradation of total non-structural carbohydrates (TNC) in temperate grasses. The content of carbohydrates increases rapidly when growth rate is declining during autumn. During the winter the content of TNC is gradually reduced.

The aim of this experiment was to investigate the influence of climate on frost hardening and carbohydrate reserves from autumn to spring, in different species and varieties. Another aim was to study if there were correlations between carbohydrate content and the degree of frost resistance.

Material and methodes

Experiments with timothy (*Phleum pratense*) and red clover (*Trifolium pratense*) were carried out during 1990/1991 and 1991/1992 in pots, flats and field trials. Frost hardening was investigated in timothy, and cabohydrate content was determined in timothy and red clover. For both timothy and red clover a southern and a northern variety were used. The southern varieties were 'Grindstad' timothy and 'Molstad' red clover, and the northern were 'Engmo' timothy and 'Bjursele' red clover.

Timothy and red clover were sown last week of May and planted in pure stands in pots or flats last week of June. The flats were 45 by 18 cm and 10 cm high, and timothyplants were planted

2.5 cm apart in the flats. Pure stands of timothy or red clover were planted in big pots, 34 cm in diameter and 40 cm high. In each pot there were ca. 22 plants evenly distributed. 100kg N/ha were applied to timothy after planting. No nitrogen was applied to red clover. The top growth was trimmed to ca. 7cm with a handclipper twice, first time in the beginning of September, and second time the last week of November, just before the pots and flats were moved to different test sites. In October plants were sprayed with Quintozen (Brassicol 75) 10kg/ha to avoid damage of low temperature fungi. Weather recordings at automatic weather stations were made at all experimental sites.

From sowing in May to the end of November all pots and flats were located in inland climate (Apelsvoll, Kapp). During the last week of November, 1/3 of the pots and flats were moved to a coastal climate (Landvik, Grimstad) and 1/3 to a highland site (Løken, Volbu). The rest remained in inland climate at Apelsvoll. Plants from field trials at Vollebekk, Ås (inland to coastal climate) were also included in freezing tests.

At each experimental site pots were placed in furrows in the field with straw between the pots. In this way the plants in the pots stood at the same level as the plants in the field around. The flats were so low that they were placed just on top of the field with straw around. Every month from January till May pots and flats were taken from each site and brought back to Apelsvoll. After the pots and flats arrived Apelsvoll, they were placed at ca. + 4°C for 7 days to thaw. Then it was possible to dig the sample plants out of the soil. The plants were washed in cold water and cut.

Plants for freezing tests were taken from flats and field at Apelsvoll, from flats brought back from Landvik and Løken, and from field at Vollebekk, Ås (inland/coastal climate).

Frost hardening was measured as frost resistance in plants after controlled freezing and regrowth. The plants were washed, cut (1 cm root and 3 cm top) and devided into single ramets. Ten ramets of each variety and site were bundled together. The bundles were put in metal trays, covered with moisty sand and placed in a computer-controlled freezer. From 0 to -3 °C the temperature declined 1 °C per hour, and then the freezer dwelled 6-14 hours (depending on working schedule) at -3 °C. Between -3 and -10 °C the temperature was lowered by 1 °C per hour, whilst from -10 °C the fall was 3 °C per hour.

Eight bundles of each variety and site were needed in every freezing. Bundles were taken out at 7 different temperature levels, with 2 or 3 °C intervals. In addition, one bundle was kept as a control at +1°C.

After removal from the freezer and thawing at +1°C till the next day, the plants were planted in cases and remained for 21 days in greenhouse for regrowth. The frost resistance of the plants was rated visually on a scale from 0 to 9, 0 for dead plants and 9 for normal looking plants. To estimate LD_{50}-values (freezing temperature which killed 50% of the plants) plants with scale values <4.5 were regarded as dead.

There were 10 ramets at each freezing temperature, LD_{50} values were calculated from the scale values of these 10 plants. Samples from fields were taken from two different repetitions in the trials.

Samples for carbohydrate analysis were taken both from timothy and red clover. Every month from January to May one pot of each clover and timothy variety and two flats of each timothy variety were brought in from Landvik, Løken and Apelsvoll for sampling. Some samples were also taken from field in autumn and spring.

For carbohydrate samples of red clover only the taproot was used. In timothy the samples were taken from shoots cut to 3-4 cm. Reducing sugars (glucose and fructose), total sugar (mostly glucose, fructose, sucrose) and fructan were analysed in timothy. Total non-structural carbohydrates (TNC) for timothy is the sum of total sugar and fructan calculated as % glucose of the dry matter. In red clover starch was determined instead of fructan. The total non-structural carbohydrates (TNC) for red clover is the sum of reducing sugar (glucose and fructose), sucrose, higher watersoluble carbohydrates and starch, calculated as % glucose of the dry matter content.

Results and discussion

So far only results from Apelsvoll and Løken are ready for presentation. In this preliminary presentation the data are just visualized through graphs.

Freezing tests

Frost hardiness was measured as LD_{50} temperatures after freezing tests from August 28 (week 4) to May 6 (week 40). There seems to to be good correlation between falling temperature and increasing frost hardiness, and between rising temperature and dehardening. The positive correlation between temperature and frost hardening (LD_{50} values) seems to exist during the whole periode (Aug. to May) at both Apelsvoll and Løken. LD_{50} values at both sites were quite similar during the winter (fig. 1 and 2).

The frost hardiness was at the same level in May as in August. At this time there was little difference in frost hardiness between the two varieties, LD_{50} values were around -6 to -7 °C for both. Between August and May the northern variety of timothy 'Engmo' was more hardy than the southern variety 'Grindstad' (fig.1 and 2).

Fig. 1. Frost resistance (LD50 temperature) in timothy, weekly temperature and %TNC (total non-structural carbohydrates) in timothy and red clover at Apelsvoll from Aug. 28 (week 4) 1991 to May 6 (week 40) 1992.

Timothy:	'Engmo'- - - - - 'Grindstad'——
Red clover:	'Bjursele'····· 'Molstad' — · — ·
TXX(+):	Weekly maximum temp. (2 m)
TD (0):	Weekly average temp. (2 m)
TNN (-):	Weekly minimum temp. (2 m)

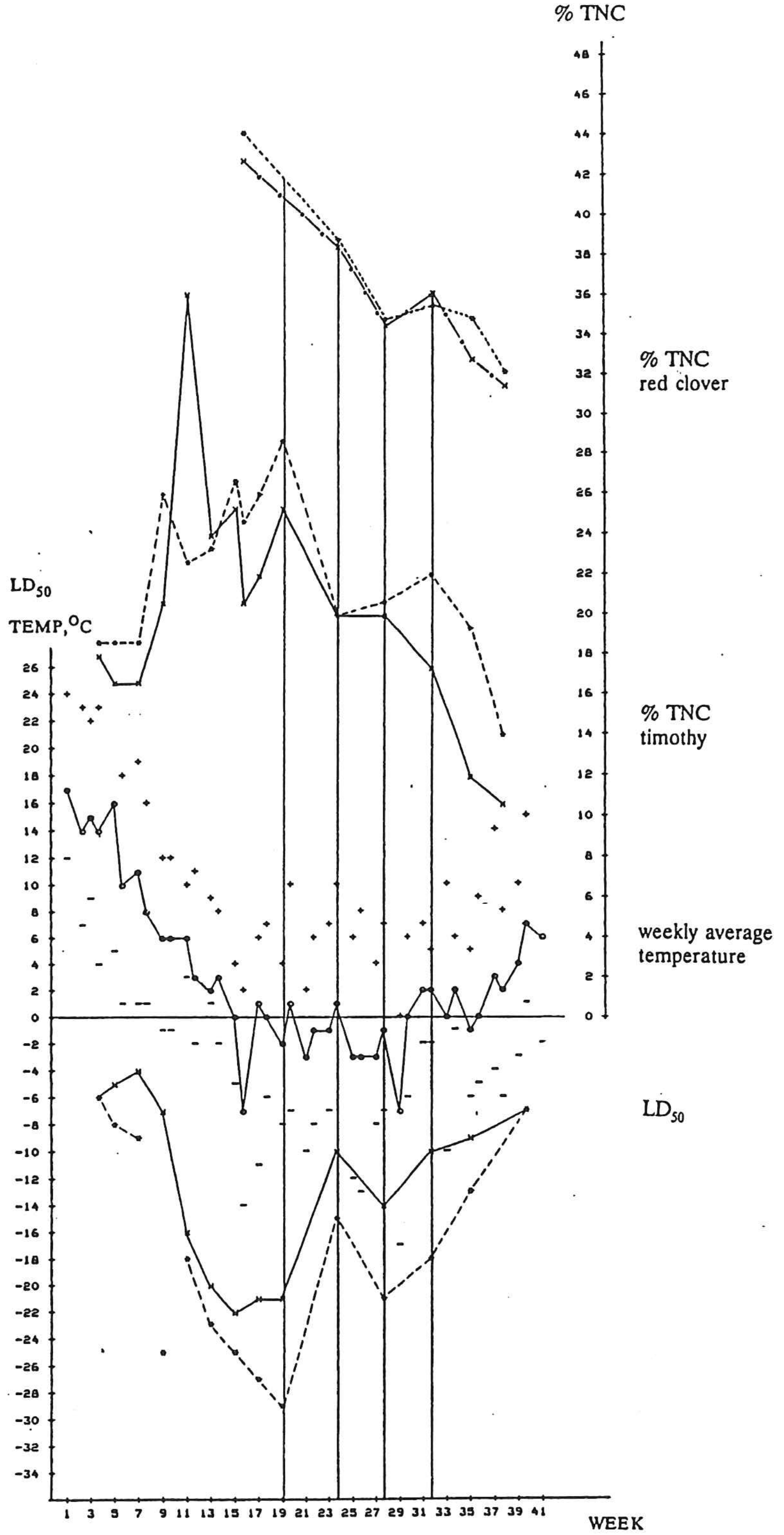
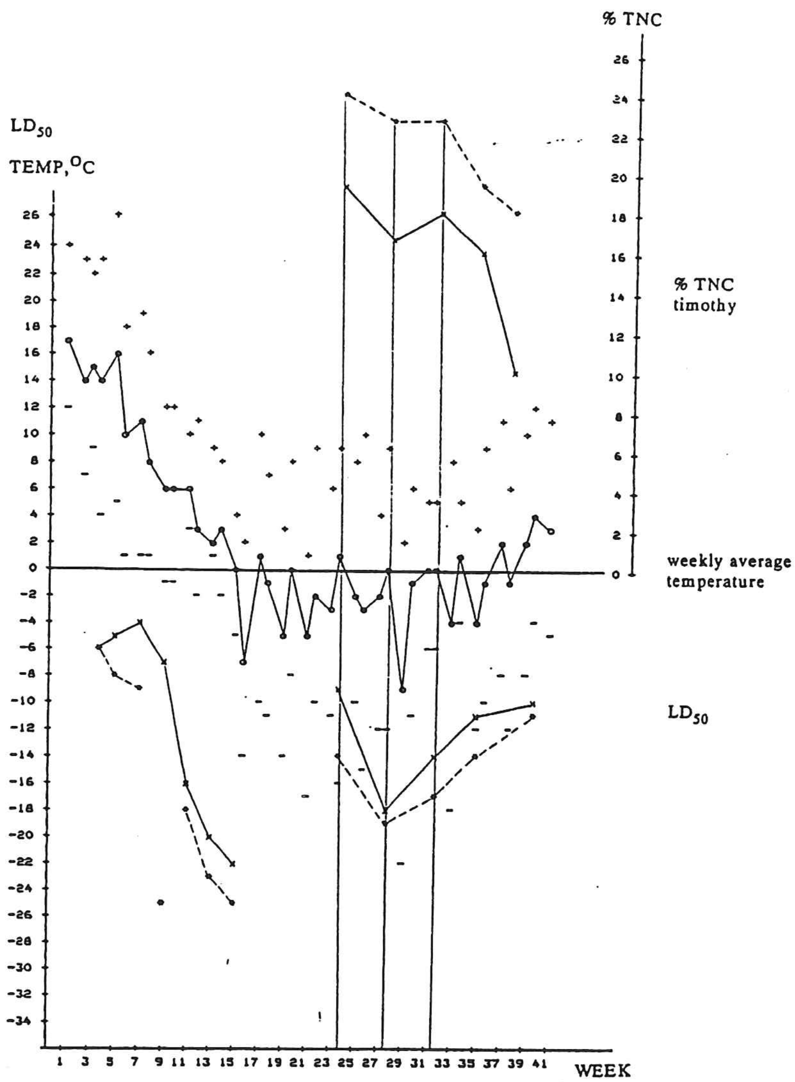


Fig. 2. Frost resistance (LD50 temperature) in timothy, weekly temperature and % TNC (total non-structural carbohydrates) in timothy at Løken from Jan. 14 (week 24) 1992 to May 6 (week 40) 1992. The plants were moved from Apelsvoll to Løken in week 16 (Nov. 20) 1991.
Timothy: 'Engmo' ------ 'Grindstad'——
TXX (+): Weekly maximum temp. (2 m)
TD (0): Weekly average temp. (2 m)
TNN (-): Weekly minimum temp. (2 m)



Both temperature and LD_{50} values were falling from week 4 to week 19. 'Grindstad' reached maximum hardiness (LD_{50} = -22.2 °C) in week 15, whereas 'Engmo' achieved maximum (LD_{50} = -28.8 °C) in the middle of December (week 19). Two weeks with very high weekly maximum-temperature (+9.6°C in week 20 and +9.7°C in week 24) might have caused the dehardening from week 19 to week 24. The rehardening from week 24 to week 28 seems to be associated to low weekly minimumtemperatures in week 25 (-12.4 °C) and week 26 (-13.2 °C). From week 29 both weekly temperature and LD_{50} values are increasing (fig. 1).

Fig. 3. % TNC (total non-structural carbohydrates) in timothy at Apelsvoll (from Nov. 16, week 16, 1990 to May 13, week 41, 1991) and at Løken (from Jan. 15, week 24, 1990 to april 22, week 38, 1991)
'Engmo' Apelsvoll ------ 'Grindstad' Apelsvoll ——
'Engmo', Løken ······· 'Grindstad' Løken — · — ·

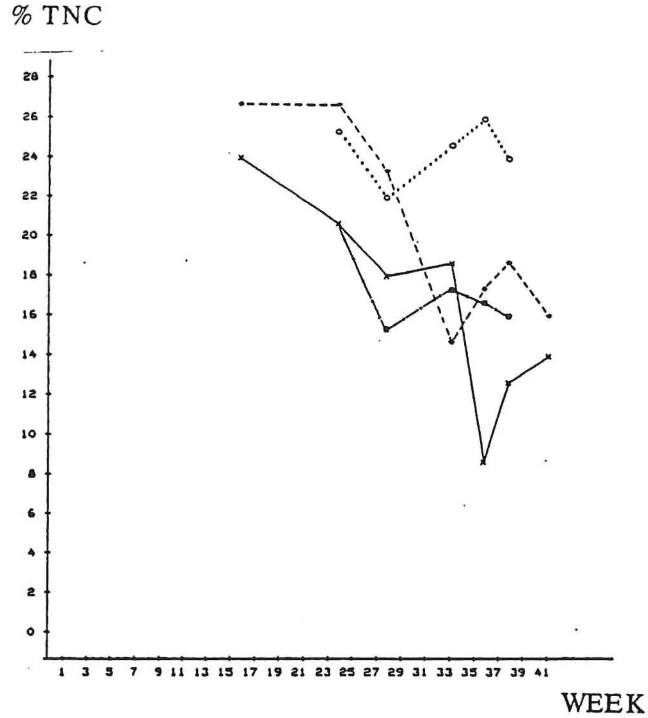
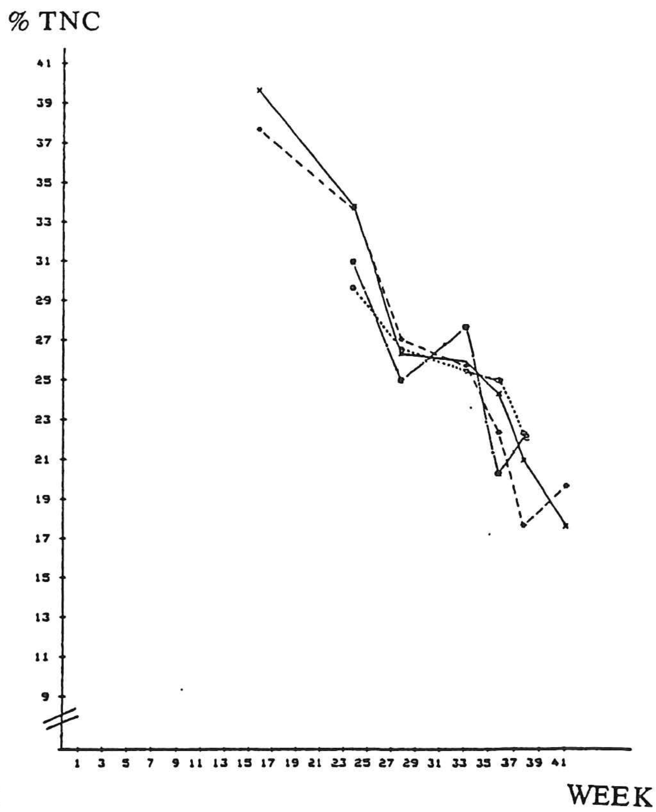


Fig. 4. % TNC (total non-structural carbohydrates) in red clover at Apelsvoll (from Nov. 16, week 16, 1990 to May 13, week 41, 1991) and at Løken (from Jan. 15, week 24, 1990 to april 22, week 38, 1991)
'Bjursele' Apelsvoll ------ 'Molstad' Apelsvoll ——
, Løken ······· Løken — · — ·



Carbohydrate content

There have been substantial losses of % TNC (total non-stuctural carbohydrates) in timothy and red clover from December (week 19) to May both at Apelsvoll and Løken (fig. 1,2,3). Although there was a decline in % TNC from autumn to spring, the graphs fluctuate with a peak in week 32, the beginning of March, with exception of 'Grindstad' at Apelsvoll (fig.1 and 2).

At both Apelsvoll and Løken, and in timothy as well as red clover, rising temperature reduced % TNC. During periodes with high weekly maximumtemperature (week 19 to 24) or increasing temperature (week 32 to 38) it seems like carbohydrates were degraded both in timothy and red clover (fig. 1 and 2).

Red clover had higher % TNC from autumn to spring than timothy at both Apelsvoll and Løken (fig. 1 and 4).

At Apelsvoll % TNC was analysed from early autumn (fig. 1). From week 4 to week 9 % TNC increased rapidly in both 'Engmo' and 'Grindstad' probably as a result of declining growth rate.

'Engmo' timothy, the most hardy variety, had generally higher carbohydrate content (% TNC) during autumn and winter than 'Grindstad' both 1990/91 and 1991/92 at Apelsvoll and Løken (fig.1,2 and 3). 'Engmo' is a northern variety and the growth terminate earlier than the growth of 'Grindstad'. When the growth rate declines and the temperature still is high enough for photosynthesis, carbohydrates can be stored in the plants. This can probably explain why 'Engmo' has a higher carbohydrate content than 'Grindstad'.

At Apelsvoll 'Engmo' had both higher %TNC and better frost resistance than 'Grindstad' (fig.1). 'Engmo' at Løken had also higher %TNC than 'Grindstad', but there was little difference in frost resistance between the two varieties (fig.2).

There seems also to be a negative correlation between carbohydrate content and hardening in timothy and red clover (fig.1 and 2). In periodes with increasing temperature and degradation of carbohydrates frost resistance is also reduced.

Conclusions

* There was an increase in frost hardiness (LD_{50} temperatures) as airtemperature fell during the autumn.

* The northern timothy cultivar 'Engmo' got lower LD_{50} temperatures than the southern cultivar 'Grindstad'.

* There were substantial losses of total non-structural carbohydrates (%TNC) from late autumn to spring in both timothy and red clover.

* Temperature rises during the winter seems to reduce %TNC in both timothy and red clover.

* A high level of %TNC seemed to be associated to high frost hardiness.

References

Eagles, C.F. 1989. Temperature-induced changes in cold tolerance of Lolium perenne. Journal of Agricultural Science, Cambridge 113:339-347.

Halling, M. 1988. Growth of Thimothy and Red Clover in Relation to Weather and Time of Autumn Cutting. Swedish J.agric.Res. 18:161-170.

Tronsmo, A.M. & A. Larsen. 1991. Utvikling av frostherdighet hos gras under naturlige klimaforhold. SFFL, FAGINFO 2:267-274.

Stig Larsson
Svalöf Weibull AB
S-268 81 Svalöv, Sverige

SOLUBLE SUGARS AND MEMBRANE LIPIDS IN WINTER WHEAT DURING COLD ACCLIMATION

Introduction

Already seventy years ago it was shown that sugar was a good indicator of cold tolerance of hardened winter crops. Comparative studies of sugar content and cold hardiness in wheat, assessed by artificial freezing tests and observations of winter survival in field was performed. It has also recently been found that membrane lipids, primarily those in the plasma membranes, are intimately involved in cold acclimation and freezing tolerance. The aim of the present study was to determine if any single component, or any combination of components of sugars and lipids, could be used as an indicator of cold hardiness to select hardier plant material of winter wheat.

Plant material

Fourteen cultivars of winter wheat of different origin and winter hardiness were used. These cultivars have been tested for cold hardiness in an artificial freezing test according to Larsson (1986). The cold hardiness ranged from very hardy, including the cultivars Norstar from Canada, Varma, Vakka and Aura from Finland, and the old Swedish cultivar Svea I, to those with medium to low hardiness, including the Swedish cultivars Holme, Hildur and Solid.

Methods

The young leaves, at the two-leaf stage, was cold acclimated (hardened) in a climate room at +2°C during 30 days. Analysis of soluble sugars and different lipid components were made from samples taken a) before the acclimation period, b) after 10 days of acclimation, c) after 30 days of acclimation and d) after 4 days of de-acclimation at +20°C (Larsson et al. 1992).

Results

The content of soluble sugars doubled during the hardening period, from 78 to 142 mg/g dw. The most drastic change was found in raffinose. Glucose, sucrose, raffinose and the total sugar content were all significantly correlated with cold hardiness. After 30 days of acclimation 84 per cent of the variation in cold hardiness could be explained by the total sugar content. The separate lipid components were only slightly correlated with the cold hardiness. Using multiple regression, higher correlation with cold hardiness were found for lipids and sugars together than for any single component. More than 75 per cent of the variation in cold hardiness could be explained by a combination of the amounts of linolenic acid, palmitic acid, sucrose and fructose already present before the acclimation period. These results indicate it may be possible to test for cold hardiness by analysis of some chemical key substances, without using expensive equipments for cold acclimation and freezing tests.

References

Larsson S. (1986). New screening methods for drought resistance and cold hardiness in cereals. In: Olsson, G (Ed.). Svalöf 1886-1986. Research and results in plant breeding. Stockholm, LTs förlag.
Larsson S., Johansson L-Å. and Svenningsson M. (1992). Soluble sugars and membrane lipids in winter wheats (Triticum aestivum L) during cold acclimation. - Eur. J. Agron: 1(2), 85-90.

METABOLIC AND CELLULAR IMPACT OF ICE ENCASEMENT ON HERBAGE PLANTS

B. E. GUDLEIFSSON
Agricultural Research Institute
Modruvellir Experimental Station
601 Akureyri
ICELAND

ABSTRACT

In northern maritime areas, with high winter precipitation and unstable winter temperatures fluctuating around zero, thaw water and precipitation freezes to form an ice layer on the ground, which is highly impermeable to gases. Litterature on the impacts of ice encasement on the metabolism and cellular damages of herbage plants is reviewed. Plants encased in ice are forced to anaerobic respiration and metabolites are formed, mainly CO_2, ethanol, lactate and malate. These metabolites are potentially toxic to the ice encased plants and primarily damage the plasma membrane. The primary site of injury on the membrane is the ion transport system while the semepermeable properties of the lipid bilayer is damaged at a higher stress level.

INTRODUCTION

Research activity on winter stresses has concentrated mostly on freezing injury to plants, indicating that freezing damage is often considered more important and having greater impact on agriculture than other winter stresses. However, in some areas, ice encasement damage is commonly experienced by herbage plants. This kind of damage has repeatedly been reported to cause large economic losses and to be a practical problem to local farmers (Brink et al., 1939; Rakitina, 1965; Andersen, 1971; Gudleifsson, 1975a, 1975b, 1989). Ice crusts may form over fields at different times during autumn, winter or spring. Most frequently, ice cover occurs when snow melts, and thaw water accumulates on the surface, especially in depressions in the landscape (Gudleifsson, 1975a; Andersen, 1976). At the end of thaw, the water refreezes to form an ice layer which is highly impermeable to gases (Hemmingsen, 1959). These weather conditions are frequently experienced in northern maritime areas with high winter precipitation and unstable winter temperatures fluctuating around zero (Gudleifsson and Larsen, 1992). Larger or smaller areas of field can be covered for different durations with layers of ice of varying thickness with resulting ice encasement damage to the plants beneath (Jónsson, 1938; Friðriksson, 1954; Andersen, 1963; Woehrle, 1963; Beard, 1964; Gudleifsson, 1971; Årsvoll, 1973; Andrews and Pomeroy, 1975; Hakamata et al., 1978; Andrews et al., 1986).

The expected global warming might reduce the probability of ice encasement damage at the locations where it dominates today. However, the expected temperature rise may also increase the probability of winter thaws and subsequent ice encasement at locations currently dominated by stable snow cover.

Plants encased in ice are readily killed by freezing temperatures that do not affect the survival of unencased plants. Nevertheless freezing is not believed to be the main cause of plant death under ice (Gudleifsson and Larsen, 1992). The effects of ice encasement in many ways are similar to those of flooding since both can impose anaerobic stress. Flooding induces the accumulation of metabolites in winter cereals similar to those also observed during icing. Although metabolic rates are slowed at the depressed temperatures of ice encasement, the development of anoxia is faster than in flooding, because of the high impermeability of ice to gases, and the absence of continous leaching of metabolites that occurs in flooding (Andrews, 1977; Pomeroy and Andrews, 1979; McKersie et al., 1982). Ice encasemant is therefore a more severe type of stress than flooding.

METABOLIC IMPACTS OF ICE ENCASEMENT

Plants encased in ice are exposed either to partial or to complete oxygen deprivation (Rakitina, 1970). As a result, their normal pathways of respiration are restricted or blocked and pathways of anaerobic respiration predominate. This results in production of potentially toxic metabolites and a decrease in production of metabolic energy (ATP). Early studies indicated that plants encased in ice become depleted of oxygen and accumulate CO_2 (Sprague and Graber, 1940, 1943; Freyman and Brink, 1967; Rakitina, 1970) in association with reduced survival (Rakitina, 1965). Later it was demonstrated that ethanol (Andrews, 1977; McKersie et al., 1982), lactate (Pomeroy and Andrews, 1978a) and malate (Andrews and Pomeroy, 1983) also accumulate during ice encasement (Fig. 1).

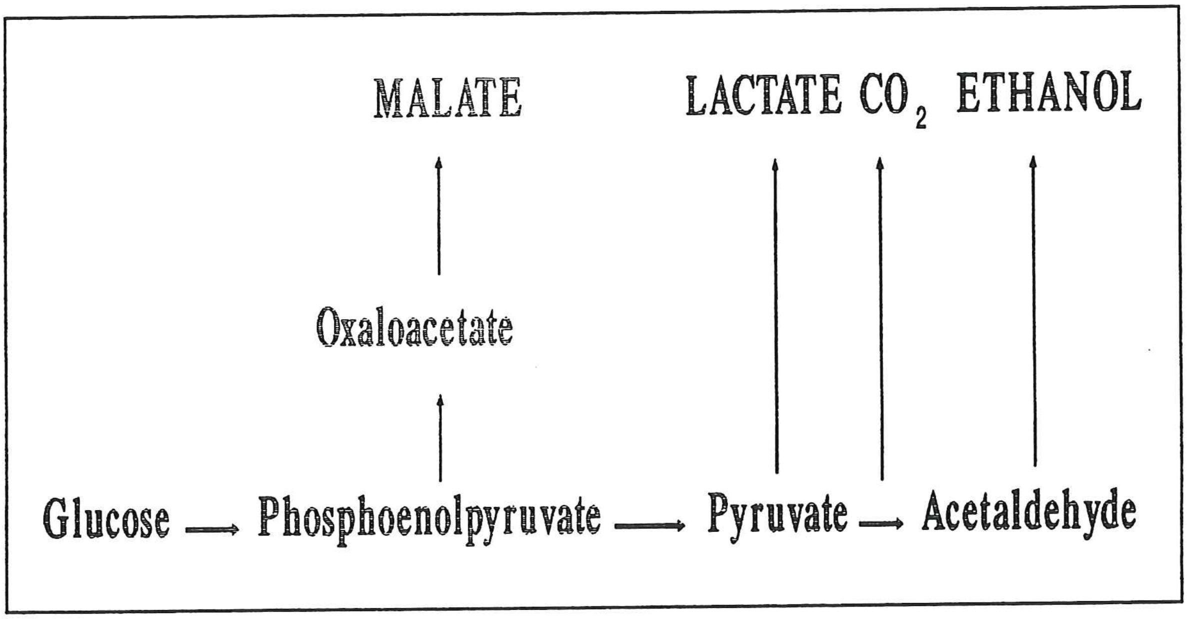


FIGURE 1. Summary diagram illustrating the major end products in anaerobic respiration (based on Crawford, 1978).

During ice encasement herbage plants utilize storage carbohydrates accumulated during prior hardening (McKersie et al., 1982; Andrews et al., 1984). McKersie et al., (1982) and Pomeroy and Andrews (1983b) found that levels of non-structural carbohydrates and ethanol-soluble carbohydrates declined during ice encasement of winter cereals while plant content of reducing sugars increased (Fig. 2). In winter cereals the increase in reducing sugars results primarily from an accumulation of fructose (Pomeroy and Andrews, 1983b). The loss of plant viability was not associated with depletion of carbohydrate reserves. Gao et al., (1983) and McKersie et al., (1982) obtained no evidence to indicate that survival, cold hardiness or ice encasement tolerance were directly related to carbohydrate levels.

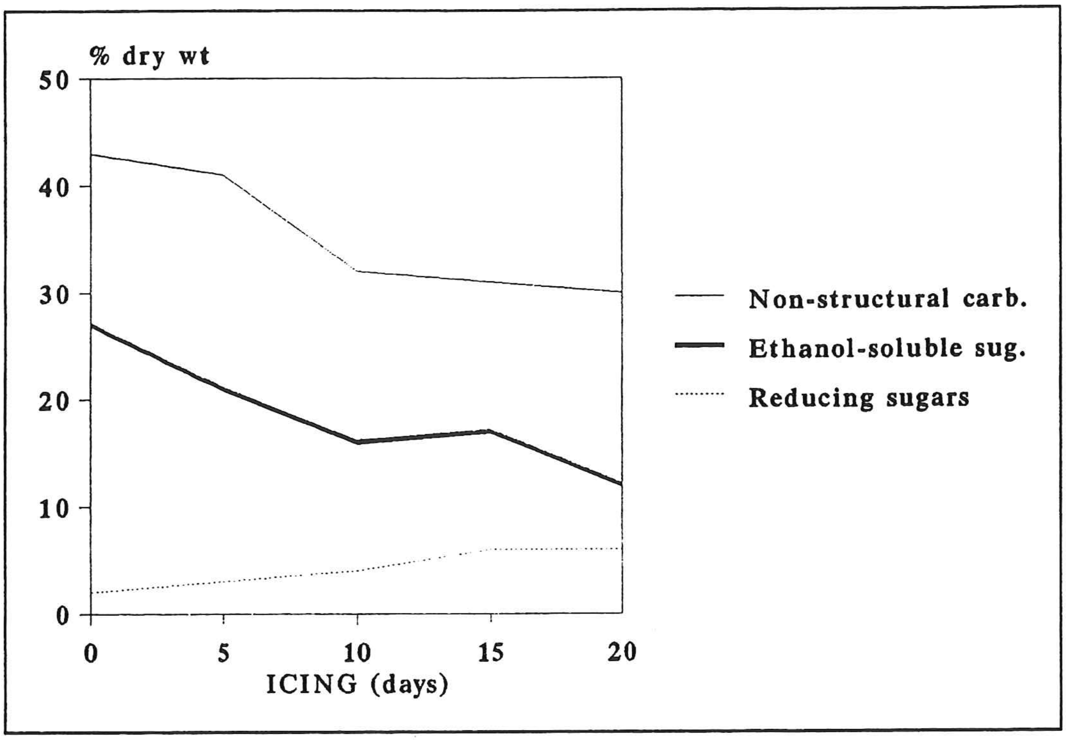


FIGURE 2. Effect of ice encasement on non-structural carbohydrates, ethanol-soluble sugars, and reducing sugars from crowns of cold acclimated Fredrick winter wheat (from Pomeroy and Andrews, 1983b, with permission).

Tissues of plants encased in ice are CO_2-enriched and O_2-impoverished, and Rakitina (1970) demonstrated that CO_2 accumulates more rapidly than oxygen is utilized. This was apparent on the first day of ice encasement at -5°C. After 5 days, the CO_2 content increased by a factor approximately 100 while oxygen decreased by 3-6. Freyman and Brink (1967) demonstrated that flushing the soil of ice encased plants or plants in tubes with CO_2 was injurious. Sprague and Graber (1943), working with alfalfa, found a direct relationship between CO_2 concentration and the injury sustained. Freyman and Brink (1967) and Rakitina (1970) concluded that injury to plants under an ice crust occurs not as a result of oxygen deficiency but because of the injurious action of CO_2 and other products of anaerobic metabolism.

Ice encasement of field plants reduces respiration and increases ethanol, which decreases again during the spring thaw

(Pomeroy and Andrews, 1978a). Andrews (1977) measured tissue and leachate ethanol after ice encasement of winter cereals. Concentrations on fresh weight basis was higher in crowns than roots or leaves and at LD_{50}, the ethanol concentration in crowns was highest in the hardiest cultivars.

Non iced field plants did not accumulate ethanol but accumulated lactate (Fig 3). The lactate concentration in field plants increases in early autumn, prior to the occurrence of significant environmental stress, indicating that lactate accumulation is not associated with stress damage (Pomeroy and Andrews, 1978a). Lactate does not accumulate to sufficiently high level in ice to account for plant death (Andrews and Pomeroy, 1979).

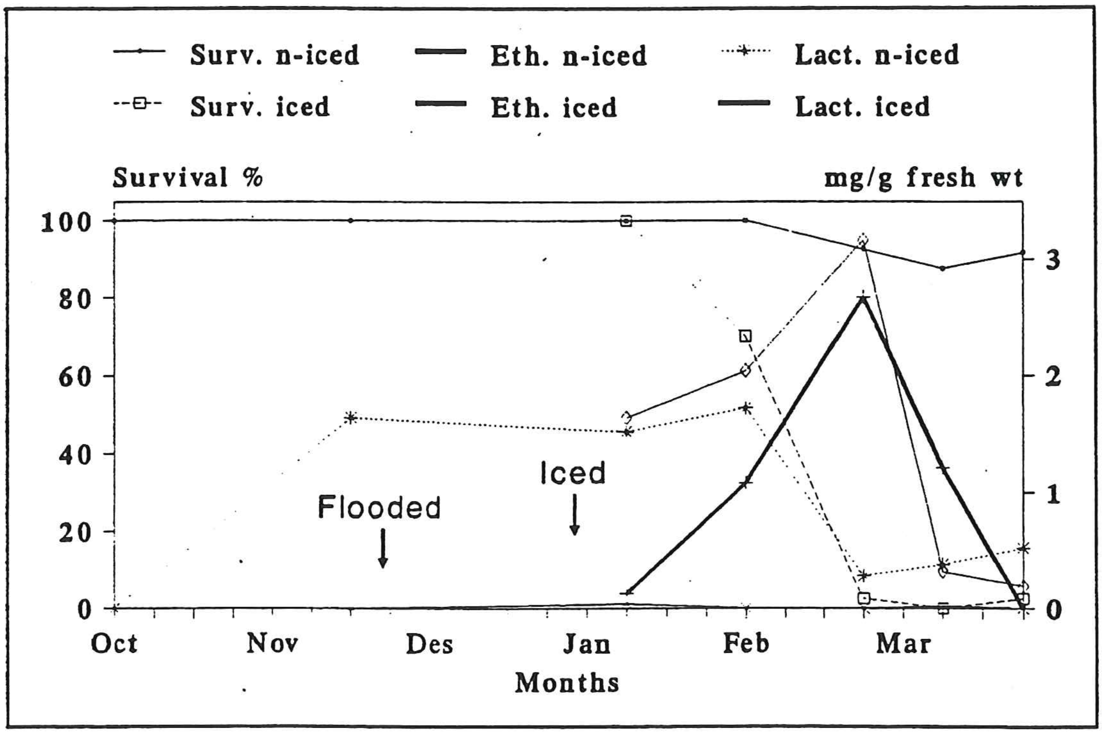


FIGURE 3. Effect of flooding and ice encasement under field conditions on survival and on ethanol and lactic acid levels in crowns of Fredrick winter wheat (redrawn from Pomeroy and Andrews, 1983a, data from Pomeroy and Andrews, 1978a, with permission).

Andrews and Pomeroy, (1983) found some malate accumulating in ice-encased winter cereal plants. This malate did not substitute for ethanol and sterilization of plants had no effect, indicating that malate was not of bacterical origin. On the other hand, McKersie et al., (1982) have demonstrated that winter cereal crowns deplete malate under icing condition. Gudleifsson (1986) demonstrated that timothy plants produce CO_2, ethanol, lactate and malate during ice encasement. Only the malate accumulation was higher in timothy than observed in winter cereals.

Comparison of the balance between carbohydrate depletion and metabolite accumulation (ethanol, CO_2, lactate) did not indicate accumulation of any major unidentified metabolite in winter wheat (Pomeroy and Andrews, 1983b). On the other hand McKersie et al. (1982) calculated the balance between carbohydrates broken down and metabolites produced. They concluded that the hardiest winter wheat cultivars must produce unidentified metabolites in addition to CO_2 and ethanol. Lactate might be one of them, and based on work with flood tolerant plants others could include succinate, σ-aminobutyrate and alanine (Crawford, 1978; Zemlianukhin and Ivanov, 1978). Accumulation of these products in herbage plants during ice encasement has not been examined. In some cases, a strong odour has been observed immediately after the thaw of ice from grasslands (Woehrle, 1963; Gudleifsson, 1977; Gudleifsson et al., 1986). This odour might originate from some of the substances produced by plants or soil when anaerobic under the ice sheet. Butyric acid has been isolated from ice encased timothy plants, but this is probably of bacterial origin (Gudleifsson, 1986).

CELLULAR DAMAGE DURING ICE ENCASEMENT

Plants killed by freezing loose their structure, tissues become flaccid, discoloured and water soaked (Palta et al., 1978; Steponkus, 1984). Microscopic studies during and after cooling to lethal, subfreezing temperatures show cell collapse and destruction (Pomeroy and Andrews, 1978b; Levitt, 1980; Singh and

Andrews, 1981). On the other hand plants killed by ice encasement often look healthy after ice escape (Gudleifsson, 1979, 1986) but wilt and die gradually during regeneration (Tanino and McKersie, 1985).

Tanino and McKersie (1985), looking at tissue viability in wheat crowns, demonstrated that most crown cells seemed to survive icing stress that was lethal to the plant. After lethal icing stress, the crown was unable to regrow and cell viability, measured as the ability to reduce triphenyltetrazolium cloride (TTC), was gradually lost during the regrowth period. Thus, in winter cereals, no specific regions within the crown were more susceptible to injury than other (Tanino and McKersie, 1985), but in timothy (Gudleifsson and Larsen, 1992) the extreme apex of the crown is the first part to be killed during ice encasement, while the basal region survives for some time after the plant has lost its ability to regenerate. The last part of the crown tissue to die is the intermediate region. The death of the apex could explain why plants fail to regrow after ice encasement, even though most crown cells survive.

Singh and Andrews (1981) observed no major structural changes in winter wheat cells during lethal ice encasement at -1°C, while during lethal freezing, cell collapse and ultrastructural dissolution of membranes were observed. This difference between freezing and ice encasement damages demonstrate that cell death during ice encasement results not from the disruptive mechanical and dehydrative stresses produced by ice formation during freezing (Pomeroy and Andrews, 1978b; Singh and Andrews, 1981). After ice encasement of field grown winter wheat plants and plants grown in controlled environments, Pomeroy and Andrews (1978a, 1978b) and Andrews and Pomeroy (1979) observed characteristic ultrastructural cellular changes in early stages of ice encasement. The changes included proliferation of the ER membrane system, wich often resulted in formation of concentric whorls of parallel membranes. They also observed membrane-bound electron-dense areas within the cytoplasm which appeared to be associated with the expanded ER. The structural integrity of many cellular organelles was relatively unaffected by ice encasement, but after 7 days of ice encasement of plants grown in controlled environments, disorganisation of internal mitocondria membranes were observed and after 14 days, when plants were killed, separation of the inner and outer membranes of the nuclear envelope and formation of large pores was observed. These ultrastructural changes disappeared rapidly in surviving plants during recovery (Pomeroy and Andrews, 1977).

By using freeze-fixation technique, Singh and Andrews (1981) were able to study cells during ice encasement. They observed neither dramatic disruption of the protoplasm nor destruction of membrane ultrastructure during the stress. During longer periods of ice encasement numerous vesicles appeared in the protoplasm, but no reorganization of the endoplasmic membranes to form concentric rings. The changes, observed by Pomeroy and Andrews (1978a, 1978b) and Andrews and Pomeroy (1979), therefore seem to happen during the post-thaw period. Singh and Andrews (1981) concluded that neither mechanical effects of encasing ice nor dehydration are factors in lethal injury during ice encasement. This indicates that death as a result of ice encasement is not a result of mechanical stress but rather a result of biochemical stress, related to an accumulation of metabolites.

To test the toxicity of the metabolites accumulated during anaerobic respiration, Andrews (1977) and Andrews and Pomeroy (1977b) exposed winter cereal plants to various concentrations of metabolites at -1°C. Plant survival was reduced by separate exposure of ethanol, lactate and CO_2 , and when all three were supplied together plants were killed at rather low concentrations. Comparison was made of the concentrations killing plants in external exposure and the concentrations measured in ice. Winter cereal crowns tolerated about 3-4 times higher external ethanol and lactate concentrations than the concentrations produced in ice, indicating that these metabolites, although potentially toxic, are not the only damaging factors in ice encasement. On the other hand the crowns tolerated much lower concentrations of CO_2 at metabolite exposure than in ice exposure (Table 1), indicating more significant role of CO_2 in ice encasement damage than for ethanol or lactate (Andrews and Pomeroy, 1979, 1990).

Exposure to external lactate alone (0,5%) had little effect on cell ultrastructure. Ethanol (10%) caused proliferation of membrane whorls and nuclear rearrangement. CO_2 (100%) did not induce proliferation of membrane but induced a condensed form of the nucleus (Andrews and Pomeroy, 1979). Exposure to CO_2 and ethanol simultaneously showed proliferation of membranes and nuclear condensation similar to that in ice encased plants and the membrane permeability increased markedly. It was concluded that cell damage caused by combined effect of accumulated ethanol and CO_2 occur at the membrane level and change permeability and destroy membrane functions.

TABLE 1. Comparison of the endogenous metabolic concentration at the 50% kill point of cold hardened seedlings of Fredrick winter wheat induced by ice encasement, or exposure to the exogenous metabolite (from Andrews and Pomeroy, 1979, with permission).

Metabolite	LD_{50} ice exposure	LD_{50} metabolite exposure
	mg/g fresh wt.	
Ethanol	2.94	14.01
Lactate	0.67	2.17
CO_2	2.45	1.24

Hetherington et al. (1987) found that electrolytic leakage increased during ice encasement, along with a decline in total microsomal protein and phospholipids, suggesting some membrane degradation. At the same time, increase in membrane microviscosity of microsomes and liposomes was observed. The increased microviscosity might be caused by changes in the lipid component or increase in free fatty acid level. These processes continued through a 6 hour post thaw period, but in contrast to the anoxic phase, the degree of fatty acid unsaturation declined markedly, indicating lipid peroxidation. During ice encasement of winter wheat plants Hetherington et al. (1987, 1988) observed two to four fold increase in the free fatty acid level of microsomal membranes and minor reduction in total fatty acid unsaturation. The composition of the free and total fatty acids did not change significantly nor did the amount of different phospholipid classes, indicating that degradation is nonspesific. Membrane injury apparently involves hydrolysis of the ester bond between glycerol and the acyl groups of the phospholipid, resulting in loss of the phosphate-containing polar head group and a concomitant accumulation of free fatty acids in the bilayer. Lipolytic enzymes may be responsible for this while free radicals possibly perturbate enzymes involved in electron transfer reactions.

Gao et al. (1983), working with winter wheat subjected to various low temperature stresses, obtained indications that stress induced metabolic changes occured before applied stress is severe enough to reduce survival. By ice encasment or ethanol treatment of isolated winter wheat cells Pomeroy et al. (1983) demonstrated that passive efflux of amino acids increased gradually but uptake of ^{86}Rb, an analogue to potassium, declined much more rapidly. Electron-spin resonance studies revealed no major changes in molecular ordering within the cell membranes following these treatments. Hetherington et al. (1988) demonstrated that membrane damage, detected as increase in microviscosity and electrolyte leakage or loss of semipermeability, started at early stage of ice encasement. Pomeroy et al. (1983) and Pomeroy and Andrews (1985), working with cell suspensions at -1°C, demonstrated on the other hand that ice encasement resulted in decline in ion uptake before any increase in ion efflux and decrease in cell viability could be detected. Thus, during ice encasement, decline in membrane transport capacity approximates with the decline in survival of intact plants or crowns, while reduction in cell viability and ion efflux occur at higher stress levels. This demonstrates that the ion transport system of these cells is the primary site of injury due to ice encasement and that damage to the ion pump system is prior to damage to the bilayer.

The ATPase enzymes of the plasma membrane might be rapidly inhibited by metabolites accumulating within tissues in the early stages of ice encasement. Working with mitochondria Andrews and Pomeroy (1977a) found a slow decline in respiratory properties during ice encasement and the activity was little impaired even when 50 % of the plants were killed. Tanino and McKersie (1985) also observed that mitochondrial activity, measured as TTC reduction, was high after ice encasement lethal to plants but was gradually reduced during regeneration. Although oxygen consumption is high in ice encased plants, the ATP supply could be low and inhibit activity of energy-dependent ATP-ase membrane ion pump, thus explaining damage to the ion transport system. During ice encasement there is a general decline in adenylate energy charge (AEC) and total adenylates (Andrews and Pomeroy, 1989b). Pomeroy and Andrews (1986) noted that the levels of the three nucleotides (ATP, ADP, AMP) decreased gradually in approximate relation to a decline in cell viability, but AEC remained high even when cell viability was severly reduced (Andrews and Pomeroy, 1989b). The reduction in ion uptake in ice is therefore not due to decreased levels of ATP or to a decline in AEC system of the cell, as is the case in anoxia, nor can it be attributed to a shortage of carbohydrate energy substrates (Gao et al., 1983). Fermentation processes provide sufficient substrate level ATP to maintain high energy charge and to maintain synthetic functions in surviving cells (Andrews and Pomeroy, 1990). Inhibition of enzyme activity or denaturation by rapidly accumulating anaerobic products remains as a possibility.

The damaging effect of CO_2 is supported by the fact that carbonate and bicarbonate ions reduce cell survival and promote amino acid leakage from cells more rapidly than anoxia alone (Table 2). The membrane ATP-ase activity is strongly inhibited by carbonate and bicarbonate ions but much less so by ethanol and lactic acid (Andrews and Pomeroy, 1989a, 1990). This indicates that CO_2 might be the main cause of damage to membrane ion pumps during ice encasement stress. Ten millimolar suspension of Ca^{++} protects isolated cells against damage (Table 2) and also reverses the inhibitory effect of ice exposure on the ^{86}Rb uptake system (Pomeroy and Andrews, 1985; Pomeroy and Andrews, 1986) while other cations such as Li^{++} and Mg^{++} do not. Calcium seems to have stabilizing effect on the plasma membranes (Andrews and Pomeroy, 1989a).

TABLE 2. Survival of isolated cells of winter wheat in various treatments at -1°C. Gas and ice treatments in 30 mM Tris Mes, pH 6.5, Ca^{++} at 10 mM. Bicarbonate and carbonate treatments in 100mM Tris Mes. Survival as percentage of freshly isolated cells (from Andrews and Pomeroy, 1989a, with permission).

Treatment	7 day	14 day
Air	93	87
Air + Ca	92	85
N_2	86	74
N_2 + Ca	86	77
CO_2	61	21
CO_2 + Ca	72	42
Iced	81	60
Iced + Ca	92	83
$KHCO_3$ 100 mM	77	51
$KHCO_3$ 500 mM	58	16
K_2CO_3 100 mM	68	27
K_2CO_3 500 mM	0	0

These results indicate that the plasma membrane is an early target and a primary site of injury due to ice encasement and that damage to the ion transport system is the earliest manifestation of this injury. This happens prior to injury of the semipermeable properties of the cell and prior to significant losses of cell viability or to changes in other physiological or metabolic properties of the cell, and is partially reversible by calcium.

REFERENCES

Andersen IL (1963) Investigation on the wintering of meadow plants in Northern Norway II. Some investigations on damages caused by ice and water choking on meadows (Overvintrings undersökelser i eng i Nord-Norge. II. Noen undersökelser over is- og vannskader i eng). Forskning og forsök i landbruket 14; 639-669.

Andersen IL (1971) Experiments on winter damages on different grass species (Overvintringsforsök med ulike grasarter). Forskning og forsök i landbruket 22; 121-134.
Andersen IL (1976) Winter damages on grasslands (Overvintrings skader på eng). Aktuelt fra Landbruksdepartementets opplysningstjeneste Nr. 2 1976; 118-126.
Andrews CJ (1977) Accumulation of ethanol in ice-encased winter cereals. Crop Science 17; 157-161.
Andrews CJ, Pomeroy MK (1975) Survival and cold hardiness of winter wheats during partial and total ice immersion. Crop Science 15; 561-566.
Andrews CJ, Pomeroy MK (1977a) Mitochondrial activity and ethanol accumulation in ice-encased winter cereal seedlings. Plant Physiology 59; 1174-1178.
Andrews CJ, Pomeroy MK (1977b) The effect of anaerobic meta bolites on survival and ultrastructure of winter wheat in relation to ice encasement. Plant Physiology 61 Supplement 17.
Andrews CJ, MK Pomeroy (1979) Toxicity of anaerobic metabolites accumulating in winter wheat seedlings during ice encasement. Plant Physiology 64; 120-125.
Andrews CJ, Pomeroy MK (1983) The influence of flooding pre treatment on metabolic changes in winter cereal seedlings during ice encasement. Canadian Journal of Botany 61; 142-147.
Andrews CJ, Pomeroy MK (1989a) Ice-encasement injury at the cellular and membrane level. Icelandic Agricultural Sciences 2; 57-61.
Andrews CJ, Pomeroy MK (1989b) Metabolic acclimation to hypoxia in winter cereals. Low temperature flooding increases adenylates and survival in ice encasement. Plant Physiology 91; 1063-1068.
Andrews CJ, Pomeroy MK (1990) Low temperature anaerobiosis in ice encasement damage to winter cereals. In: Jackson MB, Davies DD, Lambers H (eds) Plant life under oxygen deprivation. SPS Academic Publishing, The Hague, pp 85-99.
Andrews CJ, Pomeroy MK, Seaman WL (1986) The response of fall-sown cereals to winter stress in eastern Ontario. Canadian Journal of Plant Science 66; 25-37.
Andrews CJ, Seaman WL, Pomeroy MK (1984) Changes in cold hardiness, ice tolerance and total carbohydrates of winter wheat under various cutting regimes. Canadian Journal of Plant Science 64; 547-558.
Årsvoll K (1973) Winter damage in Norwegian grasslands, 1968-1971. Meldinger fra Norges landbrukshögskole 52(3), 21p.
Beard JB (1964) Effects of ice, snow and water covers on Kentucky bluegrass, annual bluegrass and creeping bentgrass. Crop Science 4; 638-640.
Brink RA, Keller W, Eisenhart C (1939) Differential survival of alfalfa strains under an ice sheet. Journal of Agricultural Research 59; 59-71.
Crawford RMM (1978) Metabolic adaptation to anoxia. In: Hook DD, Crawford RMM (eds) Plant life in anaerobic environments. Ann Arbor Science, Ann Arbor, pp 119-136.
Freyman S, Brink VC (1967) Nature of ice-sheet injury to alfalfa. Agronomy Journal 59; 557-560.
Fridriksson S (1954) Investigations on winter injury of plants in Icelandic hayfields 1951-1952 (Rannsóknir á kali túna

árin 1951 og 1952). Deptartment of Agriculture, Report Serie B No 7, 72p.
Gao J-Y, Andrews CJ, Pomeroy MK (1983) Interactions among flooding, freezing, and ice encasement in winter wheat. Plant Physiology 72; 303-307.
Gudleifsson BE (1971) Winter damages on grasslands in Iceland, extent and causes (Overvintringsskadar i grasmark på Island, omfang og årsaker). Lisensiatoppgave, Norges landbruks-högskole, 130p.
Gudleifsson BE (1975a) Winter damages in grasslands (Over vintringsskadar i grasmark). Nordisk Jordbruksforskning 58; 498-504.
Gudleifsson BE (1975b) On winter damages IV. Relationship between climate and winter damages (Um kal og kalskemmdir IV. Samband veðurfars og kalskemmda. Ársrit Ræktunarfélags Norðurlands 72; 45-64.
Gudleifsson BE (1977) Ice encasement damages and smell from damaged fields (Svellkal og kallykt). Ársrit Ræktunarfélags Norðurlands 74; 70-76.
Gudleifsson BE (1979) Descriptions of grass diseases No 11. Ice and water damage. Weibulls Gräs-tips 22.
Gudleifsson BE (1986) Ice encasement damages on grasses and winter cereals. NJF seminar Nr. 84 Lantbruksväxternas övervintring 59-65.
Gudleifsson BE (1989) Extent and importance of ice-encasement damages on gramineous plants in the Nordic countries. Icelandic Agricultural Sciences 2; 7-14.
Gudleifsson BE, Andrews CJ, Björnsson H (1986) Cold hardiness and ice tolerance of pasture grasses grown and tested in controlled environments. Canadian Journal of Plant Science 66; 601-608.
Gudleifsson BE, Larsen A (1992). Ice encasement as a component of winterkill of herbage plants. In: Li PH, Christersson L (eds) Advances in plant cold hardiness. CRC Press, Boca Ratoon, Ann Arbor, London, Tokyo, pp 229-249.
Hakamata T, Noshiro M, Hirashima T, Nose I (1978) (Investigation of actual condition on the winter killing of pasture species in the Nemuro-Kushiro district. - Exploration of factors by the quantification No 1). Journal of Japanese Grassland Science 23; 280-288.
Hemmingsen E (1959) Permeation of gases through ice. Tellus 11; 355-359.
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 post-thaw period. Plant Physiology 85; 1068-1072.
Hetherington PR, Broughton HL, McKersie BD (1988) Ice-encasement injury to microsomal membranes isolated from winter wheat crowns. II. Changes in membrane lipids during ice encasement. Plant Physiology 86; 740-743.
Jónsson Ó (1938) Kal. Ársrit Ræktunarfélags Norðurlands 34; 19-47.
Levitt J (1980) Responses of plants to environmental stresses. I. Chilling, freezing, and high temperature stresses. Academic Press, New York.
McKersie BD, McDermott BM, Hunt LA, Poysa V (1982) Changes in carbohydrate levels during ice encasement and flooding of

winter cereals. Canadian Journal of Botany 60; 1822-1826.
Palta JP, Levitt J, Stadelmann EJ (1978) Plant viability assay. Cryobiology 15; 249-255.
Pomeroy MK, Andrews CJ (1978a) Metabolic and ultrastructural changes in winter wheat during ice encasement under field conditions. Plant Physiology 61; 806-811.
Pomeroy MK, Andrews CJ (1978b) Ultrastructural changes in shoot apex cells of winter wheat seedlings during ice encasement. Canadian Journal of Botany 56; 786-794.
Pomeroy MK, Andrews CJ (1979) Metabolic and ultrastructural changes associated with flooding at low temperature in winter wheat and barley. Plant Physiology 64; 635-639.
Pomeroy MK, Andrews CJ (1983a) Flooding and ice encasement damage to winter wheat. In: Fowler DB, Gusta LV, Slinkard AE, Hobin BA (eds) New frontiers in winter wheat production. Western Canada Winter Wheat Conference 1983. pp 39-56.
Pomeroy MK, Andrews CJ (1983b) Responses of winter cereals to various low temperature stresses. In: Randall DD, Blewins DG, Larson RL, Rapp BJ (eds) Current topics in plant biochemistry and physiology, Vol 2. University of Missoury Columbia, pp 96-106.
Pomeroy MK, Andrews CJ (1985) Effect of low temperature and calcium on survival and membrane properties of isolated winter wheat cells. Plant Physiology 78; 484-488.
Pomeroy MK, Andrews CJ (1986) Changes in adenine nucleotides and energy charge in isolated winter wheat cells during low temperature stress. Plant Physiology 81; 361-366.
Pomeroy MK, Pihakaski SJ, Andrews CJ (1983) Membrane properties of isolated winter wheat cells in relation to icing stress. Plant Physiology 72; 535-539.
Rakitina ZG (1965) The permeability of ice for O_2 and CO_2 in connection with a study of the reasons for winter cereal mortality under the ice crust. Soviet Plant Physiology 12; 795-803.
Rakitina ZG (1970) Effect of an ice crust on gas composition of the internal atmosphere in winter wheat. Soviet Plant Physiology 17; 755-759.
Singh J, Andrews CJ (1981) Comparisons of ultrastructural changes during extracellular freezing at -10°C and ice- encasement at -1°C in winter wheat crown cells by the method of freeze-fixation. Cryo-Letters 2; 117-124.
Sprague VG, Graber LF (1940) Physiological factors operative in ice-sheet injury of alfalfa. Plant Physiology 15; 661-673.
Sprague MA, Graber LF (1943) Ice sheet injury to alfalfa. Journal of American Society of Agronomy 35; 881-894.
Steponkus PL (1984) Role of the plasma membrane in freezing injury and cold acclimation. Annual Review of Plant Physiology 35; 543- 584.
Tanino KK, McKersie BD (1985) Injury within the crovn of winter wheat seedlings after freezing and icing stress. Canadian Journal of Botany 63; 432-436.
Woehrle T (1963) Ice sheet damage. The golf course reporter 31; 22-24,26.
Zemlianukhin AA, Ivanov BF (1978) Metabolism of organic acids of plants in the condition of hypoxia. In: Hook DD, Crawford RMM (eds) Plant life in anaerobic environments. Ann Arbor Science, Ann Arbor, pp 203-268.

Ilgvars Krumins, Peteris Berzins, Elmars Bunga
Latvia State Scientific Research Institute
of Agriculture "Agra", Skriveri-1,
Aizkraukle reg., Lettland, L, LV 5126

CROP OVERWINTERING PROBLEMS IN LATVIA

General information

The main overwintering crops in Latvia are winter cereals and perennial grasses. Winter cereals cover about 200-250 thousand ha and contribute for 30% of cereals or 15% of arable land. Perennial grasses cover about 1 400 000 ha or 55% of the agricultural land but there are large differences in grasslands. The most intensively used grasslands are in crop rotation and they cover about 550 000 ha. Permanent grasslands, pastures and meadows, cover about 850 000 ha. From 600 000 ha pastures only some 250 000 are enclosed and intensively used, however this figure can decrease due to the fact that many large cattleshed complexes are closed down recently. From 250 000 ha meadows, some 150 000 are regularly harvested and fertilized. So we can say that only about half of the permanent grassland is actually used in agricultural production, the other half is so called native grasslands - open area with unsown species used from time to time.

Winters in Latvia are quite different and almost always with changeable weather conditions. Usually the soil is frozen from December till March. Thaw in January is quite often and we have 7-15 days with positive temperatures. Snow cover lasts in average 120-130 days in the eastern regions, about 100 days near Riga and in the western regions, about 85 days in the central southern part and only about 65 days in the western coast. Winters without snow cover also occur. The main dangerous factors for crops are snow mould and ice crust.

Winter cereals

Winter cereals are considered to be the most important overwintering crops. The largest area, about 55%, is covered by winter rye and this is the most reliable crop. Winter wheat covers about 45% of the winter cereals and is more vulnerable. Only a few thousand hectares are under triticale and winter barley. Triticale is a new crop in Latvia, winter barley was introduced during the last years when actually we had no normal winters. Winter rye dominates in the northern and eastern part of Latvia, winter wheat in the southern, central and western parts.

About 10% of winter cereals are badly damaged during the winter and have to be resown by spring cereals each year. This figure has large deviations over the years and regions. For example the percentage of resown winter cereal was 25% in 1982, 16.4% in 1980, 14.7% in 1981, 14% in 1987, but only 2.6% in 1983 and 3% in 1986. The differences among regions (Figure 1) reflect both unfavorable weather conditions and management levels. Failures in management usually are too late sowing dates, too deep seed incorporation into the soil, too high seed rates and bad soil seedbed preparation. As regards sowing time it is well known that optimal dates are 1-15 September but despite of this, for example, the last year more than half of the area was sown after 15 September.

A work with State Statistic Service data was done some years ago to evaluate the influence of different weather factors on the overwintering of cereals by Dr. Elmars Bunga. He found close correlation between rainfall in September-November and percentage of resowed winter cereals. The average sum of precipitation in this period varies from 135-140 mm in the Bauska, Daugavpils, Aluksne regions till more than 220 mm in the Madona and Valmiera regions. If the actual rainfall sum is more than 200 mm, a sharp increase in winter damage is expected. Close correlation was found also between the precipitation in July and winter damage extent (Figure 2). This calculation was made on five region (Valmiera, Madona, Saldus, Preili and Jekabpils) data for years 1981-88. Snow cover influences the overwintering negatively if it lasts more than 105 days. Such situations occurred in 1981, 1982 and 1987.

Grasslands

Winter hardiness of grasses is not an important problem in Latvia and little attention is paid to this item, however the acreage of some species is limited exactly due to low winter hardiness.

Fourteen perennial grass species are grown in Latvia and they include four legumes and ten grasses. The legumes are red clover (Trifolium pratense L.), alsike clover (Trifolium hybridum L.), white clover (Trifolium repens L.) and lucerne (Medicago sativa L.). The grasses are timothy (Phleum pratense L.), meadow fescue (Festuca pratensis Huds.), cocksfoot (Dactylis glome-rata L.), perennial ryegrass (Lolium perenne L.), meadow grass (Poa pratensis L.), foxtail (Alopecurus pratensis L.), red fescue (Festuca rubra L.), bromgrass (Bromus inermis Leys.), tall fescue (Festuca arundinacea Schreb.) and reed canarygrass (Phalaris arundinacea L.).

Not all of these species are of the same importance. Red clover is the dominant legume and it is usually included in mixtures for leys. Alsike clover is grown under less favorable conditions. The area under lucerne is limited due to the soil conditions - lucerne is the favorite species in the central southern part of Latvia on carbonate soils. White clover is included in mixtures for pastures and we expect that use of this species could increase in the future. Most important Poaceae species are timothy and meadow fescue which are common companion grasses for legumes in leys. Cocksfoot is good as an early grass both in pastures and leys and is recommended to be grown in pure stands. The area under other grasses is quite small and species are used for specific purposes.

Usually there are no problems with overwintering of clovers in Latvia however some years swards suffer from Sclerotinia sp. and Fusarium sp. in certain areas. There are attempts to increase the disease resistance in the new variety breeding. Local varieties of lucerne have good winter hardiness on suitable soils. Experience with foreign varieties from former USSR southern regions is very bad, this lucerne is completely killed in the first winters.

The most vulnerable grass is perennial ryegrass and many western origin varieties tried in Latvia are not suitable. According to field trial results at Skriveri the highest winter hardiness have varieties Valinge (included in recommended list in Estonia), Priekulu-59 (Latvia), Veja (Lituania) and Pashavi (Byelorussia). From Priekulu-59 plants a new tetraploid variety Spidola was bred (treatment with colchicine) with better winter hardiness. There were attempts to create interspecies hybrids between perennial ryegrass and meadow fescue with the aim to increase also winter hardiness. Some tetraploid (n=28) ryegrass-fescue hybrids seem to be promising. They have higher winter hardiness than perennial ryegrass but less than meadow fescue, good seed yields (600-700 kg per hectare) and dry matter yields even 150% of that of parent species.

Cocksfoot has good winter hardiness but sometimes the swards are damaged under heavy fertilizer application and intensive management.

Timothy, meadow fescue, red fescue, meadow grass and foxtail varieties (Baltic origin) have high winter hardiness, no attention is paid to sward management with the aim to improve overwintering of these species and no problems in practice have arisen.

Tall fescue (var. Baltika, Zapadnaja) and reed canarygrass (var. Pedja) are new species in production. They are grown on limited areas for specific purposes and no problems with winter hardiness has appeared.

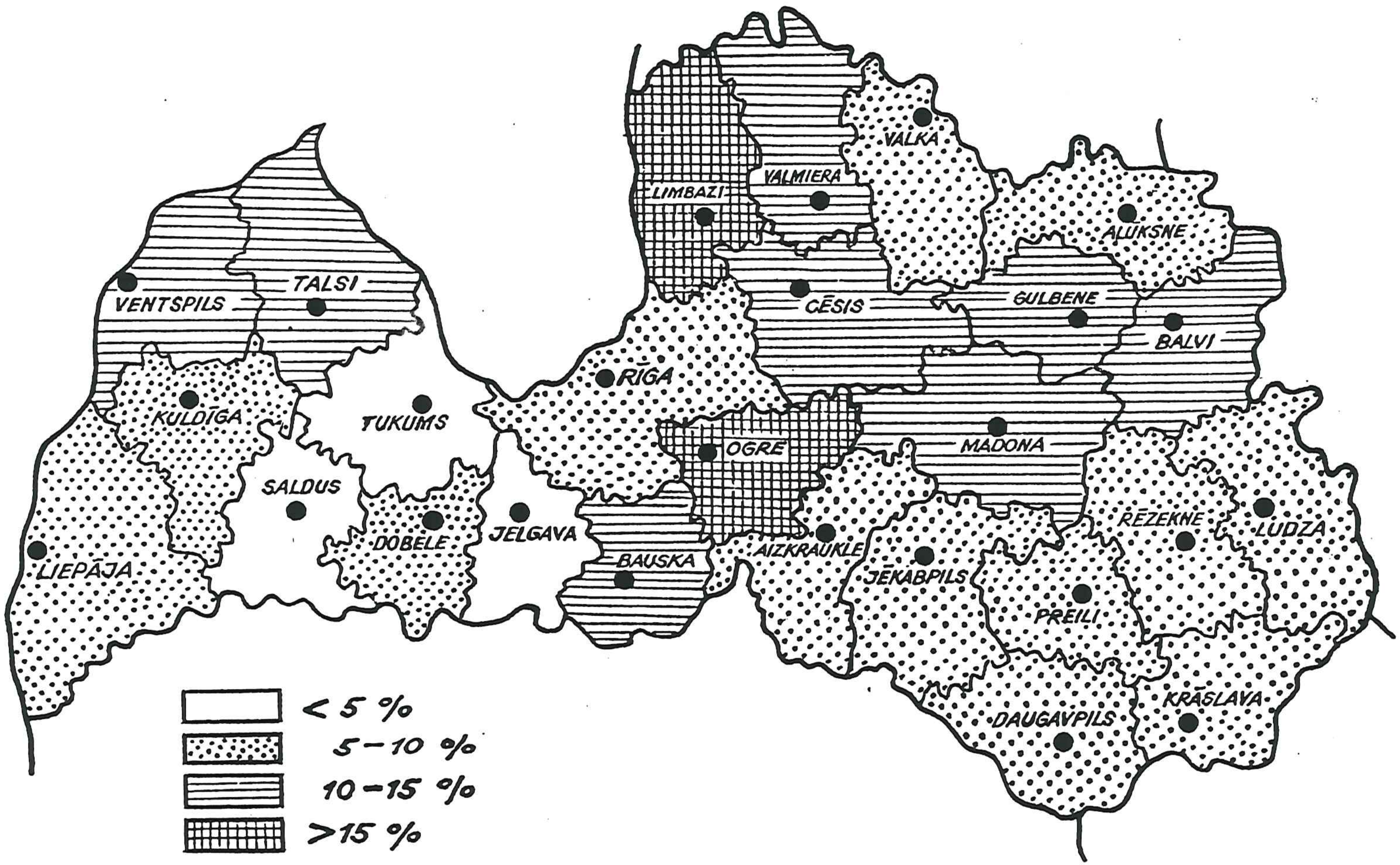


FIGURE 1.
PERCENTAGE OF RESOWED WINTER CEREALS /1980 – 88/

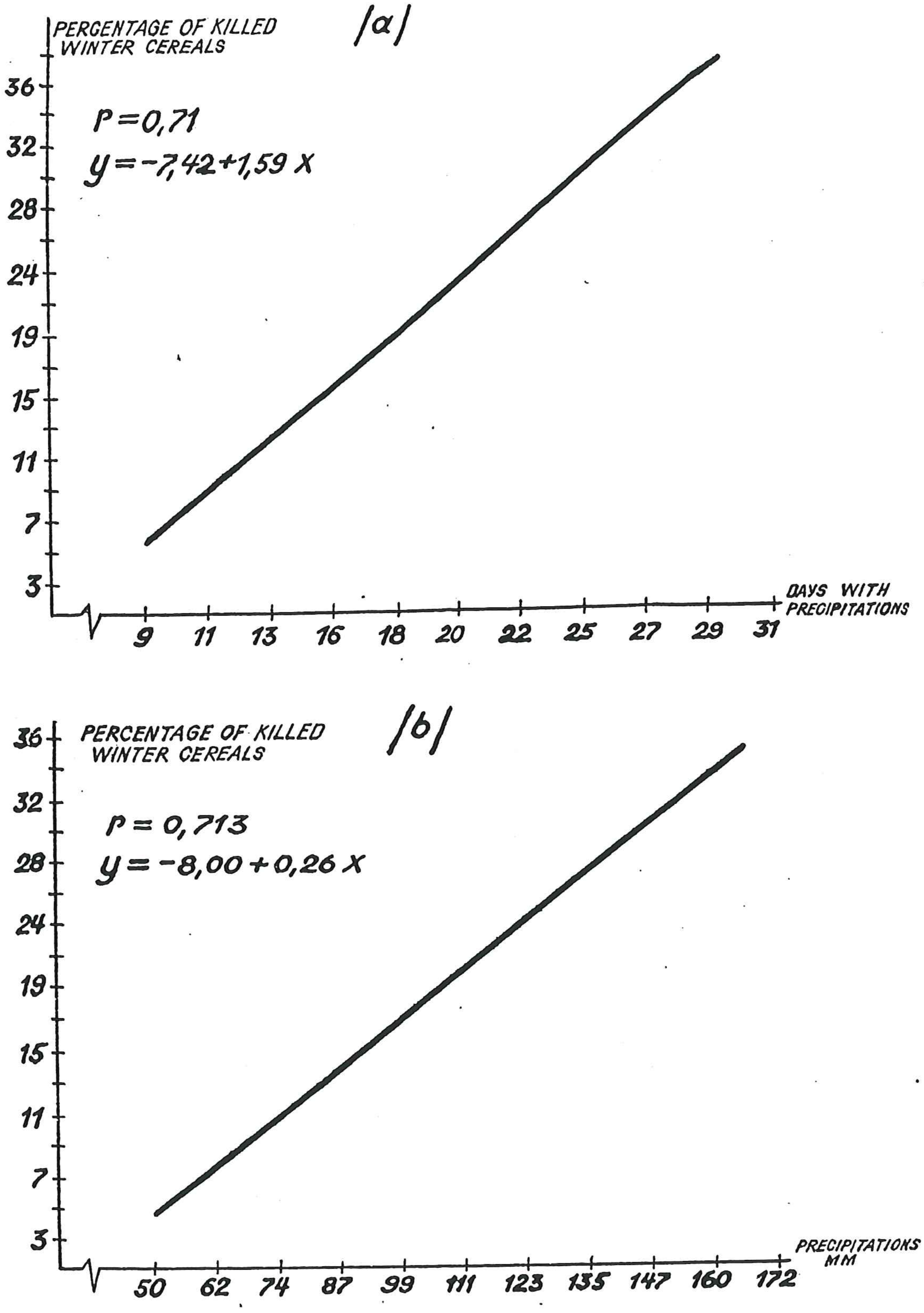


FIGURE 2.
CORRELATION BETWEEN PRECIPITATIONS IN JULY AND PERCENTAGE OF KILLED WINTER CEREALS /1981-88/

Heino Lõiveke, Hindrek Older
Estonian Research Institute of
Agriculture and Land Improvement
Saku Harju District, EE 3400 Estonia

THE WINTERHARDINESS OF PERENNIAL PLANTS IN ESTONIA

In order for presenting this article the research data of the Estonian Institute of Agriculture in 1980-1990 is used. In addition the materials of the Estonian Meteorological and Hydrological Institute, and Prognosis Department of Estonian Plant Protection Station are used.

Sincere thanks to the workers of the above-mentioned establishments for their friendly help.

There was the great need for our own Estonian produced rye and wheat crops. Our main research work was concentrated on investigating the problems of hibernation of winter crops. There is only some field observation data about the red clover and grasses.

The reasons for the winter damage of winter corn in 1986-1992 (Table 1) were the formation of ice cover and during the snow melting the water-logging caused by excessive dampness. Snow mould and rotting occured more seldom which is connected with the thin snow cover during the last years. When the thick snow cover lies on the unfrozen ground and the temperature is above zero, the conditions for developing of snow mould exist. The snow mould occured on rotten corn sprouts too. The freezing and frostraising of corn sprouts occured seldom and only locally.

Earlier in winters 1976/1977, 1978/1979 and in 1981/1982 when the snow cover was thick the snow mould occured epiphytotically and due the large damages 30...42 % of the area of winter crops (rye about 80 %) was substituted by summer crops (P. Talvoja, H. Löiveke, 1985, P. Talvoja, 1986). Later years (1983-1992) the snow cover was thinner in winter time and the development of snow mould has been less. Only in winter 1988/1989 the snow mould spred widely and developed intensivly. It was determined in the end of February (Table 1).

The aim of our research work was to find out the casual organisms of snow mould and to develop the chemical control methods.

Analysing the damaged plants, Fusarium nivale (Fr.) Ces., F. nivale (Fr.) Ces var. majus Wr., F. culmorum (W.G.Sm.) Sacc. and F. avenaceum (Fr.) Sacc. were isolated and they were pathogenic for winterwheat and rye. The similar to snow mould pathogenic sympthoms were caused by Typhula incarnata Jasch. Fr. and Typhyla spp. (Typhula blight).

For control of snow mould different fungicides as Benlate, Fundosol, Uzghen, Topsin-M, BMK were tested, spraying the corn sprouts in autumn. Agrocyt, Baytan universal, Topsin-M and Fundosol were used for dressing the seeds. The suitable dosage for spraying was 0,5 kg per hectare. With smaller dosage 0,25 kg per hectare the effectiveness was smaller. By dressing

Table 1. The winter damages of cereals in Estonia in 1986 - 1992

Culture	Year	Average damage, %	Damaged plants % in February 0-10	11-20	21-30	31-35	Main causes of damage	Yield, ts/ha
Rye	1986	6,7	72	25	3	0	IC,WL,SM	29,0
	1987	5,1	88	10	2	0	IC,WL,SM,FR	32,6
	1988	6,0	78	15	5	2	WL,IC,SM,FR	25,4
	1989	9,9	64	28	4	4	SM,R,WL	25,0
	1990	6,6	77	13	7	3	WL,IC,SM	27,0
	1991	3,3	95	5	0	0	WL,R,FR	21,7
	1992	2,6	94	6	0	0	WL,IC	24,2
	X	5,7	81,1	14,6	3,0	1,3	-	-
Winter wheat	1986	3,7	94	3	0	3	IC,WL,SM	29,7
	1987	5,2	91	9	0	0	IC,WL,SM,FR	33,9
	1988	4,1	81	19	0	0	WL,IC,SM,FR	21,7
	1989	4,9	82	18	0	0	SM,R,WL	27,5
	1990	3,3	89	11	0	0	WL,IC,SM	25,0
	1991	5,8	70	24	6	0	WL,R,FR	24,8
	1992	2,3	96	4	0	0	WL,IC	
	X	4,2	86,1	12,6	0,9	0,4	-	-
Winter cereals (total)	1986	5,8	78	19	2	1	IC,WL,SM	29,2
	1987	5,1	89	10	1	0	IC,WL,SM,FR	32,9
	1988	5,6	79	16	4	1	WL,IC,SM,FR	24,8
	1989	8,8	68	26	3	3	SM,R,WL	25,5
	1990	5,1	82	12	4	2	WL,IC,SM	26,5
	1991	4,0	88	10	2	0	WL,R,FR	22,4
	1992	2,4	95	5	0	0	WL,IC	
	X	5,3	82,7	14,0	2,3	1,0	-	-

Renotes: IC - ice cover, WL - water-logging, SM - snow mould, FR - frostraising, R - rotting.

of seeds the suitable effectivness was achieved by the dosage - 2 kg per ton, except Topsin-M (optimal dosage - 2...3 kg per ton) (H. Lõiveke, 1985, 1986, 1987).

It is easier to carry out the dressing of seeds. It does not depend on climatic conditions and on the state of the field. Also it is 2,5 times cheaper.

Spraying and dressing preparations with the same active ingredients and dosage per hectare were compared (0,5 kg per hectare was sprayed, or 2 kg per ton was dressed and 250 kg seeds per hectare were sown). In this case the dressing and spraying had the same technical and economical effectiveness by preparations with benomyl active ingredient (Agrocyt, Fundosol, Benlate). By Topsin-M the spraying with dosage 0,5 kg per hectare and the dressing 2...3 kg per ton were equally effective.

On dependent of snow mould developing conditions the use of fungicides and seed dressers reduced the number of damaged plants 1,5...10 times, intensity of the disease 1,5...2,6 times and yield increased 10...118 % in comparition with nontreated variant.

In case of mixed infection snow mould and Typhula blight the dressing effectiveness of preparations with benomyl active ingredient improved when TMTD or Vitavax was added. For control of mixed infection dresser Baytan universal had better effectiveness than benomyl-preparations Agrocyt, Fundosol, Benlate. By dressing with Baytan universal in 1986-1990 intensity of diseases reduced 1,5...1,7 times, the number of damaged plants reduced 3,4...14,0 times and the yield increased 6,8...28,8 %. By Agrocyt, Fundosol and Benlate the same data was worse therefore benomyl had less influence on Typhula blight.

Rye 'Custro' and winter barley which have low disease resistance needs complex control of diseases. Baytan universal for seed dressing integrated with Benlate (Fundosol) or Topsin-M for spraying of sprouts in autumn. In nontreated variant of winter barley 76,0 % of plants were damaged in spring 1989, in case of complex method accordingly 8,6 %.

In spring, after the snow melting when the damping climatic conditions continued, the spraying with benomyl-preparations reduced the intensity of snow mould and number of damaged plants. By developing of mixed infection of Fusarium and Septoria spp. the preparation Bayleton with dosage 0,5 per hectare has been effective.

The rate of damages, caused during the period of hibernation, depended on agroecological conditions and on resistance to diseases. More resistant to snow mould (Table 2) were varieties on rye Vambo, Sangaste, Tulvi and Viku, more susceptible were rye variety Custro and winter wheat variety Nadeja.

The flooding and formation of ice cover in some places on the fields are possible to prevent by using of agromeliorative measures. To control the snow mould and Typhula blight the preparations for seed dressing and spraying are needed. In the years, when hibernation diseases is low developed, the increase of yield would be about 10 %, in the years of higher intensity of diseases the yield increase would arise up to 50 %. In case of very widely spread and high intensity of diseases accordingly more than 100 %.

Table 2. The damage of different varieties of winter cereals in Estonia (average in 1989-1992)

	The affected area, %	Snow mould affected plants, %	Snow mould damaged plants, %	Typhula blight affected plants, %	Typhula blight damaged plants, %
RYE-VARIETY					
Vambo	46,0	17,0	2,0	0,5	1,0
Sangaste	47,2	15,3	1,4	0,02	0
Talovskaja-12	44,2	21,7	2,7	0,3	0
Custro	55,2	21,3	3,8	2,5	0,1
Tulvi	38,7	18,4	3,0	0,6	0,01
Viku	1,1	0,6	0	0,1	0
WHEAT-VARIETY					
Mironovs-kaja-808	41,9	8,4	1,6	0,003	0
Sirvinta	17,6	7,8	0	0	0
Holme	39,9	7,4	1,8	0,14	0
Kalvi	39,1	10,9	5,3	0	0
Donskaja	29,2	3,3	0	0,003	0
Polikar-Likovaja					
Nadeja	66,7	14,3	1,0	0	0

In 1986-1992 there were only 2,4 % of plants damaged in grasshay samples, which were taken in the end of February. The reasons for the winter damage were the water-logging and the formation of ice cover. Snow mould and Pythium spp. occured only locally. During the winter of 1988/1989 sclerotinia crown of clover caused by Sclerotinia trifoliorum Eriksson, was the reason of winter damages, especially in case of pure sward.

Bibliograafia

1. Löiveke, H. Topsiin lumiseene tõrjel. EMMI teadlaste soovitused tootmisele. ENSV RATK Info- ja Juurutusvalitsus. Tallinn, 1987, lk 60-64.
2. Talvoja, P., Löiveke, H. Fundosooli kasutamine taliteraviljade lumiseene tõrjel. EMMI teadlaste soovitused tootmisele. ENSV ATK Info- ja Juurutusvalitsus. Tallinn, 1985, lk. 22-25.
3. Talvoja, P. Talvekahjustused ja nende kahjulikkus taliteraviljadele. Teaduse saavutusi ja eesrindlikke kogemusi põllumajanduses. Nr. 7. Teraviljakasvatus. ENSV RATK Info- ja Juurutusvalitsus. Tallinn, 1986, lk. 24-27.
4. .Х.Э. Лыйвеке. Эффективность системных фунгицидов в защите озимой ржи и пшеницы от снежной плесени. Тезисы докладов Всесоюзной научно-технической конференции. "Комплексное использование пестицидов и других средств химизации в земледелии". М, 1986. стр. 61-62.
5. .Х.Э. Лыйвеке . Системные фунгициды в борьбе со снежной плесенью ржи. Защита растений в республиках Прибалтики и Белоруссии. Тезисы докладов научно-производственной конференции. Часть I, Таллин, 1985, стр. 88-89.

Arild Larsen,
SFL Vågønes,
N-8010 BODØ

METHODS FOR CONTROLLED HARDENING OF GRAMINEOUS PLANTS

Summary
In nature plants possess a low resistance to freezing and ice cover in active growth. Hardening develops during autumn with decreasing temperature and light conditions, according to the genetic capacity of the plants. Maximum hardening is reached in mid winter and dehardening occur during late winter and spring. Varietal differences in freezing tolerance appear to be greatest in the dehardening stage.

In controlled environments hardening is most effective at temperatures near to 0°C, with a long photoperiod (16 to 18 h), and good light intensity. Hardening is most rapid during the first week, but will continue for 3 to 4 weeks. A period with temperatures below 0°C is needed to reach maximum hardening.

Winter hardy cultivars tended to start hardening at higher threshold temperatures than do more susceptible cultivars. Hardier cultivars also maintain hardening better under dehardening conditions.

Maximum hardening may not show the best differences in resistance between genetical different plants as the varietal differences appear to be greatest during dehardening.

A long and complex hardening period will reduce test capacity and increase the cost of artificial testing of freezing and ice cover resistance.

Introduction

When considering methods for acclimation or hardening to freezing and ice cover resistance tests in a controlled environment we should study what happens under natural conditions. Three aspects should be examined. (1) Climatic conditions for effective hardening, (2) to allow good separation between plant material; the level of hardening according to actual natural conditions and (3) the hardening state of plants.

The artificial tests have to be effective allowing a large amount of plant material to be tested at a low cost. The hardening conditions should give the hardening level and the hardening differences that those plants obtain in nature, and a state of hardening corresponding to the stage when damage is most likely to occur in nature.

The plant material discussed will be mainly herbaceous plants, cereals and grasses in connection to plant breeding.

Hardening under natural conditions

Gramineous plants possess low resistance to freezing (-2 to -5 °C) and ice cover in a stage of active growth (Gusta et al. 1983, Larsen and Tronsmo 1991). Li and Weiser (1969) stated that a hardening message, presumably mRNA, exists in the plant system and functions only under hardening conditions. The resistance and genetic governed differences in resistance, develops during hardening or acclimation in autumn at decreasing temperature, photoperiod and light intensity. The conditions needed for acclimation both to freezing and to ice cover seems to be similar (Gudleifsson et al 1986).

Hardening is considered to develop in three stages (Kacperska-Palacz 1978). 1) In autumn at temperatures of 2 to 5°C under normal daylight. Cell extension and cell division are inhibited, and carbohydrates accumulate. 2) In late autumn and early winter at 0 to -3°C. Physical and chemical modifications in the cell membrane occur. Membrane fluidity and stability are maintained over a broad range of temperatures. 3) During winter at prolonged freezing. Frost induces dehydration of cells. Maximum hardiness is reached at the latter stage.

Development of resistance to freezing in winter wheat from autumn to spring on the Canadian northern great plains have been studied by Fowler at al. (1983). Plant growth slows down and cold acclimation increases when soil temperature at crown depth drop to below 7 and 10°C in morning and afternoon, respectively. After this period, a period of continuous frost (-2 to -3°C) is required to fully harden plants. Exposure of plants to warm temperatures at this stage induces rapid dehardening, but they will quickly reharden at low temperatures. A high level of hardiness can be maintained, provided temperatures remain below freezing. Prolonged exposure of hardened plants to temperatures slightly above freezing or slightly above lethal temperature, and alternate freezing and thawing will gradually reduce the plants cold hardiness. Return to favorable growth conditions in spring will result in complete dehardening. At this stage the plants are unable to rehardening (Figure 1).

Similar observations have béen made for grasses in south eastern and northern Norway (Larsen & Tronsmo 1991). In mid August timothy possessed low resistance to freezing. Hardening increased steadily to early November and remained stable to the end of the year. At Vågønes in coastal north Norway freezing resistance decreased from January and was low in March. At Ås in south-east Norway pronounced increase in resistance occurred in the beginning of December and the plants stayed very hardy to mid February. This increased resistance was associated with a spell of hard frost on bare ground, and must relate to the 3th stage of hardening. A maritime climate and very mild test winters with altering

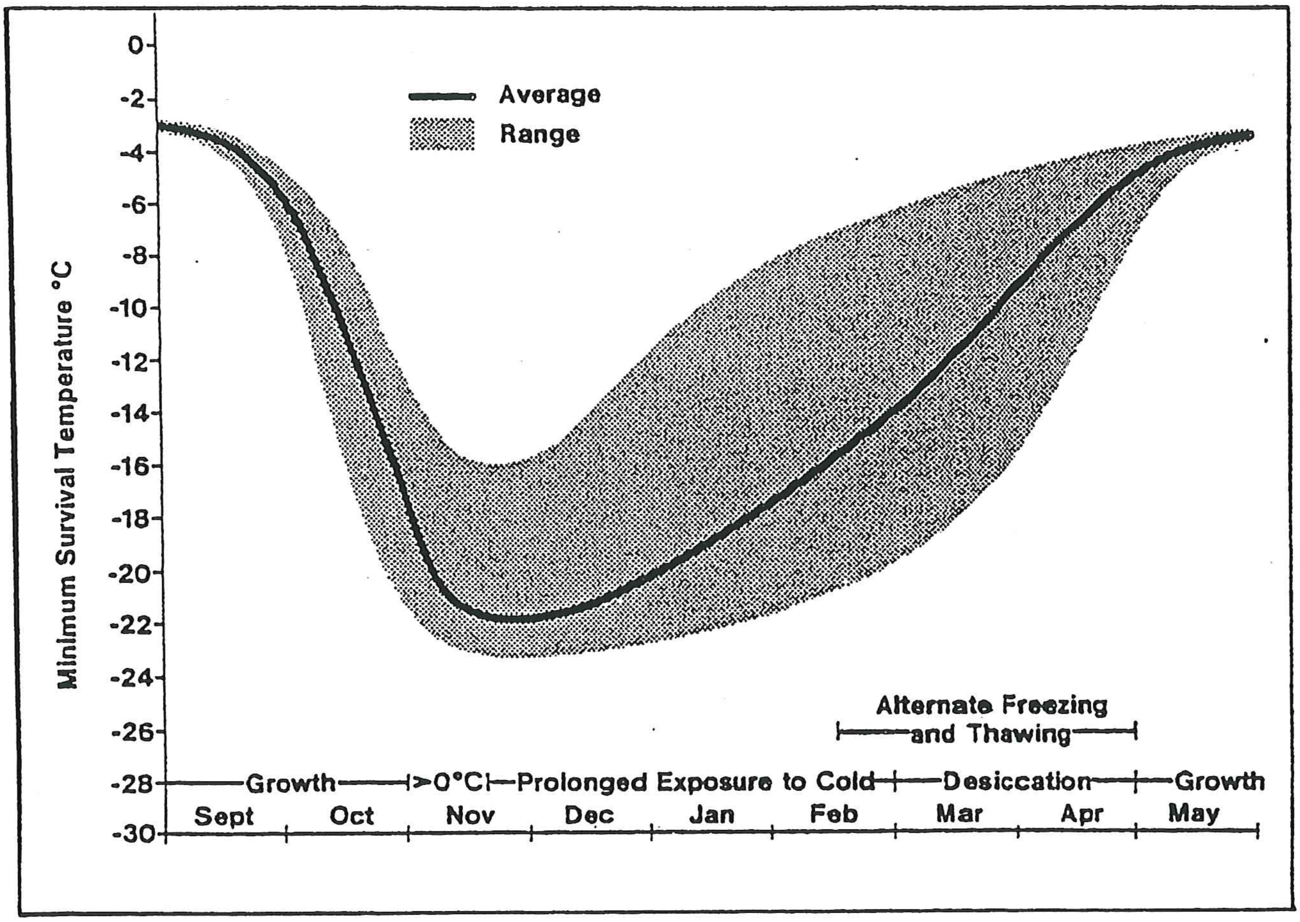


Figure 1. Change in cold hardiness of Norstar winter wheat for the period September to May. The primary factors responsible for these changes are shown at the bottom of the graph. (Fowler et al. 1983).

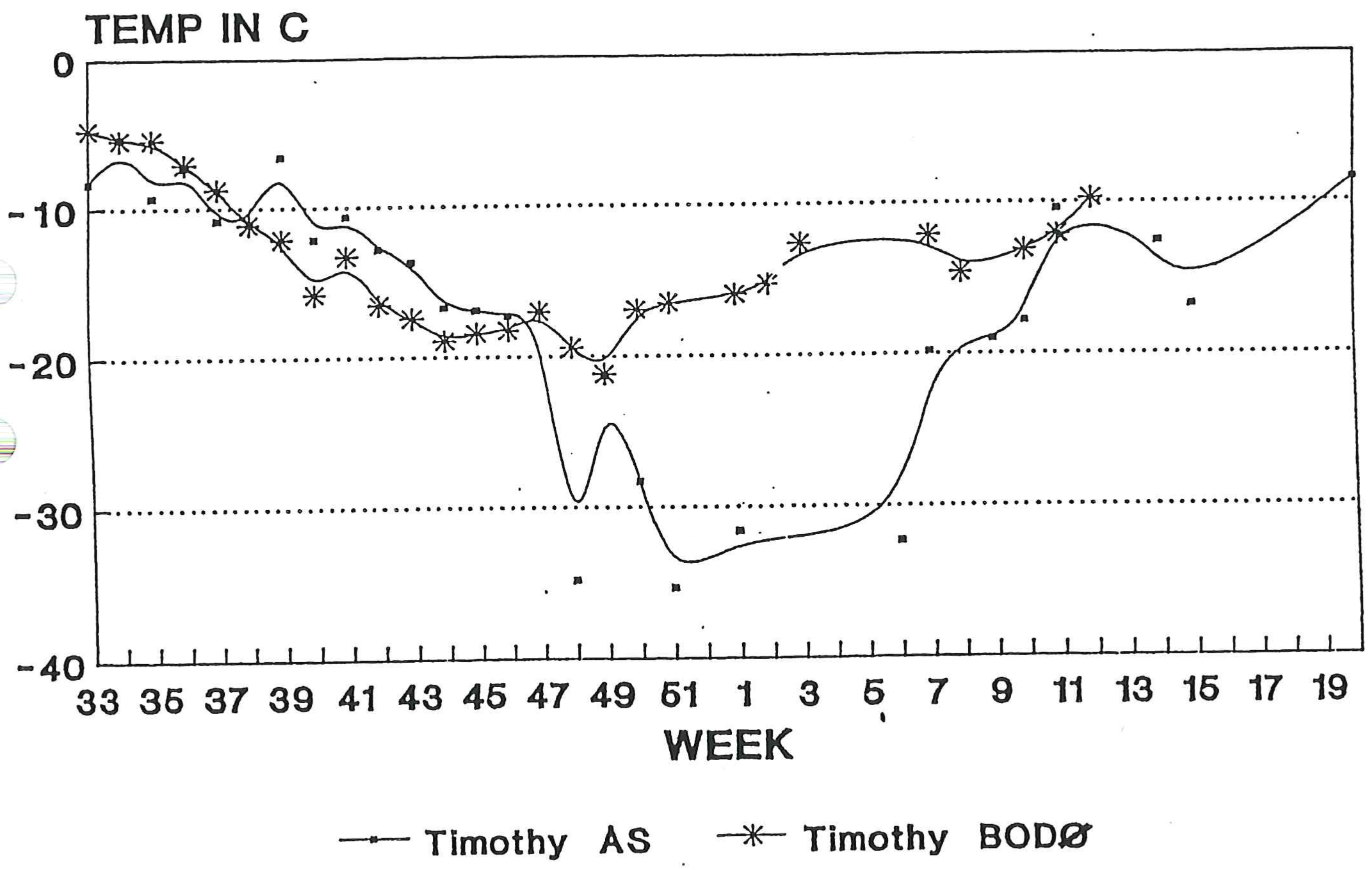


Figure 2. Development of natural hardening of timothy (<u>Phleum pratense</u>) tested as freezing resistance. LT-50 values of plants grown at Ås and at Bodø. (Larsen and Tronsmo 1991).

temperatures prevented development of a 3th stage of hardening at Vågønes (Figure 2).

Three timothy cultivars differing in winter hardiness were studied at Vågønes. In mid August no significant differences occurred. The hardiest cultivar 'Engmo' started hardening earliest, and possessed the greatest freezing resistance through the winter period. At the most resistant stage from late October to late December the cultivars were significantly different in freezing resistance. However, the varietal differences were much more pronounced during the dehardening period from late December to mid March. 'Engmo' kept its resistance much better than the two more southern cultivars 'Grindstad' and 'S352' (Figure 3).

Climatic conditions for artificial hardening

Adaption to natural hardening has been tried in controlled climate. For instance Limin and Fowler (1982) recommended the following decrease in temperature for winter wheat plants at the 3 leaf stage with a 12 h photoperiod: The first 7 days; 5°C at light, 2°C at dark. The next 10 days; 3°C at light, -2°C at dark. However, Gusta et al (1982) hardened 'Nordstar' winter wheat for five weeks at gradually decreasing temperatures and photoperiods in four and two steps, compared to continuous low temperature (2°C/0°C) and a 12 h photoperiod. The latter condition resulted in faster hardening and higher freezing resistance (Figure 4).

Experiments with fluctuating temperature conditions during hardening indicated additional freezing resistance in cultivars of *Lolium perenne*. Low temperature enhancement of hardening occurred irrespective of whether the low temperature exposure was during the light or dark period. Minimum required time of low temperature exposure was 4-8 h for enhanced hardening (Eagles and Williams 1992).

The effect of photoperiod on hardening will depend on temperature (*L. perenne* - Lorenzetti et al. 1971, *Poa pratensis* - Larsen 1978). At low temperatures (0-4°C) the hardening will increase with photoperiod up to 16-18 h. At higher temperatures a photoperiod of 8-12 h will be more effective because of the reduction in growth rate (Figure 5).

Increased light intensity was observed to improve freezing resistance of *L. perenne* at both 8 and 16 h photoperiods (Lorenzetti et al. (1971). Pollock et al. (1988) showed increasing daily radiation integrals during hardening to increase freezing resistance of contrasting cultivars of perennial ryegrass up to 10 mol m^{-2} d^{-1} but not above this value.

Plant genotypes and populations may react differently to hardening conditions. In *L. perenne* Fuller and Eagles (1980, 1981) identified a significant interaction of cultivar x hardening temperature. Hardier cultivars showing a threshold

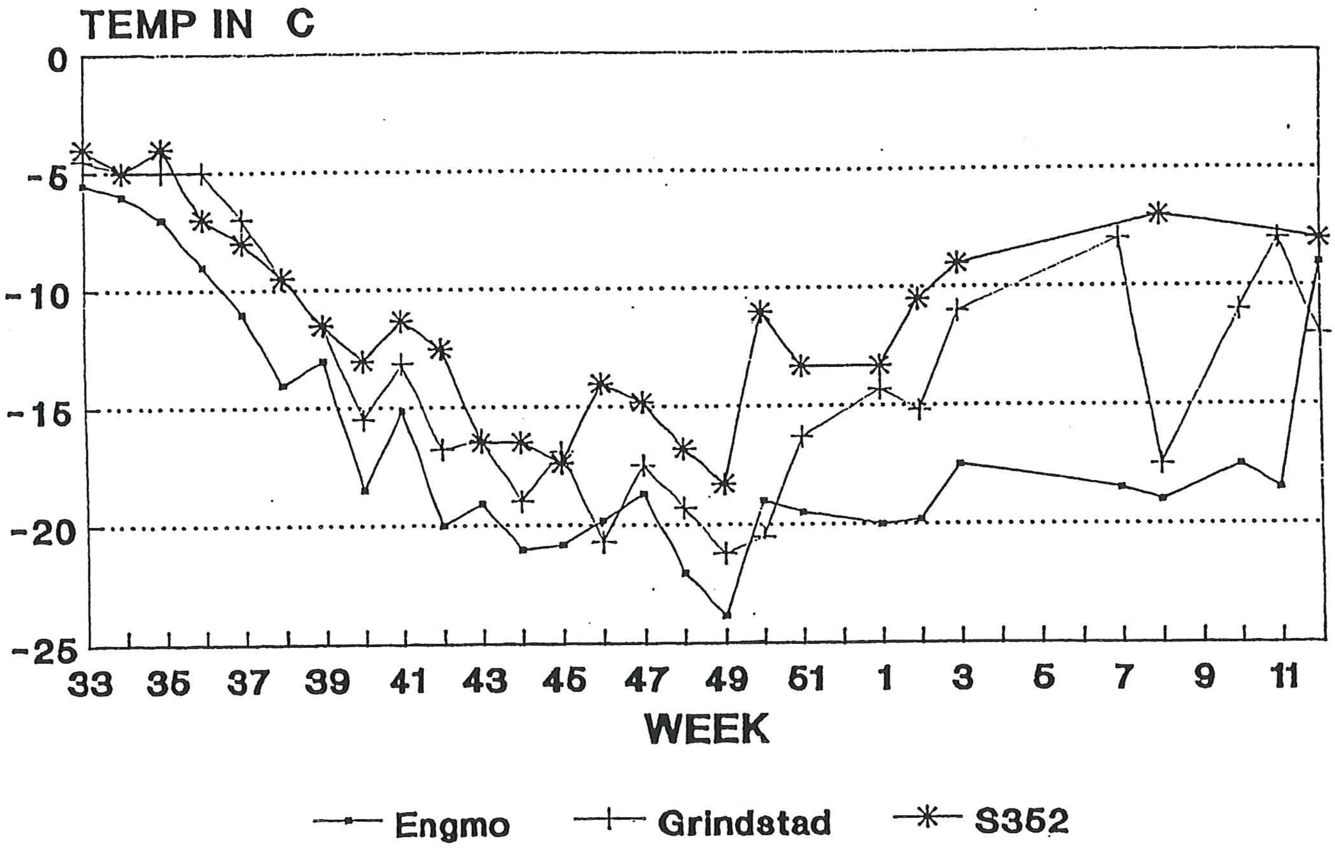


Figure 3. Development of natural hardening of three cultivars of timothy (Phleum pratense) tested as freezing resistance. LT-50 values of plants grown at Bodø. (Larsen and Tronsmo 1991).

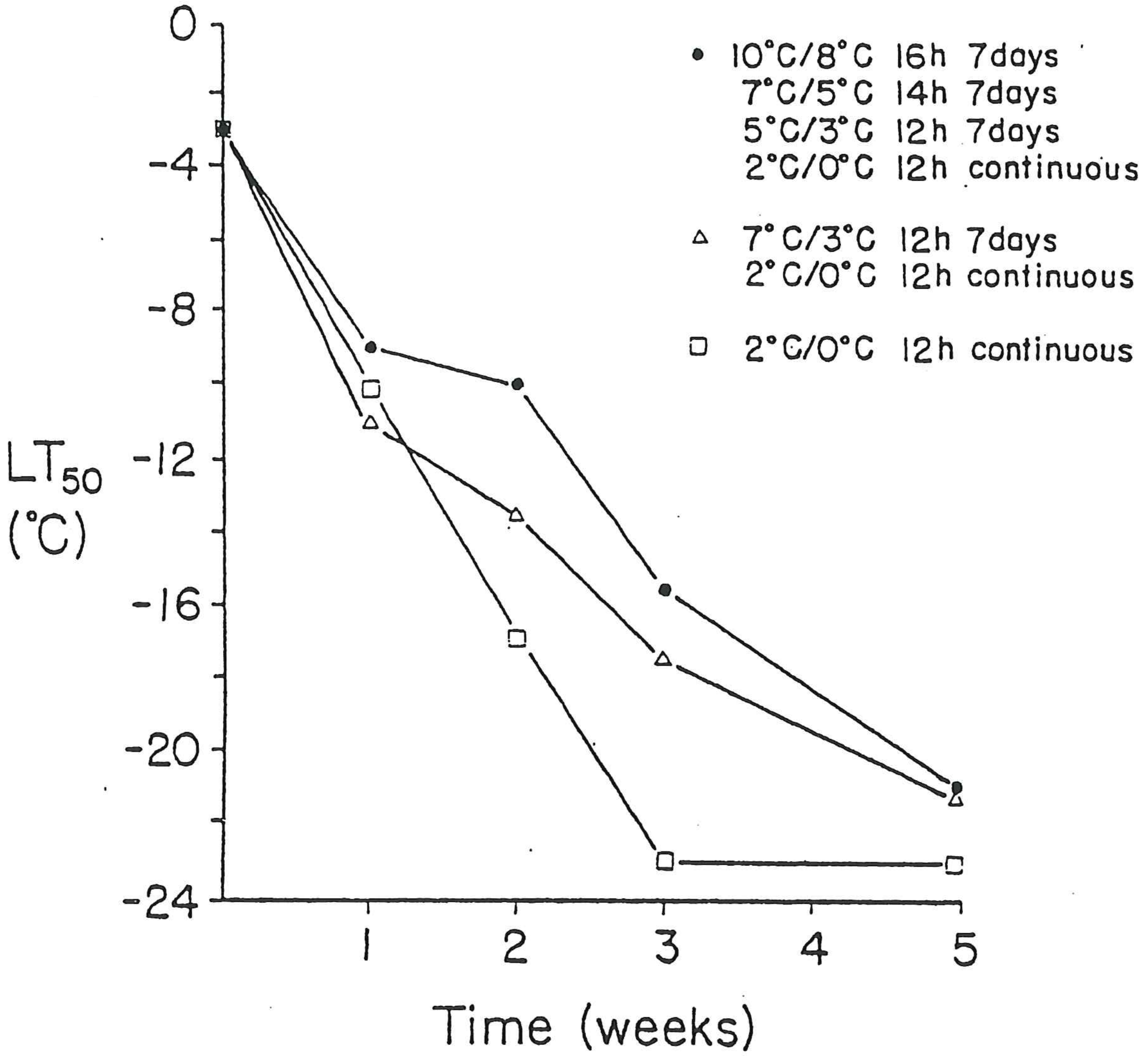


Figure 4. Hardening of Norstar winter wheat crowns at different temperatures. (Gusta et al 1982).

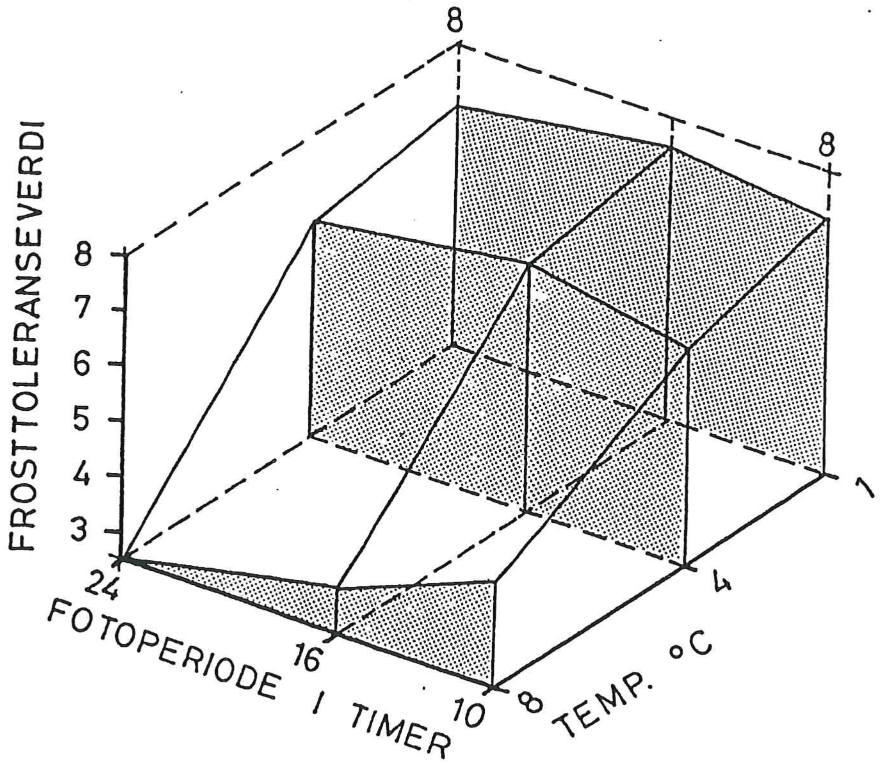


Figure 5. Freezing resistance (0 to 9) of <u>Poa pratensis</u> after hardening in 42 days at three temperatures and three photoperiods. (Larsen 1978).

temperature for hardening between 5°C and 7°C while for a susceptible one the threshold was between 2°C and 5°C. Ecotypes of *P. pratensis* showed a significant interaction with photoperiod when hardened at 6°C. The most northern ecotype obtained highest freezing resistance at 16 h photoperiod, while more southern and less hardy ecotypes showed highest resistance at 10 h. At lower hardening temperatures no such interaction occurred (Larsen 1978).

<u>Stage of hardening at testing</u>
In controlled tests the plants should probably have a similar state of hardening as when the damage in field is most likely to occur. Hard frost and prolonged ice cover will be most harmful in late winter when the plants are in the dehardening stage. More tender plants, such as some winter cereals, may also be killed by frost and ice cover even at the maximum stage of hardening. The tests should also be conducted at a hardening stage when the differences in the test material are most obvious.

Fully hardened hardy winter wheat cultivars such as 'Norstar', can be held at -3 to -5 °C with little or no loss of hardiness (Gusta et al 1983). Less winter hardy cultivars may tolerate very low temperatures in the autumn, but readily deharden when stored at -3 to -5°C. Cultivars of *L. perenne* differed in their dehardening responses to temperature, hardier cultivars maintaining hardiness at temperatures about 10°C whereas a susceptible one showed appreciable loss of hardiness at 10°C (Fuller and Eagles 1980, 1981).

Significant increased genetic variability was observed between genotypes in populations of *L. perenne* when hardening was followed by a dehardening period (Larsen 1978). Similarly, Eagles (1984) showed greater differences between varieties after dehardening at 4 to 8°C, mainly because the least hardy variety lost hardening.

<u>Recommendations</u>
Some days with a short photoperiod (8 to 10 h) between the growing and hardening period should be used to reduce the growth intensity.

At hardening the plants should be exposed directly to low fluctuating temperatures, 0 to 4°C, and with an extended photoperiod, 16 to 18 h.

At such conditions a sufficient level of hardening is obtained after 2 to 3 weeks. If freezing resistance near the maximum level is required, the hardening period should be prolonged with a week at -3°C.

The best measure of hardiness seems to be the plant materials ability to keep their resistance during dehardening conditions. Therefore, a week of dehardening at approximately

10°C will increase the genetic variation for freezing tolerance. The cost of such treatment compared to its advantages should be investigated.

References

Eagles, C.F. 1984. Effect of temperature on hardening and dehardening responses in *Lolium*. In The Impact of Climate on Grass Production and Quality. Pros. of the 10th general meeting of EGF. H. Riley and A.O. Skjelvåg, eds. The Norwegian State Agricultural Research Stations: 287 - 291.

Eagles, and J. Williams, 1992. Hardening and Dehardening of *Lolium perenne* in Response to Fluctuating Temperatures. Annals of Botany 70: 333 - 338.

Fowler, D.B., A.E. Limin and L.V. Gusta, 1983. Breeding for winter hardines in wheat. In New Frontiers in winter wheat Production. The Univ. of Saskatchewan Printing Service: 136 - 184.

Fuller, M.P. and C.F. Eagles, 1980. The effect of temperature on cold hardening of *Lolium perenne* seedlings. J. agric. Sci., Camb. 95: 77 - 81.

Fuller, M.P. and C.F. Eagles, 1981. The effect of temperature on cold dehardening of *Lolium perenne* L. seedlings. J. agric. Sci., Camb. 96: 55 - 59.

Gudleifsson, B. E., C.J. Andrews, H. Björnsson, 1986. Cold hardiness and ice tolerance of pasture grasses grown and tested in controlled environments. Can J. Plant Sci. 66: 601 -608.

Gusta, L.V., D.B. Fowler and N.J. Tyler, 1982. Factors influencing hardening and survival in winter wheat. In Plant Cold Hardiness and Freezing Stress Mechanisms and Crop Implications. Vol. II. P.H. Li and A. Sakai, eds. Academic Press, New York: 23 - 40.

Gusta, L.V., T.H.H. Chen and D.B. Fowler, 1983. Factors affecting the cold hardiness of winter wheat. In New Frontiers in winter wheat Production. The Univ. of Saskatchewan Printing Service: 1 - 25.

Kacperska-Palacz, A. 1978. Mechanism of cold acclimation in herbaceous plants. In Plant cold hardiness and freezing stress. P.H. Li and A. Sakai, eds. Academic press, New York: 139 - 152.

Larsen, A. 1978. Freezing Tolerance in Grasses. Methods for testing in controlled environments. Meld. Norg. LandbrHøgsk. 57 (23): 56 pp.

Larsen, A. and A.M. Tronsmö, 1991. Natural hardening in grasses. Sveriges Lantbruksuniversitet Raporter Nr.53. The 4th Plant Cold Hardiness Seminar: 26.

Li, P.H. and C.J. Weiser, 1969. Metabolism of nucleic acids in one-year old apple twig during cold hardening and dehardening. Plant Cell Physiol. 10: 21 - 30.

Limin, A.E. and D.B. Fowler, 1982. The expression of cold hardiness in Triticum species amphiploids. Can. J. Genet. Cytol. 24: 51 - 56.

Lorenzetti, F., B.F. Tyler, J.P. Cooper and E.L. Breese, 1971. Cold tolerance and winter hardiness in *Loliun perenne*. I. Development of screening techniques for cold tolerance and survey of geographical variation. J. agric. Sci., Camb. 76: 199 - 209.

Pollock, C.J., C.F. Eagles and I.M.Sims, 1988. Effect of photoperiod and irradiance changes upon development of freezing tolerance and accumulation of soluble carbohydrate in seedlings of *Lolium perenne* grown at 2°C. Annals of Botany 62: 95 - 100.

Magnus Halling
Swedish University of Agricultural Science
Department of Crop Production Science
P.O. Box 7043
S-750 07 UPPSALA

AUTUMN TREATMENT OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

Introduction

Perennial ryegrass (*Lolium perenne* L.) is widely used as a forage grass, both for cutting and grazing in Europe. Under Swedish conditions, despite a large cultivar material, it is less used. Many farmers would like to grow more perennial ryegrass because it is easy to establish, can be used both for grazing and cutting, and has a high production capacity with a high nutrient content, especially energy. Results show that an initial high production of perennial ryegrass has largely been reduced in older leys (Frankow-Lindberg, 1993), mainly due to winter damage caused by pink snow mould (*Fusarium nivale* (Fr.) Ces.) and other fungi. This problem may be very severe in perennial ryegrass, especially during winters with prolonged snow cover. Experiences from Scotland have shown that a late autumn cut can reduce the risk of snow mould damage. In grasses, winter survival and spring growth are also greatly influenced by the date of autumn cut and the length of the last regrowth period (Torstensson, 1938; Østgård, 1962; Thomson, 1974; Andersson, 1984). With the above background, a study was started with the objective to investigate if a late autumn cut could improve the winter survival of perennial ryegrass under Swedish conditions, leading to a higher production in older leys. By using experiments in the official testing programme the reaction of the current varieties would be assessed.

Description of varieties

Bonita, Condesa and **Madera**: Tetraploid varieties from the Netherlands, mainly for grazing. **Patora, Sisu** and **Verna:** Diploid varieties from Denmark; Patora is late, Verna is early, and Sisu has shown high endurance in Denmark. **Rikka:** Diploid variety from Finland. **Fredrik:** Tetraploid italian ryegrass (*Lolium multiflorum* Lam.) from Sweden with fairly good winter hardiness. **Gunne, Svea** and **Viris:** Diploid varieties from Sweden; Gunne and Svea have high winter hardiness, especially Svea. Viris is very susceptible to snow mould fungi. **Helmer** and **Leia:** Tetraploid varieties from Sweden, Helmer is more winter-hardy than Leia. Leia has high resistance against leaf spot diseases. **Lorry:** A tetraploid hybrid between perennial and italian ryegrass, originating from Sweden, with moderate winter hardiness. **Taptoe:**

Tetraploid variety from the Netherlands. **Tonga** and **Tove**: Tetraploid varieties from Denmark, both have shown good winter-hardiness.

Methods

Field experiments.

Experiments in the official variety testing programme in perennial ryegrass were used in this study. Two of the four blocks were cut as late as possible before cessation of growth, the other two were left untreated. The cut was done in late October to early November (Table 1). Cutting height was about 5 cm. Experimental details are presented in Table 1. The experimental sites were: Uppsala (59°49'N and 17°39'E) in eastern part of central Sweden and Lillerud (59°24'N and 13°16'E), Bjertorp (58°16'N and 13°7'E) and Rådde (57°35'N and 13°16'E) in central part of western Sweden. Potassium (K) and phosphorus (P) were applied according to the soil surveying, generally 30 kg of P and 100 kg of K per ha each year of ley. Nitrogen (N) was supplied with totally 200 kg N per ha and year, 100 kg in spring, 60 kg after first harvest and 40 kg after second harvest. Harvest figures presented are dry matter of pure grass. The distribution of varieties in trial 2 at Uppsala (Table 1) was changed when analysing the data in comparision with the distribution according to the project outline. Stand density was scored as percent of optimal stand in late autumn and early spring.

Weather conditions.

The weather during the winters 90/91 and 91/92 was much warmer than normal with less snow cover and of shorter duration than normal. Monthly maximum snow depths from the nearest regional weather station to the experimental sites are shown in Table 2.

Pathological studies.

Early in spring, trials were examined for snow mould fungi, the most common being pink snow mould (*Fusarium nivale* (Fr.) Ces.) but grey snow mould (*Thyphula incarnata* Lasch ex Fr.) could also be found. Occurrences of leaf spot diseases were also examined. The most common on perennial ryegrass are brown blight (*Drechslera siccans* (Drechs.) Shoemaker) and net blotch (*Drechslera dictyoides* (Drechsler) Shoemaker). Fifteen shoots were taken randomly from each plot. The areas covered by leaf spots were classified for each leaf from each shoot in a six degree scale. The mean occurrence of leaf spots was then calculated in percent for the first and second leaves to developed.

Statistical procedures.

The autumn treatment was not strictly randomized in the experiment since it was applied on every second block. However, in the statistical analysis, the design was regarded as a randomized split plot. In experiments with 18 varieties the mean values were adjusted for the variance within and between main and sub-blocks (there are three sub-blocks in a main block). Data reported in Table 3 to 7 are least-square-means, which means that values are adjusted to what would be expected if the design had been balanced for all experiments. The procedures Varcomp and Matrix in SAS (Statistical analysing system) were used.

Results

The autumn cut has significantly reduced the following spring yield, by about 25 percent, both in the second and third year leys (Table 3). In the second and third harvests in the second year ley, there were no significant differences between the autumn treatments. In the individual analysis of sites, significant interactions between autumn treatment and variety were found in spring harvest at many sites, especially in the third year ley, but no consistent reactions for different varieties were found in the analysis of sites. High CV values of the autumn treatment in the first harvest indicate a large variation between sites and years. Aftermath effects of the autumn cut on the spring yield of different varieties are shown in Table 4. The diploid varieties Gunne and Verna gave highest yields in the spring, being significantly different from many other varieties. The varieties Condesa, Madera and Lorry gave the lowest spring yields. Condesa, Madera and Patora are late varieties, and therefore a lower spring yield is to be expected. There is a tendency that Sisu, Leia, Lorry and Viris were affected most by the autumn cut, and that Condesa and Madera were less affected. Mean yields of varieties in spring declined sharply from the second to the third year ley.

The yield of the autumn cut was not recorded in 1990 and 1991, but only in the autumn of 1992 (Table 5). Yield figures indicate that what was obtained by the late harvest in the autumn was lost in the following spring yield. Stand density was negatively affected, but not significantly, by the autumn treatment (Table 6).

The examinations of snow mould fungi showed that no damage occurred in the spring of 1991 and 1992. Examinations of leaf spots are reported in Table 7. The most common fungus causing leaf spots was brown blight. The results show that the highest rate of leaf spots was on the first leaf, but generally there was a low degree of leaf spots. The highest rates were recorded in the autumn, where also significant varietal differences were found. The varieties Bonita, Condesa, Madera, Patora, Gunne and Viris have significantly higher rates of leaf spots on the first leaf than Fredrik and Leia. In the third year ley, the autumn treatment significantly reduced the amount of leaf spots on the first leaf to developed in the spring. There is a tendency that Helmer has a good resistance against leaf spot fungi.

Discussion

Because of extremely mild winters, with no damage caused by snow mould fungi, the effect of the autumn cut on the over-wintering ability was not as expected. Instead, the effect has been the opposite, an autumn cut has largely lowered the spring yield for all varieties. This finding is consistent with Thomson (1974). Assuming that yields obtained at the autumn cut in 1990 and 1991 were similar to those in 1992, the amount received by cutting late in autumn was lost from the following spring harvest. But subsequent harvests were not affected. Consequently, low spring yields after the autumn cut were not mainley caused by winter damage. The low influence of the autumn cut on stand density also confirms this. Preliminary results (unpublished) show that the autumn cut mostly affected the size and not the number of shoots.

It appears that there is a large variation between sites and years which needs to further analysed. One method availiable is to use the growth model described by Torssell & Kornher (1983) to separate weather effects from the growth potential of the stand.

The feeding value of the late autumn cut depends on the amount of senescent material. The content of energy should be high since the grass has grown under low temperatures. A late autumn cut may also damage the ley as a result of having to use heavy machines under wet conditions.

The interactions between autumn treatment and variety for the first harvest at several sites seems to have been more of a random nature since no consistent general reactions for varieties were found between experiments. Differences in the spring yield should reflect both earliness and winter-hardiness of the varieties. However, spring yield is a rough measurement of winter hardiness. Applying this, it seems that, on average, the tetraploid varieties have a lower degree of winter-hardiness than diploid varieties. Among diploid varieties, Sisu and Viris had lowest winter-hardiness. In the group of tetraploid varieties, Leia and Taptoe were less winter-hardy than the others.

Since the degree of diseases was generally low in the leaf spot screening, differences in resistance between varieties could not be fully revealed. Some varietal differences were detected in the autumn but not in the spring. The age of the leaf also seems to be of importance, low CV-values indicate that varietal differences are best shown by oldest leaves corresponding to Stegmark (1979). However, even under favourable winters with low snow mould pressure, perennial ryegrass is losing its spring production capacity in older leys. Other factors than fungi must be involved in the degeneration of the stand.

To choose an optimum time for the autumn cut could be difficult. Important factors are, e. g, the growth period between the last harvest and the autumn cut, weather conditions during the autumn, the time when growth cessation occurs. Translocation of carbohydrates from the leaves to the basal part of the shoots should have finished at the time of the autumn cut. This implies that the amount of standing biomass and the cutting height used should also influence the effect of the autumn treatment. There are several reports of a detrimental period during autumn when a cut should be avoided (Fulkerson, 1974; Andersson, 1984; Halling, 1986). The timing of this period may vary due to weather conditions, especially when the time of growth cessation occurs. Finally, the risk of an autumn cut must be balanced against the positive effects obtained if there is a severe winter with high snow mould pressure. Considering the above-mentioned conditions, future studies of this question should involve several different autumn cutting times and cutting heights, and more detailed observations of the stand, e.g. number of shoots in autumn and spring, amount of carbohydrates in over-wintering plant parts.

References

Østgård, O. 1962. Different cutting times in timothy. *Forskn. fors. Lantbr. 13*, 1-13.

Andersson, S. 1984. *Skördetidens inverkan på vallens övervintring* (Rapport från Norrlands Lantbruksförsöksanstalt Röbäcksdalen, Röbäcksdalen meddelar, Växtodling 1984:10). Umeå.

Frankow-Lindberg, B. 1993. Personal communication. (Swedish Univ. Agric. Sci., Dept. of Plant Production Science). Uppsala.

Fulkerson, R. 1974. *Stop alfalfa winterkill* (Ministry of Agr. and Food, Ontario, Factsheet, April 1974). Canada.

Halling, M. 1986. Growth of timothy and red clover in relation to weather and time of autumn cutting. 1. Yield and initial relative growth rate (in the) subsequent spring. *Swedish J. agric. Res 16*, 153-160.

SAS/SAS USER'S GUIDE: Basic and Statistics. 1985. Version 5 edition. SAS Institute Inc., Cary, North Carolina 27511-8000. USA.

Stegmark. R. 1979. *Förekomst av bladfläcksjuka* (Drechslera sp.= Helminthosporium sp.) *i olika sorter av engelskt rajgräs* (Lolium perenne) *och ängssvingel* (Festuca pratensis). (Swedish Univ. Agric. Sci., Dept. of Plant and Forest Protection, Växtskyddsrapporter, Jordbruk 6). Uppsala.

Thomson, A. J. 1974. The effect of autumn management on winter damage and subsequent spring production of six varieties of *Lolium perenne* grown at Cambridge. *J. Br. Grassld. Soc. 29*, 275-284.

Torssell, B. W. R. & Kornher, A. 1983. Validation of a yield prediction model for temporary grasslands. *Swedish J. agric. Res. 13*, 125-135.

Torstensson, G. 1938. Skördetidens och stubbhöjdens inverkan på avkastning och rotutveckling hos gräs. *Svenska betes- och Vallföreningens Medd. no. 5.*

Table 1. Experimental details of time of autumn treatments, harvesting times and sampling times for leaf spots

	Sites Uppsala			Lillerud			Bjertorp		Rådde
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 1
Number of varieties	18	18	9	18	18	9	18	9	9
Trial seeded	1988	1989	1990	1988	1989	1990	1989	1990	1990
First year ley									
Autumn cut	-	1990-11-05	1991-10-23	-	1990-11-07	1991-10-24	1990-10-23	1991-11-04	1991-11-14
Second year ley									
Sampling for leaf spots	-	-	1992-06-02	-	-	1992-05-25	-	1992-05-18	-
First harvest	-	1991-06-20	1992-06-11	-	1991-06-17	1992-06-12	1991-06-18	1992-06-04	1992-06-10
Second harvest	-	1991-07-19	1992-07-14	-	1991-07-23	1992-07-29	1991-07-22	1992-07-27	1992-07-15
Third harvest	-	1991-08-27	1992-09-02	-	1991-09-26	1992-09-22	1991-09-03	1992-09-07	1992-09-09
Autumn cut	1990-11-06	1991-10-23	1992-10-26	1990-11-07	1991-10-24	1992-11-23	1991-11-04	1992-10-21	1992-10-19
Sampling for leaf spots		{1991-08-27 1991-10-23	1992-10-19						
Third year ley									
Sampling for leaf spots		1992-06-02			1992-05-25		1992-05-18		
First harvest	1991-06-20	1992-06-11		1991-06-20	1992-06-20		1992-06-04		

Table 2. Monthly maximum snow depth[1)] (cm) at the experimental sites

B = Bare ground

Site	Month: Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Uppsala							
Winter 90/91	B	5	11	7	26	6	1
Winter 91/92	B	B	6	3	5	1	14
Lillerud							
Winter 90/91	B	8	9	2	8	3	B
Winter 91/92	B	B	B	1	5	2	9
Bjertorp							
Winter 90/91	B	1	7	B	10	3	B
Winter 91/92	B	B	8	21	6	2	16
Rådde							
Winter 90/91	B	B	B	B	6	B	B
Winter 91/92	B	B	1	22	20	B	17

1)Data have been obtained from the nearest regional weather station.

Table 3. Results[1)] of the autumn cut, as a mean of varieties in harvests during the second and third year leys (kg ha^{-1})

CV = Coefficient of variation, LSD = Least signifficant difference

Treatment	Second year ley: First harvest	Second harvest	Third harvest	Total harvest	Third year ley: First harvest
Mean					
With autumn cut	3 310	1 941	2 561	7 811	2 395
Without autumn cut	4 457	1 922	2 706	9 114	3 248
LSD at 5%	569	203	186	835	361
CV %	43.4	26.4	20.2	27.9	43.1
Number of trials	7	7	7	7	5
2) Significance					
Autumn cut (A)	**	NS	NS	*	**
Variety (V)	***	***	***	**	***
A*V	NS	NS	NS	NS	NS

1) Values are calculated as least significant means.
2) Significance: NS $p>0.05$ Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 4. Aftermath effect of the autumn cut on the spring yield[1)] of the varieties in the second and third year leys (kg ha^{-1})

CV = Coefficient of variation, LSD = Least signifficant difference

Treatment	Second year ley First harvest With autumn cut	Second year ley First harvest Without autumn cut	LSD for variety at 5%	Third year ley First harvest With autumn cut	Third year ley First harvest Without autumn cut	LSD for variety at 5%
Variety						
Bonita	3 368	4 300	386	2 408	3 134	331
Condesa	2 807	3 600	386	1 863	2 492	331
Madera	2 789	3 718	386	2 065	2 670	331
Patora	2 459	3 443	386	1 599	2 245	331
Rikka	3 780	5 101	386	2 660	3 645	331
Sisu	3 041	4 432	386	2 114	3 118	331
Sv Fredrik	3 133	4 287	246	2 273	3 096	331
Sv Gunne	3 945	5 143	246	2 845	3 866	331
Sv Helmer	3 384	4 698	246	2 491	3 307	331
Sv Svea	3 925	5 035	246	2 752	3 704	331
Taptoe	3 225	4 164	386	2 236	3 075	331
Tonga	3 577	4 832	386	2 801	3 791	331
Tove	3 516	4 828	246	2 557	3 391	331
Verna	3 954	5 025	386	3 248	4 046	432
WW Leia	3 109	4 352	246	2 129	3 068	331
WW Lorry	2 859	3 934	246	2 071	2 840	331
WW Viris	3 169	4 454	246	2 230	3 230	331
CV %	8.3	8.3		13.4	13.4	
Mean	3 310	4 457		2 395	3 248	

1) Values are calculated as least significant means.

Table 5. Autumn harvest, 1992, mean[1)] of 5 sites

CV = Coefficient of variation, LSD = Least signifficant difference

Variety	Harvest kg ha^{-1}
Sv Fredrik	944
Sv Gunne	910
Sv Helmer	1 044
Sv Svea	906
Tove	708
WW Leia	879
WW Lorry	958
WW Viris	850
Mean	896
LSD at 5%	107
CV %	13.0

1) Values are calculated as least significant means.

Table 6. Stand density in autumn and spring. Mean[1)] of varieties

Treatment	Stand density, % Autumn, year before	Spring
Second year ley		
With autumn cut	96	89
Without autumn cut	97	93
Number of trials	7	7
[2)] **Significance**		
Autumn cut (A)	NS	NS
Variety (V)	***	***
A*V	NS	NS
Third year ley		
With autumn cut	97	76
Without autumn cut	98	83
Number of trials	4	5
[2)] **Significance**		
Autumn cut (A)	NS	NS
Variety (V)	***	***
A*V	NS	NS

1) Values are calculated as least significant means.

2) Significance: NS p>0.05 Not significant; * p<0.05; ** p<0.01; *** p<0.001.

Table 7. Mean occurrence[1] (%) of leaf spots on the first and second leaves of the different varieties to develop

CV = Coefficient of variation, LSD = Least signifficant difference

Treatment	Second year ley Spring First leaf	Second leaf	Autumn First leaf	Second leaf	Third year ley Spring First leaf	Second leaf	Autumn First leaf	Second leaf
Variety								
Bonita			2.50	1.28	0.28	0.02		
Condesa			2.33	0.78	0.07	0.01		
Madera			3.24	0.43	0.50	0.03		
Patora			3.65	0.94	0.39	0.07		
Rikka					0.43	0.10		
Sisu					0.66	0.07		
Sv Fredrik	1.07	0.15	0.54	0.73	0.77	0.11	5.93	3.56
Sv Gunne	0.51	0.09	1.07	0.70	0.86	0.04	4.28	1.20
Sv Helmer	0.25	0.05	0.77	0.71	0.74	0.04	0.66	0.13
Sv Svea	0.49	0.09	0.98	0.86	0.72	0.12	2.67	0.66
Taptoe					0.43	0.06		
Tonga					1.13	0.16		
Tove	1.10	0.03	0.67	0.68	1.08	0.05	1.39	1.12
Verna					0.77	0.10		
WW Leia	0.77	0.05	0.44	0.69	0.36	0.07	9.95	1.25
WW Lorry	1.23	0.14	0.60	0.70	0.72	0.07	3.92	1.78
WW Viris	0.98	0.17	1.00	0.69	0.51	0.10	3.99	1.10
LSD at 5%	0.65	0.08	0.55	0.10	0.53	0.11		
CV %	89.0	113.4	40.1	19.0	95.5	170.6		
Mean								
With autumn cut	0.91	0.10	1.43	0.75	0.57	0.09	3.58	1.37
Without autumn cut	0.72	0.07	1.42	0.77	0.67	0.07	3.10	0.79
LSD at 5%	0.25	0.04	0.44	2.80	0.07	0.01		
CV %	36.2	51.7	11.6	200.0	15.1	30.0		
2) Significance								
Autumn cut (A)	NS	NS	NS	NS	*	NS		
Variety (V)	NS	NS	***	***	NS	NS		
A*V	NS	NS	NS	***	NS	NS		
Number of trials	3	3	2	2	3	3	1	1

1) Values are calculated as least significant means.
2) Significance: NS $p>0.05$ Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Ilgvars Krumins
Latvia State Scientific Research Institute
of Agriculture "Agra", Skriveri-1
Aizkraukle reg., Lettland, LV 5126

AUTUMN MANAGEMENT INFLUENCE ON COCKSFOOT

Introduction

A little attention is paid to autumn management decisions for grasslands in Latvia. The problem arose only under heavy fertilizer application and when three or four harvests per season were taken. The grass which every year provides at least three harvests in Latvia is cocksfoot (Dactylis glomerata L.) and for high enough crude protein (CP) content it also requests heavy nitrogen application.

Materials and methods

The results described in this paper were obtained in field trial laid out to estimate the productivity of cocksfoot depending on time and height of final harvest. Experimental site was located at Skriveri - 57°N and 25°E. Average date of start of growth in spring is 16 April, average date of the end of vegetation season is 18 October.

The influence of three factors were investigated in split-split-plot design with three fertilizer levels on main plots, two final cutting heights on sub-plots and four final harvest dates on sub-sub-plots. Three fertilizer levels were low (L) N 150, P 26 and K 100, medium (M) two times higher than L and high (H) three times higher than L. Two final cutting heights were 4 cm (S) and 8 cm (T). Four final harvest dates were 26 August (A), 10 September (B), 25 September (C) and 10 October (D). Factorial design included 24 variants in four replicates.

Cocksfoot was cut three times per season. The first cut was made in the stage of full heading, the second - in 40-50 days interval. Cutting height in the first and in the second harvest was 4 cm. Nitrogen fertilizer was split in three equal dressings. Pure cocksfoot was sown in May 1983, the treatments were imposed from spring 1984 till autumn 1988. At the start of the experiment in May 1984 in all plots there was pure cocksfoot stand with 1800-2000 tillers m^{-2} and percentage of cocksfoot was 96-97 % of grass tillers.

Weather conditions in Latvia are very changeable in autumn and it was necessary to decide what date was to be considered as the end of vegetation season. It was assumed that the end of vegetation is the first day when average daily air temperature durably falls below 5°C.

Durable fall of temperature means, that if 5°C is assumed to be a 0 value, the sum of positive temperatures after the date does not exceeds the sum of negative temperatures before the date.

Sward condition was examined at the end of vegetation season and several measurements took place - sward height, accumulated DM after final harvest, WSC content in tillering nodes, tiller density in fixed plots etc. The next spring there were estimations of WSC content in tillering nodes, visual estimation of sward condition a week after the start of vegetation, tiller density in fixed plots.

WSC content was analyzed in colourimetric way by fenol-sulphuric acid method. Tillering nodes represented 5 cm long plant parts with 2.5 cm roots and 2.5 cm stem bases.

Results and discussion

Autumn management decisions influence the first harvest in the next year but at the same time they also affect the last (third) harvest, too. In this experiment the third harvest gave about 25 % of annual yield and all years there was the same picture - at L treatments DM increased during 50-55 regrowth days (till date B), at M and H treatments - during 65-70 days (Table 1). However maximum in CP and metabolizable energy (ME) was obtained two weeks earlier.

Table 1. DM yield in the third harvest (t ha^{-1}, 1984-88)

Treatments	A	B	C	D	Average
L	1.96	2.36	2.31	2.12	2.18
M	2.70	3.25	3.51	3.17	3.16
H	2.90	3.58	3.76	3.55	3.45
Average	2.52	3.06	3.19	2.95	

LSD_{05}: for average values of fertilizer levels = 0.09 t ha^{-1}
for average values of final cutting times = 0.08 t ha^{-1}
for interactions of both factors = 0.14 t ha^{-1}

Autumn management influence on the first harvest depended on the weather conditions and differed from year to year (Table 2). Very pronounced influence was in 1986 when regrowth period of the first harvest was ten days shorter than average - start of vegetation season was seven days later and the end of April was dry and very warm what resulted in short tillering period in spring. Besides them in spring 1986 there was almost pure cocksfoot stand, till the autumn 1986 and in 1987-88 coach grass (Agropyron repens L.) compensated the reduction of cocksfoot's tillers.

The influence of A and B treatments was similar in all years but C and D treatments effect depended on sward condition at the end of vegetation season (Table 3). The D treatment gave higher yields in 1987-88, when there was no significant regrowth after the final cut, but C treatment was better in 1985-86, when regrowth after the final cut was little but significant.

Table 2. DM yield in the firs tharvest (t ha^{-1})

Treatments	A	B	C	D	LSD_{05}
1985	5.17	5.09	4.64	4.33	0.19
1986	3.80	3.15	2.87	2.48	0.22
1987	5.09	4.83	4.26	5.09	0.17
1988	4.43	4.42	3.97	4.26	0.21
Average	4.62	4.38	3.94	4.04	0.10

Table 3. Regrowth after the final cut (DM g m^{-2}) and degree days in regrowth period

Treatments	1984	1985	1986	1987
SA	51.9	52.6	26.2	38.4
SB	19.2	12.0	11.0	9.6
SC	11.2	9.9	5.7	6.1
SD	7.5	6.5	4.1	2.6
LSD_{05}	6.1	6.3	5.3	4.3
	Degree days			
A	638	572	453	555
B	453	392	283	355
C	303	253	158	210
D	144	93	92	61

In years 1985-88 there was significant interaction between cutting times and fertilizer levels but tendencies in 1985-86 were different to that in 1987-88. Influence of delay in final harvest was more pronounced at higher fertilizer levels (M and H). Treatments MD and HD gave the lowest yields in 1985-86 (Table 4) and every earlier date was better at M and H treatments. No significant differences occurred between LA, LB, LC and LD.

Tabel 4. DM yield in the first harvest (t ha^{-1}, 1985-86)

Treatments	A	B	C	D	Average
L	3.73	3.80	3.39	3.23	3.54
M	4.79	4.16	3.88	3.40	4.06
H	4.94	4.40	4.00	3.58	4.23
Average	4.49	4.12	3.76	3.40	

LSD_{05}: for average values of fertilizer levels = 0.21 t ha^{-1}
for average values of final cutting times = 0.15 t ha^{-1}
for interactions of both factors = 0.27 t ha^{-1}

The lowest yields in 1987-88 were observed at treatment C (Table 5). Treatment D gave higher yields than C. Difference between A and B treatments was pronounced only at the highest fertilizer level (H).

Table 5. DM yield in the first harvest (t ha^{-1}, 1987-88)

Treatments	A	B	C	D	Average
L	4.30	4.27	3.92	4.70	4.30
M	4.85	4.85	4.00	4.40	4.52
H	5.13	4.76	4.44	4.92	4.81
Average	4.76	4.63	4.12	4.68	

LSD_{05}: for average values of fertilizer levels = 0.23 t ha^{-1}
for average valus of final cutting times = 0.15 t ha^{-1}
for interactions of both factors = 0.25 t ha^{-1}

The height of final cut was less important than the date. At treatment T in comparison with S there was average loss of DM 0.52 t ha^{-1} in the third harvest in 1984-88. In the first harvest treatment T resulted in higher yields in 1985 and 1986 0.24 and 0.47 t ha^{-1} DM respectively. Significant interaction between final harvest date and cutting height was observed only in 1985. In the first harvest there was a reduction of DM yield in treatment SD in comparison to SC but no difference between treatments TD and TC.

Sward regrowth showed in Table 3 was estimated by cutting of adjacent area in each sub-sub-plot. Simultaneously the measurements of sward height were taken with rising-plate meter (Castle M.E., 1976). Sward height was measured in ten points per plot and in each point two measurements were taken - the first contact between plate and leave (simple sward height) and when the plate was laid down onto the sward. Rising-plate meter measurements described the actual sward mass better than simple sward height. Equations were calculated for rising-plate meter:

$y = -49.3 + 9.0x$; $r = 0.907$; s.d. = 9.7 g m^{-2}

and for simple sward height:

$y = -47.0 + 5.3x$; $r = 0.815$; s.d. = 13.4 g m^{-2}

WSC content in tillering nodes in autumn was in rage from 30 % till 16 % in DM. The greatest differences were among cutting times with the highest values in treatments A and B. WSC content was approximately the same between S and T treatments and differs a little among L, M and H treatments with slightly higher values in treatment M.

WSC content in spring was from 4 % till 9 % in DM and the tendencies were similar to autumn. Number of WSC analyses were too few for statistical calculations.

Cocksfoot tiller density on fixed plots is shown in Table 6 in actual tiller number per unit of area. Statistical analysis was made with relative tiller density in comparison to 1984, it means that deviations from the initial tiller value were analysed.

Table 6. Cocksfoof tiller density on fixed plots at the start of vegetation season (number m^{-2})

Treatments	1984	1985	1986	1987	1988
L	1770	2290	1450	1710	1070
M	1950	2320	1430	1810	1080
H	2040	2080+	1230	1330	1000
S	2013	2280	1413	1613	947*
T	1827	2187	1333	1613	1147
A	1880	2320	1880	1960	1227
B	1867	2653	1720	1840	1413
C	2080	2267**	1027**	1333**	827**
D	1853	1693**	853**	1333**	707**

The largest differences occurred between two latest cutting times (C and D) and two earliest ones (A and B), however interaction between cutting times and fertilizer levels was significant in 1985-87 (Table 7). No significant differences in tiller density were observed at the lowest fertilizer level (treatment L) but late final cutting dates decreased tiller density at medium and high (M and H) fertilizer levels.

Table 7. Cocksfoot tiller density in fixed plots in spring (number m^{-2}, average in 1985-87)

Treatments	A	B	C	D	Average
L	1820	2090	1870	1480	1820
M	2250	2020	1590**	1560*	1850
H	2080	2100	1160**	830**	1540
Average	2050	2070	1540	1290	

Total grass tiller amount consisted of 96-97 % cocksfoot in 1984 and cocksfoot was replaced with coach grass in treatments MC, MD, HC and HD starting from 1986. Cocksfoot and coach grass together contributed for 85-95 % of grass tiller amount during all experimental period but at treatments MC, MD, HC and HD coach grass percentage reached 25-60 % in 1988. At low fertilizer level (L) coach grass ingress was not observed.

Conclusions

Autumn management decisions - time and height of final cutting have more pronounced influence at the higher fertilizer levels. For cocksfoot time of final harvest is more important than height of cutting.

A good indicator of sward condition at the end of vegetation season is accumulated DM after final harvest. Good overwintering could be expected if there is no significant regrowth after final harvest - less than 100 degree days over 5°C or less than 5 g m^{-2} accumulated DM after final harvest. Regrowth after final harvest at more than 400 degree days over 5°C which provides accumulation of more than 15-20 g m^{-2} DM also contributes to good overwintering. Most dangerous time of final harvest for cocksfoot is that, what results in little but significant regrowth at 100-200 degree days and accumulation of 6-7 g m^{-2} DM.

Changeable weather conditions in autumn do not allow to rely on late final harvest at apparent end of vegetation because after short cessation of vegetation growth could resume for some extra weeks. Then intended harvest at the end of vegetation could prove to be done at the most dangerous time. Late autumn harvest time under heavy ferlitizer application causes reduction of cocksfoot tiller density and subsequent loss of DM yield but reduction in DM yield does not follows the cocksfoot tiller density because ingress of voluntary grasses (coach grass in this experiment) have an effect of compensation.

Early harvest in autumn in late August - early September results in substantial regrowth till the end of vegetation and serves to accumulation of higher WSC content in overwintering parts, helps to keep on higher tiller density and provides more reliable yields. On the other hand there are now reasons for late final harvest of cocksfoot in three-cutting regime in Latvia. Vegatation period is long enough for good third harvest already in early dates and delay with third harvest only worsens the grass quality. Besides them N rates more than 200-250 kg per season are not common for commercial farms and negative effects connected with late final harvest under such conditions are not so important for farmers.

Acknowledgements

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References

Castle, M.E. 1976. A simple disc instrument for estimating herbage yield. Journal of the British Grassland Society, vol. 31, 37-40.

Harald Elbek-Pedersen
The Danish Agricultural Advisory Centre
Udkaersvej 15, Skejby, DK-8200 Aarhus N.

OUTWINTERING OF WINTER CEREALS IN DENMARK

Summary

In Denmark, there is an increasing interest in the growing of winter cereals, especially winter wheat.

In 1 out of 10 years winter cereals are so badly injured by frost that areas have to be ploughed in.

Provided the crops have been sown in due time and no winter barley has been grown after winter barley for 3 successive years, winter fungal diseases are of no economic importance. To obtain a good wintering it is important that the plants are well-supplied with manganese.

Introduction

In Denmark, the winter cereal areas have been heavily increasing in the period 1983-93 (figure 1). Winter wheat accounts for the highest increase (figure 2). The same period saw the start of winter barley growing which was not permitted until 1980.

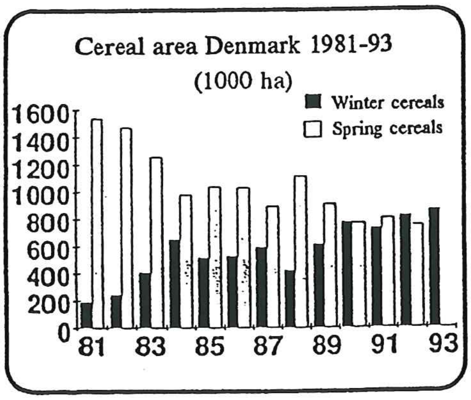


Figure 1.

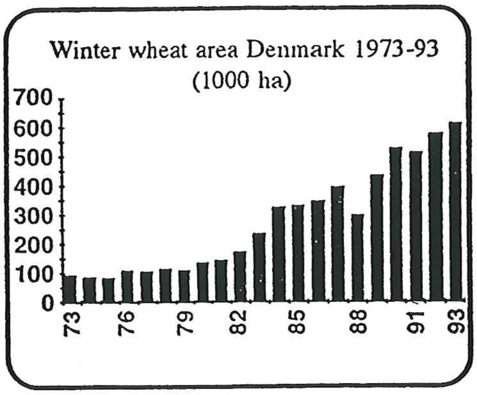


Figure 2.

The dramatic rise in winter crop areas in the late 1980s accelerated as it became compulsory in 1990 that 65% of the land of each individual holding has to be "green" for six months in the winter.

Winter wheat

Frost damages are the most important cause of outwintering in winter wheat crops in Denmark but serious outwintering occurs in maximum 1 out of 10 years. In the two winters, 1984-85 and 1985-86, outwintering caused 24% and 9%, respectively, of the winter wheat area to be resown.

The following 6 winters can all be characterized as mild, involving no outwintering.

Hard frost combined with windy weather and no snow cover are the main causes of outwintering. A minor snow cover isolates, thus reducing injuries.

Winter fungal diseases like *Typhula incarnata* (snow rot) and *Gerlachia nivale/Fusarium nivale* (brown foot rot) are normally of no economic importance in Denmark

Winter rye

All the rye varieties grown have a good frost resistance. During the two hard winters in the mid-1980s about 1-2% of the rye area outwintered.

Gerlachia nivale (brown foot rot) is the main cause of outwintering in rye crops. The most serious injuries occur after a snow cover on non-frozen soil and they are often seen along hedges and forests.

Typhula incarnata (snow rot) is of no economic importance in rye crops in Denmark.

Winter barley

Not until 1980 was winter barley growing permitted in Denmark. Over the past 4 years the area sown to winter barley has been about 150,000 ha annually.

Frost damage is the most significant cause of outwintering. The varieties grown have turned out to have a poor frost resistance. The best overwintering ability is found in the six-row barley varieties. Due to the lack of frost resistance in the Danish winter barley varieties, the three winters from the autumn of 1984 to the spring of 1987 saw outwintering percentages of 75%, 64% and 50%, respectively. The late harvest and wet autumn of 1987 combined with fear of a fourth year of serious outwintering reduced the area sown to winter barley. The past 5 winters have been mild and with no frost so outwintering has been at a minimum.

Calculations show that with an outwintering in the range of 60% it costs the Danish farmers about 100 mill. Danish kroner to establish a new crop.

Typhula incarnata (snow rot) can be found in winter barly every year. In normal years (e.g. the past 6 winters) this disease is of no economic importance but in areas where winter barley succeeds winter barley Typhula may be of economic importance.

Gerlachia nivale (brown foot rot) is of no economic importance in winter barley crops in Denmark.

Postscript

Danish trials show that outwintering increases if the plants suffer from a manganese deficiency. Therefore many areas receive an autumn application of 3-4 kg manganous sulphate ($MnSo_4$) per ha.

Per Ruuth
Svalöf Weibull AB
Box 4097, Röbäcksdalen
S-904 03 Umeå, Sverige

SELECTION METHODS FOR WINTER HARDINESS IN GRASS AND CLOVER.

Introduction

Climate adapted varieties are a primary purpose in ley crop breeding. In northern Sweden good overwintering capacity is an important condition that must be fulfilled for good economy in ley production. Because of a deep snow cover, damage due to freezing is rare but heavy losses are often caused by ice encasement particulary in the coastal region. However, timothy ecotypes with a high level of ice tolerance developed by natural selection can be found as shown by Andrews & Gudleifsson (1983).

Some years snow mould fungi cause great economic losses. Typhula blight (*Typhula ishikariensis*) and snow rot (*Myriosclerotinia borealis*) on grasses and crown rot (*Sclerotinia trifoliorum*) on clover are the most important species. Clover root rot is a syndrome of great complexity caused by patogenic fungi of Fusarium, Cylindrocarpon and Phoma species. It may indirectly contribute to winter losses in red clover stands by causing enfeebled plants.

At Svalöf Weibulls northern department breeding for better overwintering capacity is in progress. Selection include ice tolerance as well as resistance to parasitic fungi in grass and clover. The purpose is to create new winterhardy varieties mainly in timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*), the most important grassland species in northern Sweden. In a breeding programme tolerance to ice encasement and snow mould resistance will be combined.

Methods for selection

Forage crop breeding means that a lot of individual plants must be handled. To limit the costs selection methods must be rational. In resistance breeding controlled environments are necessary. For that reason selection work mainly takes place in greenhouse and climate chambers.

The grass breeding material is hardened in a hardening chamber before treatment. The influence of photoperiod, light intensity and temperature upon hardening has earlier been studied by Paulsen (1967) and Sjøseth (1971). Hardening is carried out at long days (16 hours) high light intensity (6000 lux) and low temperature (1°±1°C). Under these circumstances sufficient hardening can be obtained after two weeks concerning timothy and meadow fescue which means that about 20 test series can be performed every year in these crops. The space in the chamber is the main limiting factor for the number of plants able to be tested. However up to 7700 plants can be hardened for each test.

Ice encasement.

Selection for ice tolerance in the field is very hazardous. At Svalöf Weibull a screening method for selection under controlled environment conditions is under development. So far the method has been tested on some timothy populations. Hardened, 8 weeks old plants grown in plant trays (Plantek 144) are placed in a plastic box and transfered to a freezing chamber at -5°C. The boxes are gradually filled with water until the plants are completely ice encased. This procedure takes about four days and nights. During the period of ice encasement temperature is kept around -2°C. An ice encasement period of 8 weeks is necessary to obtain a satisfactory selection effect in the populations tested.

Typhula blight.

Breeding for resistance to Typhula ishikariensis has so far been concentrated to timothy. Selection to a limited extent has also been done in meadow fescue and perennial ryegrass. The laboratory technique corresponds to Bruel et.al.(1966).

Typhula is grown on potato based clear soup. Mycelium is mixed in water and spread over the plants with a compressed-air sprayer. Plants are grown in plant boxes (60*40*9 cm) on peat soil. They are inoculated after hardening at 8 weeks age. The boxes are covered with moist web and plastic film to keep humidity and stored at about +2°C for 16 - 20 weeks. Selektion occur after three weeks of regeneration in green house.

A modification of the method is under evaluation. Plants are grown in plant trays (Plantek 144) instead of boxes. After inoculation the trays are placed in plastic boxes able to pile up. In this way time as well as space are saved and the number of plants in each test serie can be increased from 4600 to 7700.

Crown rot.

Red clover populations are selected for resistance to the crown rot fungus, Sclerotinia trifoliorum. The fungus is reared on barley kernels and plants are infected by using dried grain inoculum as described by Kreitlov (1951). Sowing is performed in July and plants are grown outdoors in plant boxes (60*40*9 cm). Plants are 6 to 8 weeks old when infected and left outdoors for hardening. The plant boxes are moved indoors in October, covered with a plant web to keep humidity and kept at a temperature about +3°C. After another 3 - 4 months, depending upon the rate of infection, regeneration starts at +15°C and 16 hours of daylight. Almost 15 000 plants are tested for crown rot every year and on average about 5 percent of the plants are selected for resistance.

Clover root rot.

Earlier investigations show that selection for resistance to Fusarium roseum increased the level of common root rot resistance in red clover populations (Andersson & Kristiansson, 1989). At Svalöf Weibull a method described by Rufelt (1986) is used by routine in resistance breeding. Red clover plants grown in pots are inoculated at 3 months of age by removing the soil-root mass from the pot, cutting through the root ball and placing the inoculum in contact with the cut taproot. After inoculation, the plants are repotted and placed in the green-house at about +18°C and 16 hours of light. Normally 6 weeks after inoculation the roots are washed and the taprots split longitudinally.Resistant plants show no or little vertical discolouration above the point of inoculation. Selection is made yearly in 5 or 6 red clover populations of 1000 plants each.

A breeding strategy.

Plant selections for fungi resistance has been repeated for two or three generations in timothy and red clover populations. Selected plants has been planted in polycross in the field. Field tests of populations with an increased level of snow mould resistance are in progress. Evaluations of winter hardiness must be repeted during several years on many sites to support the effect of selection upon economic yield. So far no significant influence upon yield have been found.

When a reliable technique for ice tolerance selections has been developed the idea is to create new populations widely adapted to winter stress factors. Plants selected for ice tolerance will be intercrossed with plants from the same original population but selected for fungi resistance. This may be a possible way for further population improvement and prodoction of new varieties.

Litterature

Andersson, B. and Kristiansson U. Proceedings XVI International Grassland Congress. Nice, France. 1989. *Breeding for resistance to root rot in red clover.*

Andrews, C.J. and Gudleifsson, B.E. 1983. *A comparision of cold hardiness and ice encasement tolerance of timothy grass and Winter wheat.* Can. J. Plant Sci. 63: 429-435.

Bruehl, G.W., Sprague, R., Fischer, W.R., Nagamitsu, M., Nelson, W.L. and Vogel, O.A. 1966. *Snow moulds of winter weat in Washington.* Wash.Agric. Exp. Bull. 677.

Kreitlow, K.W. 1951. *Infection studies with dried grain inoculum of Sclerotinia trifoliorum.* Phytopathology XLI No 6: 553-558.

Paulsen, G.M. 1968. *Effect of photoperiod and temperature on cold hardening in winter wheat.* Crop. Sci. 8: 29-32.

Rufelt, S. 1986. *Studies on Fusarium root rot of red clover (Trifilium pratense L.) and the potential for its control.* Plant protection reports. Dept. of Plant and Forest Protection. Swedish University of Agricultural Sciences.

Sjøseth, H. 1971. *Winter hardiness in different meadow and pasture plants.* Scientific reports of the Agricultural College of Norway. Vol.50 No 13: 11-38.

Seppo Pulli
Agricultural Research Centre of Finland
Institute of Plant Breeding
SF-3100 Jokioinen, Finland

CONDUCTIVITY TESTING FOR SCREENING THE WINTERHARDINESS OF CEREALS, GRASSES AND LEGUMES

Introduction

SNP (Samnordisk planteförädling) have financed the Co-Nordic overwintering project "Utveckling och utvärdering av laboratoriemetoder, som används i praktisk förädling mot vinterskador i höstsäd och vallväxter". Accordingly, laboratory tests and field trials on winter cereals commenced in 1989 and trials on grassses and legumes in 1991. The project will reach completion in 1994. Conductivity measurements describing hardening changes and degree of plant injuries following frost treatment were included in the test performed at the Institute of Plant Breeding in Jokioinen, Finland. The conductivity (resistance) measurements obtained from both hardened and the injured plants after the freezing tests were then compared with the results from the freezing and field tests. Root reserved carbohydrate measurements were thought to also describe the results of the plant hardening process.

Materials and methods

Nine winter wheat varieties and 10 winter rye varieties were hardened as follows. The pre-hardening treatment of 3-leaf stage plants was one week at 10°C and 10 h daylength.

After pre-hardening, the plants were hardened in +4°C for four weeks at 10 h daylength. At the end of the hardening period the hardening stage was determined by a Hewlett-Packard 34401A multimeter. The measurement point was the growth point of the joint of the stem and leaves. Resistance was measured as mohms. The resistance measurement describing the conductivity between the measurement points about 1 cm apart was then compared with the results of the freezing and field trials of the 3-year SNP project. Root and lower stem carbohydrate reserves were determined by the modified Somogyi-Nelson method.

Twelve timothy, English ryegrass and red clover varieties were hardened for one week at +10°C and 10 h daylength. The first phase of hardening occured at +2°C for two weeks and the second phase at ±0°C for two weeks. Daylength during the hardening period was 16 h. The conductivity of growing points was measured before and after the plants were frozen. The above results were then compared with the results of freezing test and with the overwintering results of grasses and legumes at three locations in Finland during the winter of 1991-92. Root CHO reserves were measured according to the modified Somogyi-Nelson method. The leaf damage scale employed was that of SNP.

Results and discussion

The results for winter wheat showed that the conductivity of the hardened plants correlated with 95 % significance with the results of the freezing and field test results of the same varieties. Artificial freezing survival and field survival correlated with 99.9 % significance. The condutivity of hardened rye plants did not show a trend similar to that observed for winter wheat, probably because of the lower degree of hardening due to the high (+4°C) hardening temperature.

In the the case of timothy the freezing temperature was -16°C. The conductivity after hardening did not reveal any correlation with the other factors. The conductivity values after freezing correlated with 95 % significance with field survival and 99.9 % significance with the number of plants that survived the freezing treatment. The correlation between freezing survival and field survival was 99.9 % significant.

The hardening stage of English ryegrass frozen -16°C correlated well with the ohm values obtained for the conductivity of the plants after freezing (99 %

significance). Moreover, the conductivity values of ryegrass had a significant correlation with the results obtained for plant survival after freezing. However, one years's wintering results were probably not enough to show correlations with the field observations.

In the case of red clover (freezing temperature -15°C), a very good correlation (99 % significance) was found between the conductivity values of the plants after hardening and freezing injury. The hardening values also correlated (99 % significance) with leaf damage and plant survival after freezing (95 % significance). Red clover also showed good correlation between the plants that survived the freezing test and those that survived the field test.

Based on the results of the first tests conductivity can be concluded to be a suitable screening method for plant breeding work.

Eduardas Lemezis
Lithuanian institute of agriculture, Dotnuva-Akademija
235051 Kedainia distr., Lithuenia

CELL CULTURE AS A METHOD OF BREEDING LOLIUM PERENNE FOR HIGHER WINTER TOLERANCE

Introduction

Several years ago it was noticed that callus tissue of cherry tree was able to survive after freezing at below zero temperatures (4). In the authors opinion by way of freezing out susceptible cells of calli it is possible to screen more freezing tolerant and regenerate of them plants.

Screening for salt, herbicide and drought in different kinds of plants using tissue culture is most often used in practical breeding (1,2).

The objectives of these investigations were in consecutive order:

- to describe the methodic of obtaining plants with a higher winter tolerance by cell culture;
- to investigate the effects of various concentrations of sucrose in medium and freezing temperatures on regenerants output of calli;
- to carry out preliminary estimation of regenerants for freezing and winter tolerance.

Materials and methods

Two genotypes G-2 and G-7 of perennial ryegrass variety "Veja" were used. Shoot apices in the 2nd and 3rd stage of organogenesis were used as explants for callus initiation. Tillering points with a 3-4 cm stem fragments were sterelized by immersion in 0,1 % sulema solution for 15 min and rinsed 3 times in sterile water. Callus tissue was induced on a Linsmaer and Skoog nutrient medium modified by Stanys and Sliesaravicius containing higher concentration 10 mg/l of 2,4-D (3). In order to study the influence of a higher sucrose contentration (12 %) on callus output of frozen calli half of calli were transferred to medium with a higher sucrose concentration. The cold acclimation and freezing tests were carried out using the method described by Tumanov (5). Under each testing temperature 20 calli were evaluated. The plants regeneration of the calli was tested in special room at 26°C with a 10000 lux light intensity and a 16-h photoperiod. All regenerated plants were taken to the greenhouse for further growth. After vegetative multiplication of regenerants freezing tolerance was tested using bundles method. Winter tolerance of regenerants was tested in the clonal nursery. All the operation with calli were carried out in test-tubes 1,6 x 15,0 cm containing 10,0 ml nutrient medium in the dark.

Results

The scheme of developing regenerants with a higher winter tolerance is provided in Table 1.

Work duration from genotype selection till the getting of regenerants is about 170 days. We had got 24 regenerants of genotype G-2 working according to the scheme. That was about 10 % of all calli number. All regenerants we had got were green and viable.

The effect of freezing temperatures and sucrose concentration in medium on the output of regenerants are shown in Fig. 1.

The highest number of regenerants at both sucrose concentrations in the media has been got at -6°C calli freezing temperature. Temperatures lower than -6°C firmly reduced the number of regenerated plants. Sucrose concentration effect on plants regeneration manifested at the lowest temperatures -21 and -24°C.

Table 1. The consecutive scheme of developing ryegrass regenerants with a higher winter tolerance by a cell culture method

Action	Duration (days)	Volume	Details of action
1. Genotype selection		1 genotype	
2. Vegetative multiplication of genotype	60-80	500 tillers	Greenhouse
3. Setting of explants	1-2	400 shoot apices	2-3 stage of organogensis
4. Induction and prolifera-tion of callus tissue (CT)	25	294	Introduction CT - 10 days proliferation CT - 15 days
5. Cold acclimation and freezing of CT	20	294	1 stage of acclim. 10 days at +2°C 2 stage of acclim. 3 days at -3°C free-zing at -6,-9,-12 ......-24°C
6. Thawing and regrowth of CT	31	294	Thawing - 1 day at +2°C regrowth - 30 days at +26°C
7. Plant regeneration	30	24 regenerants	
8. Vegetative multiplication of regenerants	60	"-	Greenhouse
9. Comparative estimation of regenerants	300	"-	Greenhouse and field

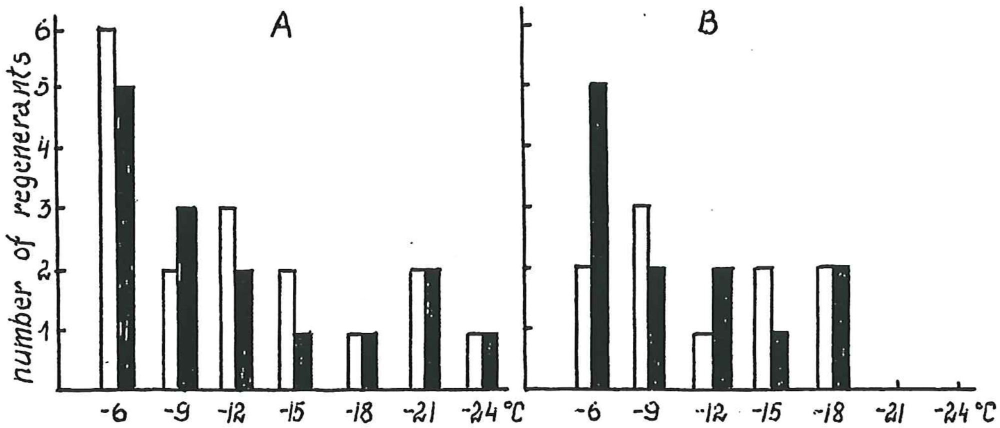


Fig. 1. Effect of freezing temperatures and sucrose concentrations in medium on regenerants output of calli of genotype G-2 and G-7

□ G-2
■ G-7

A. Acclimation of calli was carried out on the medium with the higher (12 %) sucrose concentration

B. Acclimation of calli was carried out on the medium with the normal (2 %) sucrose concentration.

Both genotypes acclimated on medium with higher (12 %) sucrose concentrations regenerated plants at these temperatures. However, callus that had been acclimated on medium with normal (2 %) sucrose concentration did not regenerate plants at this temperatures. In that way the data indicate that higher concentration (12 %) of sucrose content in medium increases plant regeneration of frozen calli.

In order to determine freezing tolerance of regenerated plants we had frozen them at -15°C (Table 2).

Frozen regenerants showed a different tolerance to the freezing test. Of all tested regenerants only 3 showed a significant difference in freezing tolerance as compared to initial forms

Table 2. Comparison of freezing tolerance of regenerated plants.

Conventional making of regenerants	Calli freezing temperature	% plant survival G-2	G-7
Genotypes G-2 and G-7 (initial form)		30,2	25,9
1	- 6	18,4	
2	- 6		5,0
3	- 9	32,3	
4	- 9		11,6
5	-15	28,0	
6	-15		10,0
7	-18	26,9	
8	-18		23,0
9	-21	37,5	
10	-21		56,8
11	-24	61,0	
12	-24		42,5
LSD 0,05		15,4	18,0

For instant, of genotype G-2 the most freezing tolerance showed two regenerants N10 and N12 (56,8 and 42,5 % of survived plants respectively). These plants were regenerated from calli which was frozen at the lowest freezing temperatures -21 and -24°C. The results of this test indicate that it is possible to regenerate more freezing tolerante plants from frozen calli.

For the preliminary estimation of regenerants by valuable characters a study of regenerants in field was conducted. In spite of that 1989-1990 winter was not severe initial forms overwintered badly and were estimated by 3,5 points. All regenerants successfully overwintered. They were estimated in 5 points. All regenerants showed very intensive regrowth in the spring. For instance after 20 days since the beginning of regrowth the height of plants was 22,0-25,0 cm. Initial forms were only 20 high at the same time. However, the regenerants had a lower green forage yield of three cuts as compared with initial form. It was ranging from 4,6 to 39,4 % among different regenerants. We did not find differences in heading date and number of chromosomes among regenerants and the initial forms. But regenerants had shown variation in plant height and leaf texture. They had a higher tolerance to the crown rust and especially to leaf spot.

These preliminary studies indicate that suitable techniques for plant regeneration give us a good chance for selecting mutant regenerants possessing higher winter tolerance from calli by freezing.

References

1. Mazin, V.V. et al, 1989. Sbornik naucnik trudov. 42: P. 66-74.
2. Smith, R.N. et al, 1985. In vitro. 21,10. P. 541-545.
3. Stanys, V. & Sliesaravicius, A., 1985. 23 p.
4. Tumanov, I.I. et al, 1968. Plant physiology, 15, 5. P. 749-756.
5. Tumanov, I.I, 1979. 338 p.

Bjarni E. Gudleifsson
Agricultural Research Institute
Mödruvellir Experimental Station
601 Akureyri
Iceland

METHODS FOR TESTING ICE ENCASEMENT TOLERANCE IN HERBAGE PLANTS.

Introduction

Winter hardiness of a plant is composed of tolerance to many different stresses. In many studies it is of interest to evaluate the tolerance of plants to each of these winter stress factors separatly, such as freezing tolerance, snow mould tolerance or ice ebcasement tolerance. Several methods have been used to accomplish this.

Studies of winter damages and ice encasement injuries have as main tasks to sort out following complexes (Baadshaug 1973b):

1. The influence of climate and plant environment on the extent of damages - impact of climate on hardening and stress intensity.
2. The effect of cultural practices on plant tolerance and injuries - impact of management on winter hardiness.
3. The genetical background of variable responses to the winter stress - breeding purposes.
4. Physiological and biochemical processes taking place in the plant during hardening and injuries - studies of the process of damage at cellular and molecular level.

Different types of studies can be used to approach these goals (Baadshaug 1973b, Larsen 1986, Sakai & Larcher 1987, Larcher 1985):

1. Sampling studies.
2. Field experiments.
3. The provocation method where stress intensity is artificially increased.
4. Laboratory experiments in controlled environmments.
5. Measurements of plant caracters related to tolerance.

These different types of studies will now be discussed.

Sampling studies

Sampling of information from farms have been used to clarify the effect of climate and soil on winter damages. This is a suitable method to map the winter damages, where great number of relatively simple data are needed. By supplementing such records with climatic data, the relationship between winter climate and the type of winter damages in Norway and Iceland have been illustrated (Andersen 1963, Årsvoll 1973, Fridriksson 1954, Gudleifsson 1971). In addition sampling of information on topography, soil conditions and management can provide a basis for investigating the effects of such factors on survival, but the confounding effects of many factors may lead to difficulties in interpretation. Hakamata et al. (1978) used sampling method to demonstrate the effect of soil conditions and management on ice encasement damages and the same applies to the studies of Fridriksson (1954), Gudleifsson (1971) and Årsvoll (1973), but the effect of management have been difficult to demonstrate this way. Samuelsen (1990) has tried to modell the overwintering of grasslands in Norway using survey data on overwintéring, winter climatic caracteristics and the information about the site and management of the fields.

Sampling can help in clarifying main lines in the impact of environment on winter damage and also the impact of management on hardiness, but more precice experiments are needed to explain details in the process of winter damage.

F i e l d   e x p e r i m e n t s

The results of ordinary field experiments, where ice encasement is involved, often indicate differences between species and cultivars of plants and the effect of management on survival (Andersen 1960, 1963). In field experiments the intensity of the winter stress often is too small or too great to separate the treatments so the experiments might be of limited value. Unfortunately the results in field experiments are often not presented as percent damage or survival but as yeald at first cut or plant cover. Larsen (1986) found rather good correlation (r= 0,67 to 0,75) between winter survival and yeald at first cut in breeding material of Dactylis glomerata in Norway, but the yield in field tests at three location was not a good criteria for selection in winter hardiness. It is quite understandable that yield of permanent grassland is not a good criterium for damages because weeds taking over the space of killed plants can partly compensate for the yeald lost by killed grasses. Therefore winter survival registrations should be included in most experiments with perennial plants where winter hardiness is of interest. In most ordinary field experiments winter climate data or field observations during winter are not inclluted and it is not obvious wich is the cause of the winter damage, i. e. if ice encasement damages are involved. In studies of ice encasemant damages in field experiments it is very important to collect such informations (Anon. 1968). The disadvantage with field survival trials is that the ice encasement stress in many cases does not occur regularly on the field. Such experiments should therefore be localized on places where ice encasement damages are commonly experienced and the field should be leveled such that the stress will be as evenly distributed as possible. Small differences in topography will affect the snow cover and ice cover and result in variable intensity of the stress over the experiment and increase the experimental error. The stress levels experienced in field trials have been shovn to change dramatically over distances as short as a few meters and the ice encasement damages often gets an patchy caracter and are unevenly distributed over the experimental area (Brink et al 1939, Huokuna 1959, Gudleifsson & Sigvaldason 1972, Fowler 1979). To minimize these errors comparisons should be restricted to plots that are in close proximity to one another.

Ordinary field experiments can be of use in ice encasement studies, but these experiments should be backed up by climatic data or field observations during winter to establish the type of winter damage. Experiments should be placed on well levelled fields to ensure as equal stress intensity as possible and on areas where ice cover might be expected to form.

The provocation method

The provocation method is performed by artificially modifying the stress in order to make field experiments more effective. Such experiments have the advantage compared to laboratory experiments that the plant material is in natural conditions. But such trials are dependent on the natural winter climate at the location and of this reason might fail. Ice cover can be established artificially by flooding of the plots during or just before frost. The ice cover can be protected from melting or extremly low temperatures by snow cower or insulating meterials. Trials, where standard winter conditions are thus simulated in the field have been used succesfully for producing ice encasement damages (Beard 1964, 1965a,b, Sjøseth 1971, Baadshaug 1973a, Andrews & Pomeroy 1977, 1990). Artificially produced ice cover has in such experiments been more damaging than natural ones (Andrews & Pomeroy 1977a). Sjøseth (1971) has described a transportable freezing equipment (115 x 115 cm) for use in the field giving the possibility of freezing the plants or producing ice cover on small plots during thaw perio-ds.

The provocation method can in many locations and in many years be used succesfully in testing ice encasement tolerance.

Laboratory methods

The ability of plants to survive ice encasement in the field might be assumed to be the best characteristic of the ice encasement tolerance. However, the nature and severity of the winter stress will vary between years and locations in the same year. The results can be difficult to interprete, and in the nature the winter damages often are a result of a mixture of stresses and the survival is a expression of "winter hardiness" rather than tolerance to a specific stress factor. Therefore scientists have been loooking for artificial and more specific methods for testing the plants tolerance to each of the winter stress factors. Then plants are cultivated and exposed to the actual stress factor in controlled environments. Testing the tolerance of herbage plants involves five steps of operation: raising, hardening, stressing, recovering and asessment of damage. In many cases there is demonstrated good agreement between results of ice encasemant in the laboratory and field survival (Sjøseth 1959, 1971, Andrews & Pomeroy 1977a, Andrews et al 1986), but in some cases the relationship is poor (Andrews et al. 1986) as could be expected where other stress factors than ice encasement are involved in the field. The laboratory tests have the advantage that great materials can be tested for specific stress factor in relatively short time. However for all laboratory tests there are many problematic questions. How should plants be raised ? How old plants should be tested, and at what stage of hardening should the test be executed and at what conditions should the plants be regenerated.? And for the ice encasement it is also a question at what temperature the plants should be encased.

For winterannual plants like the winter cereals it is natural to test young seedlings, because in field they enter winter as seedlings. For perennial plants as grasses and legumes the age of plants might affect results, but in most cases also in these species rather young seedlings are prefered in controlled environments in order to save time and space. There is no certain indication that species and cultivars rank differently with increasing age of plants, but cutting and regrowth might have more negative effects on some species than other. Grass plants of first year seedlings often respond differently from plants after later production years (Gudleifsson 1971), but this was not the case with alfalfa plants (Bowley & McKersie 1990).

Plants seem to increase their ice encasement tolerance at the same **hardening conditions** as induce the freezing tolerance (Sjøseth 1971), but timothy and winter wheat seemed to increase ice encasement tolerance faster than freezing tolerance at 2/0 C and 16 hour day (Andrews & Gudleifsson 1983). Plants hardened in controlled environments usually do not reach the same level of tolerance as field derived plants (Sjøseth 1959, Pomeroy & Fowler 1973, Andrews & Gudleifsson 1983). There are some indications that species and cultivars of winter cereals might rank differently according to ice encasement tolerance at different levels of hardening (Andrews & Pomeroy 1975, McKersie 1983), but it is still a question at what level of hardening plants should be tested for ice encasement tolerance. As pointed out by Larsen (1986) plants at coastal regions with variable climate do not always reach maximum of hardening before winter, and also ice encasement stress often occurs in late winter and eary spring when plants are at reduced tolerance. McKersie (1983) suggested field plants of winter cereals should be tested in laboratory in the spring, prior to soil temperature rising, because of better separation of cultivars at that time than in the fall. As demonstrated by Andrews & Gudleifsson (1983) and Tronsmo & Svendsen (1989) cereals and especially grasses induce ice encasement tolerance quickly at low temperature hardening conditions, so 2-7 weeks of hardening at controlled environments have commonly been used. Similar pattern of increase is observed in freezing tolerance of winter cereals in field and controlled environment conditions (Pomeroy & Fowler 1973) and the same is supposed also to apply to ice encasement tolerance. This suggests that an accurate reflection of cultivar tolerance might be obtained through the use of controlled environments. In all experiments the environmental conditions during raising and hardening should be standardized. Hetherington et al. (1990) demonstrated that different levels of fertilization did not reverse the ranking of winter wheat cultivars according to ice encasement tolerance and freezing tolerance.

Often plants are taken from the field after hardening and then tested for ice encasement tolerance in controlled environments (Sprague & Graber 1940, 1943, Smith 1952, Beard 1964, Freyman & Brink 1967, Andrews & al 1974, Andrews & Gudleifsson 1983, Andrews et al 1986, Gudleifsson 1986b, Bowley & McKersie 1990). By staining with TTC Suzuki (1972) tested the survival of field plants dug out of the soil during winter. Hardened plants are easely taken from field only in fall before the soil freezes, and also in spring in more or less dehardened state. Sometimes plants are removed from flats prepared before the onset of winter, but this involves modification of the environment. Snow and soil freezing during winter make plant removal directly from field difficult. This has been achieved with concrete chipper (Andrews et al 1974, 1986) and Bolduc (1976) described tecnique for large scale study of plants during cold season.

In some cases **the ice encasement stress** has been simulated by placing the plants under N_2 environment at low temperatures (Smillie & Hetherington 1983, Samygin et al. 1971, 1973, Rakitina 1967). This simulation has the disadvantage that gaseous substances produced by plants during anoxia will be able to evaporate from the plant to the surroundings. Thus the toxic stress of the anaerobic respiration products is not as intense in N2-atmosphere as in ice. Most laboratory experiments with ice encasement have therefore been performed in ice at subzero temperatures.

The ice encasement tests have been executed in boxes or flats where the plants are covered with ice (Sjøseth 1959, 1971, Smith 1952, Hope et al. 1984) or the whole pot with plants is submerged in bigger boxes (Larsen 1985, Gudleifsson & Bjørnsson 1989). The removal of soil from plants before ice exposure assists in incresing the uniformity of stress but adds to the labour (Andrews & Pomeroy 1989, Gudleifsson & Bjørnsson 1989). After trimming of root and top plants or crown segments are submerged in water in beaker wich is frozen to solid ice (Andrews & Pomeroy 1975, Gudleifsson et al 1986, Gudleifsson & Bjørnsson 1989, Tronsmo & Svendsen 1989, McKersie et al. 1982, Andrews 1977, Rakitina 1970). In some cases (Beard 1964, Freyman & Brink 1967, Tanino & McKersie 1985) field or greenhouse grown plants are encased with the root system in the soil medium, in order to reduce labour and to simulate the natural conditions. This method increases the requirement for freezing space and has not reduced the variability between paralelles (Gudleifsson & Bjørnsson 1989) and there is a possibility af variable air inclutions in the soil thus reducing the anaerobic stress.

The temperature during encasemnet has variated:

- -1,0 °C (Andrews & Pomeroy 1975, McKersie et al 1982 Poysa 1984),
- -2,0 °C (Mckersie 1983, Hope et al 1984, Larsen 1985, McKersie & Hunt 1987, Gudleifsson 1986, Gudleifsson & Bjørnsson 1989, Tronsmo & Svendsen 1989)
- -2,5 °C (Smith 1952, Sjøseth 1957, 1959)
- -3,0 °C (Sprague & Graber 1943, Larsen 1985)
- -4,0 °C (Beard 1964, 1965, Freyman 1969)
- -5,0 °C (Rakitina 1970, Bowley & McKersie 1990).

The temperature should secure complete freezing of the surrounding water without freezing the plants. Hardened winter cereal plants start freezing at - 6 °C (Pukacki & McKersie 1990). It is demonstrated that survival decreases as the ice encasement temperature is lovered (Andrews & Pomeroy 1975, Pomeroy & Andrews 1985, Gudleifsson 1989).

In laboratory experiments with ice encasement, plants are often subject to certain period of encasement, and survival is presented as persentage surviving plants (Sjøseth 1959). This can be done with plant material of fairly well known tolerance, but in most cases it is safer to sample ice encased plants at several different intervals, where few plants are killed at the lowest stress and few are surviving the highest stress. this method offers the possibility to express the results as LD_{50} i. e. the number of days required to kill 50% of the plant population (Pomeroy & Fowler 1973). The different sampling times increase the accuracy of the survival determination, but increase the need for plant material and ice encasement space. The lewel of hardening is not as critical when LD_{50} is determined as when survival is measured at single stress level (Pomeroy & Fowler 1973, Brule-Babel & Fowler 1989).

Laboratory tests for ice encasement tolerance have been used on winter cereals (Andrews & Pomeroy 1975,1981), grasses (Gudleifsson et al. 1986) and legumes (Bowley & McKersie 1990) and demonstrated a vide variation in ice encasement tolerance. Even though these methods demonstrate differences between plant species in ice encasement tolerance, the variability within replicates and paralelles has been considerable and limited their use in breeding programmes (Tronsmo & Svendsen 1989, Bjørnsson 1986). Efforts have been made to reducte this variability (Gudleifsson & Björnsson 1989).

Andrews (1988) has demonstrated that ice encasement **testing in light** gives higher survival than testing in dark as has been most common. In the presence of light there was lesser utilization of total nonstructural carbohydrates, less consumed O_2, less accumulated ethanol but more CO_2. The effect of light offers partly an explanation for the substantially higher ice encasement tolerance of plants under field conditions than laboratory tests which have routinely been carried out in dark (Andrews & Pomeroy 1990). The presence of light probably closer mimics the natural winter conditions in some areas, but in countries at higher latitudes the light through snow and ice during winter is of little importance. Because ranking of cultivars was different in the presence and absence of light (Andrews 1988) it is a question if further selection should be made in light as proposed by Andrews & Pomeroy (1990).

The next step after ice encasement treatment is to **assess the injury**. Some methods have been developed to determine the injury quickly after stressing. Snillie & Hetherington (1983) used the clorophyll fluorescence on wheat leaves and demonstrated that FR, the maximal rate of the induced rise in clorophyll fluorescence, decreased more in nonhardy cultivars than hardy ones during anaerobic stress. Tanino & McKersie (1985) tried to determine the cell viability after ice encasement of winter wheat by tetrazolium staining and observed that crown cells were viable first after ice encasement which was lethal to the plant. The viability of the cells was lost during the first days of regrowth. Therefore percent surviving plants is probably the most reliable determination of plant injury and it is also most commonly used. It should be noted that bacteria might be involved in the damages of plants during regeneration after freezing (Olien & Smith 1981) or ice encasement (Gudleifsson 1983), but the role of them is still not known. Many scientists have also used plant regrowth or dry matter yield as a measure of ice encasement injury (Rakitina 1977, Bowley & McKersie 1990, Baadshaug 1973a). The dry matter yield should be more indicative to sublethal stress injury and more sensitive than simply survival. The dry matter yield is not a good criterium of damages when plants of different species or different ages are compared (Bowley & McKersie 1990), but as pointed out by Larsen (1986) when comparing material with little genetic variation in tolerance the scoring of vigour or amount of regrowth seems to express plant differences better. Baadshaug (1973a) also found that althogh there was a good agreement between yield and percentage surviving plants the effect of any experimental factor manifested itself much more strongly in the yield than in persentage surviving plants.

Larsen (1988) has compaired laboratory tests for freezing tolerance, snow mould resistance and ice encasemant tolerance to field survival in timothy. The best separation in the material was achieved in freezing tolerance. The freezing tolerance was the best prediction of field survival, snow mould resistance showed some relationship to survival, but the ice encasemant tolerance measured in the laboratory did not show any correlation to the field survival. Laboratory results of freezing tolerance and snow mould resistance showed significant correlation (r=0,79) to spring field cover and ice encasement tolerance did not improve this relationship.

Measurements of plant caracters

Ideally a method for assessing the level of stress tolerance should be rapid, sensitive, repeatable and if possible non-destructive to the plant material. Instead of using a direct stressing tecnique, methods have been developed where empirical correlations between tolerance and some chemical, physical or anatomical caracters are used. Many such methods have been used for measuring freezing tolerance (Levitt 1980, Sakai & Larcher 1987). Fowler et al. (1981) and Gusta et al. (1983) tested the relationship between a number of chemical, biochemical, physiological and morphological caracters and field survival in winter wheat under prairie conditions. Most of the caracters were associated to field survival but of little practical use. Crown LT_{50} was best correlated and the best prediction of field survival. Plant erectness combined with tissue water content provided nearly as much information as LT_{50} did and is in addition nondestructive to the plants. Many plant caracters have been related to winter survival or freezing tolerance, but no such tests have yet been related directly to ice encasement tolerance.

Conclusions

All the methods described above are useful for giving informations on ice encasement damage. For breeding purposes laboratory methods would be most suitable because they are quick and make it possible to treat many entries at the same time. As mentioned, there has been too great variability between paralelles in these experiments and they have therefore still been of limited use in practical breedeng programmes. Efforts are made to reduce this variability by improving the experimental procedure. Following steps have been taken lately:

1. Plants are now raised and hardened in rockwool inert growth medium which reduces labour and decreases washing stress on plants

by removing soil.

2. When plants are put into the beakers ice is put in the water to initate ice formation. Also the beakers are closed by caps as evaporation from the ice surface tends to reduce the ice temperature during icing. Care must be taken of location of beakers in the freezer.

3. Assessment of damage by judging dead and living plants is not precice enough. Therefore assessment is now don on a scale from 0 to 9 and time from thaw is standardized.

References

Andersen I. L. 1960. Overvintringsundersøkelser i eng i Nord-Norge. I. Forskn. fors. Landbr. 11, 635-660.

Andersen I. L. 1963. Overvintringsundersøkelser i eng i Nord-Norge. II. Noen undersøkelser over is og vannskader i eng. Forsk. Fors. Landbr. 14, 639-669.

Andrews C. J. 1977. Accumulation of ethanol in ice-encased winter cereals. Crop Sci. 17, 157-161.

Andrews C. J. 1988. The increase in survival of winter cereal seedlings due to light exposure during ice encasement. Can. J. Bot. 66, 409-413.

Andrews, C. J. & B. E. Gudleifsson. 1983. A coparison of cold hardiness and ice encasement tolerance of timothy grass and winter wheat. Can. J. Plant Sci. 63, 429-435.

Andrews, C. J. & Pomeroy, M. K. 1975. Survival and cold hardiness of winter wheats during partial and total ice immersion. Crop Sci. 15, 561-566.

Andrews, C. J. & M. K. Pomeroy, 1977. Changes in survival of winter cereals due to ice cover and other simulated winter conditions. Can. J. Plant Sci. 57, 1141-1149.

Andrews, C. J. & Pomeroy, M. K. 1981. The effect of flooding pretreatment on cold hardiness and survival of winter cereals in ice encasement. Can, J. Plant Sci. 61, 507-513.

Andrews, C. J. & M. K. Pomeroy. 1990. Low temperature anaerobiosis in ice encasement damage to winter cereals. In: Plant life under oxygen deprivation (Eds: M. B. Jackson, D. D. Davies & H. Lambers). SPS Academic publishing p. 85-99.

Andrews, C. J., M. K. Pomeroy & I. A. De la Roche. 1974. Changes in cold hardiness of overwintering winter wheat. Can. J. Plant Sci. 54, 9-15.

Andrews, C. J., M. K. Pomeroy & W. L. Seaman. 1986. The response of fall-sown cereals to winter stress in eastern Ontario. Can. J. Plant Sci. 66, 25-37.

Anonym. 1968. Schema før bedømning av utvintringsskador i førsøk rørande øvervintrande åkerbruksväkster. Nord. Jordbruksforskn. 50, 360-364.

Baadshaug, O. H. 1973a. Effects of soil type and soil compaction on the wintering of three grass species under different wintering conditions. Acta Agric. Scand. 23, 77-86.

Baadshaug, O. H. 1973b. En vurdering av forskjellige metoder for overvintringsundersøkelser i eng og beitevekster. Forskn. fors. landbr. 24, 221-234.

Beard, J. B. 1964. Effects of ice, snow and water covers on Kentucky bluegrass, annual bluegrass and creeping bentgrass.

Crop Sci. 4, 638-640.
Beard, J. B. 1965a. Bentgrass (Agrostis spp.) varietal tolerance to ice cover injury. Agron. J. 57, 513.
Beard, J. B. 1965b. Effects of ice covers in the field on two perennial grasses. Crop Sci. 5, 139-140.
Beard, J. B. & Martin, D. P. 1970. Influence of water temperature on submersion tolerance of four grasses. Agron. J. 62, 257-259.
Bjørnsson, H. Statistisk analys och planläggning av laboratorieførsøk med frost- och isresistens. NJF seminar Nr. 84 Lantbruksväxternas øvervintring. 176- 179.
Bolduc, R. 1976. Tecnique pour echantillonner les racines de plantes dans le sol gele et enneige. Can. J. Plant Sci. 56, 633-638.
Bowley, S. R. & B. D. McKersie. 1990. Relationships among freezing, low temperature flooding, and ice encasement tolerance in alfalfa. Can. J. Plant Sci. 70, 227-235.
Brink, R. A. W. keller & C. eisenhart. 1939. Differential survival of alfalfa under an ice sheet. Jour. Agr. Res. 59, 59-71.
Brule-Babel, A. L. & D. B. Fowler 1989. Use of controlled environments for winter cereal cold hardiness evaluation: Controlled freeze tests and tissue water content as prediction tests. Can. J. Plant Sci. 69, 355-366.
Fowler, D. B. 1979. Selection for winterhardiness in wheat. II. Variation in field trials. Crop Sci. 19, 773-775.
Fowler, D. B., L. V. Gusta & N. J. Tyler. 1981. Selection for winterhardiness in wheat. III. Screening methods. Crop Sci. 21, 896-901.
Freyman, S. 1969. Role of stubble in the survival of certain ice-covered forages. Agron. J. 61, 105-107.
Freyman, S. & Brink, V. C. 1967. Nature of Ice-sheet injury to alfalfa. Agron. J. 59, 557-560.
Fridriksson, S. 1954. Rannsòknir à kali tùna àrin 1951 og 1952. Rit Landb.Deild. B-7, 72 s.
Gudleifsson, B. E. 1971. Overvintringsskadar i grasmark på Island, omfang og årsaker. Lisensiatoppgave, Norges landbrukshøgskole. 130p.
Gudleifsson B. E. 1983. Isdekkeresistens og frostherdsle hjå enggras. Nord. Jordbruksforskn 65, 3.
Gudleifsson, B. E. 1986a. Ice encasement damages on grasses and winter cereals. NJF seminar Nr. 84 Lantbruksväxternas øvervintring. 59-65.
Gudleifsson, B. E. 1986b. Måling av isdekketoleranse hos gras i laboratoriet. NJF seminar Nr. 84 Lantbruksväxternas øvervintring. 171-175.
Gudleifsson, B. E. 1989. Extent and importance of ice-encasement damages on gramineous plants in the Nordic countries. Icel. Agr. Sci. 2, 7-14.
Gudleifsson, B. E. & H. Bjørnsson. 1989. Methods for estimating ice-encasement tolerance of grasses in the laboratory. Icel. Agr. Sci. 2, 99-103.
Gudleifsson, B. E., C. J. Andrews & H. Bjørnsson. 1986. Cold hardiness and ice tolerance of pasture grasses grown and tested in controlled environments. Can. J. Plant Sci. 66, 601-

608.
Gudleifsson B. E. & J. Sigvaldason. 1972. Um kal og kalskemmdir. III. Kalskemmdir ì tilraunareitum Tilraunastødvarinnar à Akureyri. Arsrit Ræktunarf. Nordurlands 69, 84-101.
Gusta, L. V., D. B. Fowler & N. J. Tyler. 1983. An evaluation of several chemical tests as possible selection measures for winterhardiness in wheat. Can. J. Plant Sci. 63, 115-119.
Hakamata, T., M. Noshiro, T. Hirashima & I. Nose. 1978. (Investigation of actual condition on the winter-killing of pasture species in the Nemuro-Kushiro district. Exploration of factors by the quantification No 1. J. Japan. Grassl. Sci. 23, 280-288.
Hetherington, P. R., B. D. McKersie & L. C. Keeler. 1990. The influence of mineral nutrition on the expression of traits associated with winterhardiness of two winter wheat (Triticum aestivum L.) cultivars. Can. J. Plant Sci. 70, 443-454.
Hope, H. J., A. Comeau & P. Hasty. 1984. Ice encasement tolerance of prairieland wild ryegrass, orbit tall wheatgrass and puma rye grown under controlled environments. Cereal Res. Commun. 12, 101-103.
Huokuna, E. 1959. Jääpoltteen tuhot viikin laidunkoenurmilla talvikautena 1956/57. Agriculture and Research 11, 305-311.
Larcher, W. 1985. Schädigung der Pflanzen durch Bodenfrost und Schneebedeckung. In: Handbuch der Pflanzenkrankheiten. I,5. Die Nichtparasitären Krankheiten (Eds: W. Larcher, H. Häckel & A. Sakai). Verlag Paul Parey. 274- 287.
Larsen, A. 1985. Testmetoder for overvintringskarakterer hos gras i Norge. Nordkalottkomiteens Promemorier 22, 33-38.
Larsen, A. 1986. Test methods for wintering characters. NJF seminar Nr.84 Lantbruksväxternas øvervintring. 149-166.
Larsen A. 1988. Kontrolled prøving for overvintringsegenskaper i offisiell verdiprøving. NLVF, SFS, Engvekstforedling V, 1988, 82-86.
Levitt, J. 1980. Responses of plants to environmental stresses. I. Chilling, freezing, and high temperature stresses. Academic Press 497p.
McKersie, B. D. 1983. Types of winter stresses - Do winter wheat cultivars respond differently ? In: New frontiers in winter wheat production. Western Canada winter wheat conference 1983. (Eds: D.B. Fowler, L. V. Gusta, A. E. Slinkard & B. A. Hobin). 26-38.
McKersie, B. D. & L. A. Hunt. 1987. Genotypic differences in tolerance of ice encasement, low temperature flooding, and freezing in winter wheat. Crop Sci. 27, 860-863.
McKersie, B. D., McDermott B. M., Hunt, L. A. & Poysa, V. Changes in carbohydrate levels during ice encasement and flooding of winter cereals. Can. J. Bot. 60, 1822-1826.
Olien, C. R. & Smith, M. N. 1981. Extension of localized freeze injury in barley by acute post-thaw bacterial disease. Cryobiology, 18, 404-409.
Pomeroy, M. K. & Andrews, C. J. 1985. Effect of low temperature and calcium on survival and membrane properties of isolated winter wheat cells. Plant Physiol. 78, 484-488.
Pomeroy, M. K. & D. B. Fowler. 1973. Use of lethal dose tempera-

ture estimates as indices of frost tolerance for wheat cold acclimated under natural and controlled environments. Can. J. Plant Sci. 53, 489-494.
Poysa, V. W. 1984. The genetic control of low temperature, ice-encasement, and flooding tolerances by chromosomes 5A, 5B and 5D in wheat. Cereal Res. Comm. 12, 135-141.
Pukacki, P. M. & B. D. McKersie. 1990. Supercooling and ice nucleation events in the crovn of winter wheat seedlings. Can. J. Plant Sci. 70, 1179-1182.
Rakitina, Z. G. 1967. Effect of the gaseous composition of the atmosphere on the frost-resistance of winter wheat plants. Soviet Plant Physiol. 14, 278-284.
Rakitina, Z. G. 1970. Effect of an ice crust on gas composition of the internal atmosphere in winter wheat. Soviet Plant Physiol. 17, 755-759.
Rakitina, Z. G. 1977. Effect of a surrounding ice crust on winter wheat plants as a function of their flooding before freezing. Soviet Plant Physiol. 24, 317-324.
Sakai, A. & W. Larcher. 1987. Frost survival of plants. responses and adaptation to freezing stress. Springer Verlag. 321p.
Samuelsen, R. T. 1990. Modelling overwintering of grassland in Norway. In: Future research in production of grass and forage crops. Workshop Proceedings 4-5th sept 1990. SFL Særheim. 79-82.
Samygin, G. A., Z. G. Rakitina & A. Z. Livshin. 1971. Resistance of winter wheat tissues to freezing and desiccation under adverse gas regime. Doklady Botanical Sci. 196-198, 77-80.
Samygin, G. A., Z. G. Rakitina & A. Z. Livshin. 1973. Influence of an adverse gaseous regime and ice-cold bark on the water-retaining capacity of the leaves and tillering nodes of hardened winter wheat. Doklady Botanical Sci. 209, 60-62.
Sjøseth, H. 1957. Undersøkelser over frostherdighet hos eng-vekster. Forskn. fors. landbr. 8, 77-98.
Sjøseth, H. 1959. Studies on ice encasement in strains of red clover (Trifolium pratense) and timothy (Phleum pratense). Acta Agric. Scand. 9, 292-298.
Sjøseth, H. 1971. Vinterhardførhet hos ulike eng- og beitevekster. Meld. Norg. LandbrHøgsk. 50(13). 39p.
Smillie, R. M. & S. E. Hetherington. 1983. Stress tolerance and stress-induced injury in crop plants measured by clorophyll fluorescence in vivo. Chilling, freezing, ice cover, heat 4, and high light. Plant Physiol. 72, 1043-1050.
Smith, D. 1952. The survival of winter-hardened legumes encased in ice. Agron. J. 44, 469-473.
Sprague, V. G. & Graber, L. F. 1940. Physiological factors operative in ice-sheet injury of alfalfa. Plant Physiol. 15, 661-673.
Sprague, M. A. & Graber, L. F. 1943. Ice sheet injury to alfalfa. J. Am. Soc. Agron. 35, 881-894.
Suzuki, M. 1972. Winterkill patters of forage crops and winter wheat in P.E.I. in 1972. Can. Plant Dis. Surv. 52, 156-159.
Tanino, K. K. & B. D. McKersie. 1985. Injury within the crovn of winter wheat seedlings after freezing and icing stress. Can. J. Bot. 63, 432-436.

Tronsmo, A. M. & S. Svendsen. 1989. Ice encasement in timothy and cocksfoot- a possible screening method for application in breeding programs. Icel. Agr. Sci. 2, 105-107.
Årsvoll, K. 1973. Winter damage in Norwegian grasslands, 1968-1971. Meld. Norges Landbr.Høgsk. 52(3), 21p.

Ivar L. Andersen
Holt forskingsstasjon
Postboks 2502, N-9002 TROMSØ

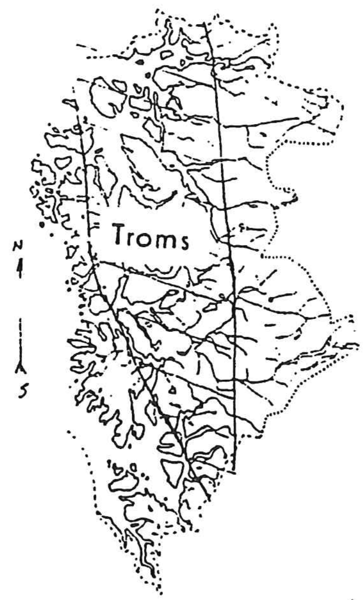
LOKALE SNØFORHOLD SETT I SAMMENHENG MED OVERVINTRINGSSKADER PÅ ENG I TROMS OG FINNMARK

Innledning

Snøforholdene i de to nordligste fylkene i Norge, Troms og Finnmark, varierer sterkt fra distrikt til distrikt, fra sør til nord og fra vest til øst. Objekter som gir le for vinden, fører i tillegg til lokal snøopphoping. Slike le er f.eks. skogskanter, hekker, snøskjermer, hus og tette gjerder m.v. Det er slike variasjoner i lokale snøforhold sett i sammenheng med ulike former for overvintringsskader en her ønsker å se noe nærmere på.

Noen virkninger av ulike snøforhold på overvintring av enga

Vinteren 1966/67 var det relativt lite snø i kyststrøkene i Sør- og Midt-Troms, mens det var mer snø i fjordstrøkene og noe inn i landet. Vinterværet var sterkt vekslende, noe som resulterte i store overvintringsskader på enga i fjordstrøkene og noe inn i landet i Sør- og Midt-Troms. I kyststrøkene der det var sparsomt med snø, forsvant den i mildværsperiodene, og derfor ble det lite av is- og vannskader i disse strøk. I de mer snørike områder i fjordstrøkene og litt inn i landet, førte mildværsperiodene til at snøen ble mer eller mindre vassmettet, og i kuldeperiodene etterpå, frøs vannet i snøbotn til is. Dette resulterte i omfattende is- og vannskader i disse områder i 1967 (Andersen 1967). Lengre nord i fylket der det var mer snø ut mot kysten, var det til dels omfattende overvintringsskader også på øyene (figur 1).

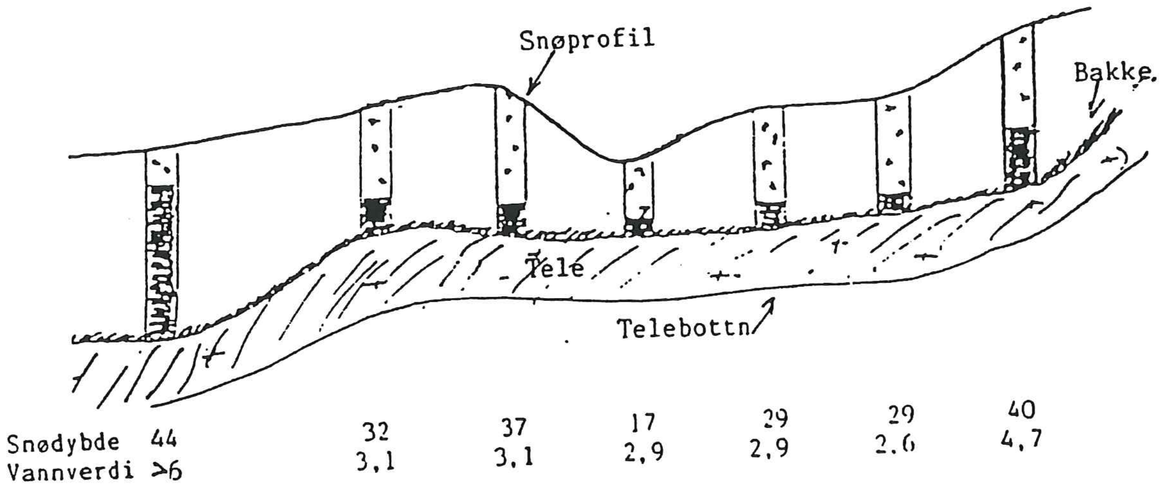


Figur 1. Det skraverte området viser tilnærmet der enga i Troms fylke hadde store overvintringsskader i 1967

Overvintringsskadene på enga i Troms i 1967 var av de mest omfattende i de siste 50 år.

Til sammenligning kan nevnes at det i Island nettopp er etter snøvintrer at de store is- og vannskader viser seg på engene (Gudleifsson 1971).

Varigheten av mildværsperioden er viktig for at det skal dannes is på eng i bakker. Er mildværsperioden kortvarig, vil det ennå ligge blaut snø igjen som kan fryse til is. Er mildværsperioden lang, vil det meste av sigevannet forsvinne nedover bakken til lavere partier, og isdannelse blir det lite av. For å vise hva som skjer med vannverdien (mm vann pr. cm snø) i snøen under en langvarig mildværsperiode, undersøkte en blant annet snødybde og snøens vannverdi langs en linje på 175 meter i hellende og flatt terreng på ei eng ved Holt forskingsstasjon. Jorda var telet, og vannet kunne derfor ikke slippe ned gjennom denne (figur 2).



Figur 2. Snøprofil med "søyler" der de mørke deler angir snøens vannverdi.

Figuren viser at mot slutten av en lang mildværsperiode (over en uke) så er snøens vannverdi lavere i hellinga enn under bakken øverst og nederst. Nede på flaten er vannverdien over 6. Sigevannet har altså på en måte stuvet seg opp under bakkene. I denne forbindelse kan det være på sin plass å nevne at registreringer ved Holt forskingsstasjon har vist at når vannverdien er på omkring 5, og det ellers er laglig for isdannelse, er faren for is- og vannskade til stede (Andersen 1976).

At eng på flate jorder er mer utsatt for is- og vannskader enn eng i hellende terreng, er vist i flere sammenhenger. Årsvoll (1973) viste i sine studier at store is- og vannskader forekom 3 ganger hyppigere på flatmark enn i hellende terreng. På lokaliteter der enga ofte blir is- og vannskadd, har høstbeiting forverret skadene of der isdekket var tykkest, ble skadene meget store (Andersen 1963).

Tabell 1. Prosent dekning av timotei om våren etter en "is-vinter"

Behandling	Maksimal istykkelse 15 - 20 cm	30 - 60 cm
Ingen høstbeiting	74 (100)	74 (100)
Høstbeiting	49 (66)	17 (23)

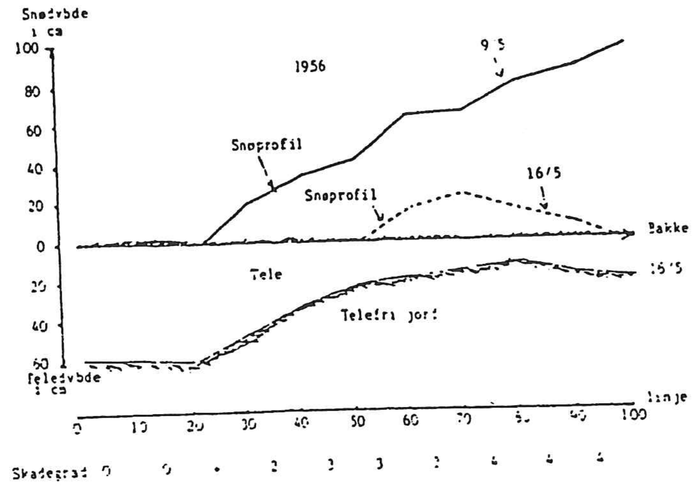
I dalfører i indre Troms der vind fra øst kan blåse snøen bort fra flate enger, kan sterk kulde om vinteren føre til store skader, frostskader. Temperaturer på minus 30 til minus 35°C om vinteren er ingen sjeldenhet i disse områder. Skadene synes å være aller størst når engene på forhånd har vært dekt av is. Lignende skader ble registrert på eng i Nord-Troms og Vest-Finnmark i 1969. Der ble engene dekt med et tynt islag med bare lite snø oppå allerede på førjulsvinteren i 1968, da det periodevis også var meget kaldt.

Overvintringsskader forårsaket av sopp er knyttet til lokaliteter/områder der det til vanlig er mye snø, og der snødekket er langvarig (Andersen 1963, 1966 og Årsvoll 1973). I sine studier over utbredelse og form av overvintringsskader i Norge, viste (Årsvoll 1973) at store skader etter overvintringssoppene _Typhula ishikariensis_ og _Sclerotinia borealis_ for det aller meste viste seg når engene var dekt med snø mere enn 150 og 180 døgn. Om en ser på hvor slike skader i hovedsak har forekommet i Nord-Norge (Andersen 1992) og sammenligner det med hvor lenge markene er dekt med snø (Lomakka 1958), så er sammenhengen innlysende.

Lokale snøforhold som gjør at overvintringsskader på eng forårsakes av nevnte sopper er meget interessante, kanskje spesielt fordi leplanting for tiden er i skuddet. Følgende eksempler forteller oss noe om hvorfor dette er så interessant.

Under de første studieår av overvintringsskadene ved Holt forskingsstasjon, så en at en lokalitet på stasjonens område var velegnet for studie av snø- og teleforhold sett i sammenheng med overvintringsskader forårsaket av sopp. Et skogholt var en god skjerm mot vind fra nordvest, og siden det nettopp under slike vindforhold oftest er snøvær i Troms, fikk en sterk snøakkumulering på lesiden av skogholtet. Undersøkelsene av snø- og teleforholdene her samt av skadene forårsaket av _T. ishikariensis_, gav meget interessante og nærmest forventede resultater (Andersen 1960). Se figur 3.

Skadeomfanget er taksert etter en skala fra 0 - 5, der 0 betyr at skadene ikke er nevneverdig (< 5 prosent), mens 4 betyr at omlag 80 prosent og 5 at alle engplantene er døde.



Figur 3. Resultater av snø- og telemålinger samt av taksering av overvintringsskader forårsaket av <u>Typhula ishikariensis</u>

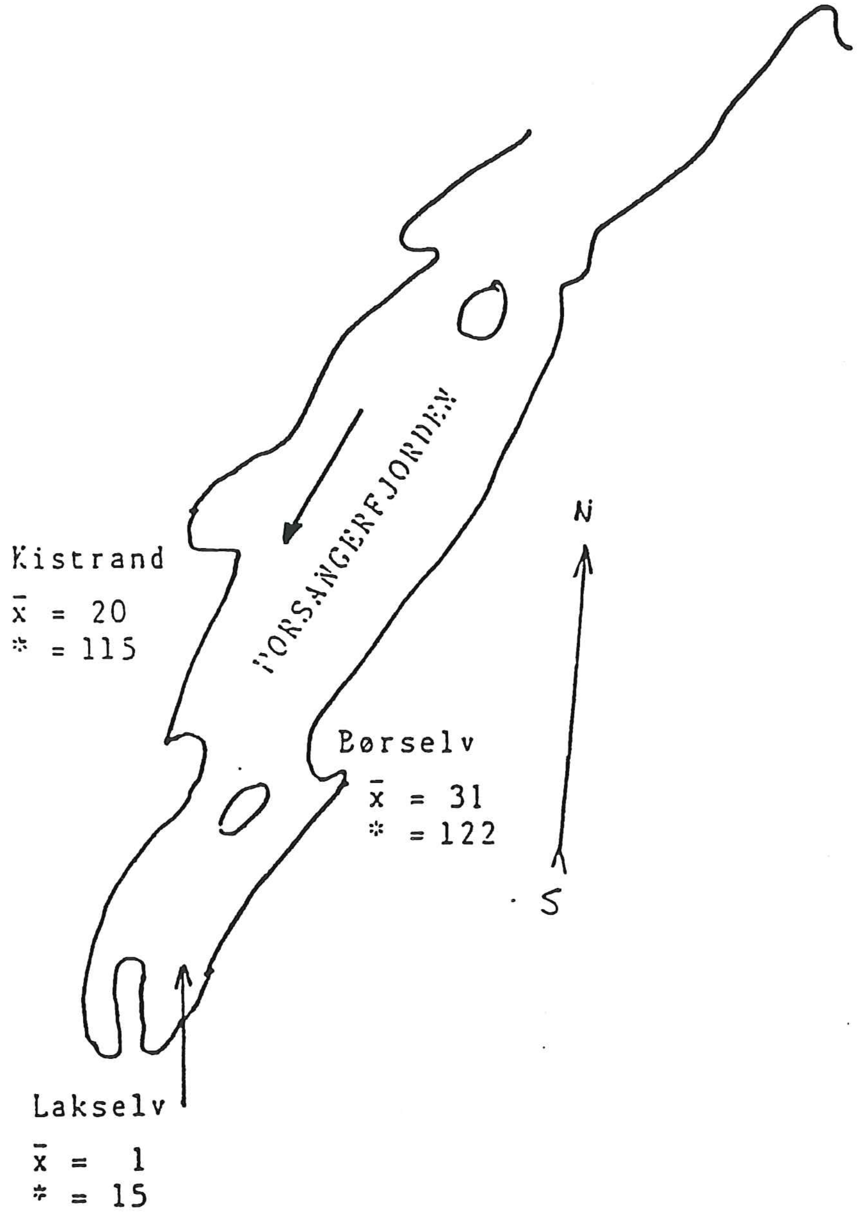
Der snøen gikk før 5. mai og jorda var sterkt telet, var der ingen overvintringsskader, mens der snøen gikk etter 16. mai, var 60-80 prosent av timoteiplantene døde. Leeffekten av skogholtet, med økt snøopphoping, har altså ført til store overvintringsskader forårsaket av nevnte overvintringssopp.

En langt større skala å se sammenhengen mellom ulike snøforhold og ulike overvintringsskader, blir det f.eks. når en ser snøforholdene i Porsanger og sammenligner disse med former og grader av overvintringsskader. For å anskueliggjøre noen eksempler på dette, har en valgt å presentere en kartskisse av Porsangerfjorden og omland med to lokaliteter der det er relativt mye snø og en med lite snø i mai måned (figur 4).

Figuren viser at det er svært lite snø i Lakselv i mai måned, mens det på Kistrand og i Børselv er langt mer snø. Årsaken til dette synes å ligge i at landvinden er den herskende i Lakselv, mens havvinden er det på de to andre lokalitetene. Se pilene på kartet som angir hovedvindretningene. Jamfør Børve og Sterten (1978). På Kistrand og i Børselv (ikke belegg i herbarier fra sistnevnte lokalitet) er det registrert langt mer overvintringsskader forårsaket av sopp enn i Lakselv, der slike skader bare opptrer der det er rent lokal snøopphoping.

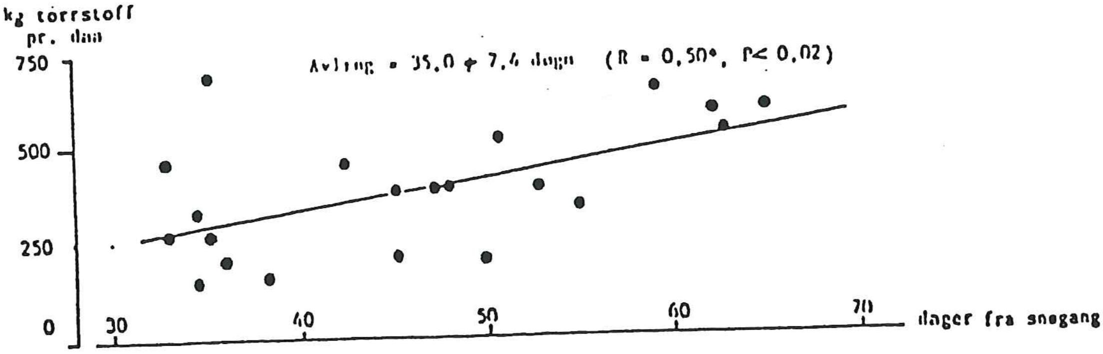
Sein snøgang forkorter veksttida, og ved Holt forskingsstasjon har en gjennom ganske mange år registrert at dette reduserer grasavlinga sjøl om ikke enga er overvintringsskadd.

Undersøkelser på felt på lesiden av snøskjermer viste at der snøen lå 8 - 10 dager lengre en vanlig, ble timoteiavlinga redusert med 14 - 20 prosent (Andersen 1988).



Figur 4. Kartskisse over Porsangerfjorden med omland som viser midlere (x) og maksimal snødybde (*) i mai måned på Kistrand og i Børselv og Lakselv.

Avlingsresaultater fra 21 års ukentlige tilvekstundersøkelser (1968 - 1988) i timoteieng ved Holt forskingsstasjon, gav muligheter for å studere hva lengden av tidsintervallet fra snøgang til høstedato betydde for avlingsstørrelsen. Som eksempel på dette, viser en her sammenhengen mellom lengden av tidsintervallet fra snøgang til 1. juli og avlingsstørrelsen i timoteieng i de nevnte 21 år.



Figur 5. Tørrstoffavling i timoteieng pr. 1. juli sett i sammenheng med antall dager fra snøgang til nevnte dato i årene 1968 - 1988.

For hvert døgn lenger perioden fra snøgang til 1. juli var, økte tørrstoffavlinga med vel 7 kg. Korrelasjonen mellom dagantall fra snøgang til nevnte dato og avlingsstørrelse var signifikant - R = 0,50 - P < 0,02 (Andersen & Johnsen 1989).

Tidlig snøgang er likevel ikke gunstig i alle sammenhenger. Spesielt gjelder det i distrikter der vår- og forsommertørken er en plage. I Lesja, en fjellbygd i Dovre kommune, ble det f.eks. tidligere satt opp skigarder (lave lebelter) på tvers av den herskende vindretning for å oppnå snøopphoping på jordene. På det viset kunne en skaffe eng- og andre kulturplanter bedre vannforsyning i den første delen av veksttida (Rognerud og Vigerust 1975).

Litteratur

Andersen, I.L. 1960. Overvintringsundersøkelser i eng i Nord-Norge I. Forskning og Forsøk i Landbruket 11, 635-660

Andersen, I.L. 1963. Overvintringsundersøkelser i eng i Nord-Norge II. Noen undersøkelser over is- og vannskader i eng. Forskning og Forsøk i Landbruket 14, 639-669

Andersen, I.L. 1967. Overvintring av enga i Troms og Finnmark vintrene 1965/66 - 1966/67. Norden 71, 714-716, 723

Andersen, I.L. 1976. Overvintringsskader på eng. Aktuelt fra Landbruksdepartementets opplysningstjeneste (LOT). Nr. 2 1976, 118-126

Andersen, I.L. 1988. Snøfonner på lesiden av skogholt, snøskjermer og andre le kan gi reduserte grasavlinger. Norden 92 (20), 8-9

Andersen, I.L. 1992. Winter injuries in grassland in Northern Norway caused by low temperatur fungi. Norwegian Journal og Agricultural Sciences. Supplement No. 7, 13-20

Andersen, I.L. & O. Johnsen 1989. Snøgang og timoteiavling i juli måned. Norden 93 (11), 12-13

Børve, A.B. & A.K. Sterten 1978. Husbygging under krevende naturtbetingelser. Fase I. Undersøkelser av de naturgitte bygge- og bobetingelser i noen kyst- og fjordområder i Finnmark. Norges byggforskningsinstitutt 1978. Arbeidsrapport 14, 93 sider

Gudleifsson, B.E. 1971. Overvintringsskader i grasmark på Island, omfang og årsaker. Avhandling for lisensiatgraden ved Norges landbrukshøgskole. 130 sider

Lomakka, L. 1958. Norra Fennoskandias jordbruk. Del I. Nordisk Jordbruksforskning 40, 131-351

Rognerud, B. & E. Vigerust 1975. Kunstig le. Hydrologiske forhold, planteproduksjon og jordtemperatur. Resultater fra Lesja 1964-1979. Den norske komitee for den internasjonale dekade. Rapport nr. 6. 66 sider

Årsvoll, K. 1973. Winter damage in Norwegian grasslands, 1968 - 1973. Meldinger fra Norges landbrukshøgskole, 52 (3), 31

Hans-Arne Jönsson & Curt Nilsson
Svalöf Weibull AB
268 81 Svalöv

VARIATION IN RESISTANCE TO TYPHULA ISHIKARIENSIS IN GRASSES OF VARIOUS ORIGIN

In previous papers (Jönsson & Nilsson 1986, 1992) we have reported the work to develop a method of selection for improved resistance to snow moulds (Fusarium nivale and Typhula ishikariensis) in perennial ryegrass, meadow fescue and timothy. We have continued these selections and here the variation in reaction to selection in timothy and meadow fescue of various origin will be shown.

Material and Methods

Fusarium nivale is the most common snow mould fungus in South Sweden with a snow cover of short duration, whereas Typhula ishikariensis is the dominating fungus of the snow mould complex in North Sweden. According to our experience there is a good correlation between the plant reaction to these two fungi which also has been confirmed by Tronsmo (1992). As the artifical cultivation of Typhula ishikariensis is less complicated we have chosen to work with this fungus.

Typhula ishikariensis is isolated from sclerotia which are surface sterilized. The fungus is grown on malt-yeast-glucose agar and multiplied on autoclaved wheat/oat kernels in glass flasks. A single aggressive isolate is used.

The plants are raised in the greenhouse and transferred to a controlled environment chamber when 12-16 weeks old. They are hardened at a decreasing temperature (down to + 2,0° C) for 3-6 weeks under a 6/18 hours day/night regime.

The plants are inoculated by spreading the wheat/oat kernels containing the fungus over the plants. Then they are covered with moist paper and a plastic sheet. They are kept in darkness at a temperature of + 2,0° C. The incubation period varies from 12 to 20 weeks depending on material and on how successful the hardening has been. With some experience the time of termination of the incubation can be determined by the softness of the plant stems. We are aiming at 5-10 % surviving plants but sometimes the survival has been up to 50 %. If too many plants are alive a second incubation period of the same plants is necessary.

The plants are left to recover under light and a gradually increasing temperature. Surviving plants are transplanted into the field for seed production. The relative survival rate is calculated as the percentage surviving plants in relation to the percentage survival of a standard variety.

This selection process has been repeated for up to five cycles.

Results

Timothy (Phleum pratense)

In the southern part of Sweden there are normally no problems with overwintering diseases in timothy. In North Sweden on the other hand severe attacks by Typhula ishikariensis may occur. Therefore, it was considered worthwhile to start a selection in the northern type of timothy and selection has also been made in southern material to increase its zone of adaptation northwards.

The selection progress in survival rate after artifical inoculation is shown in figure 1. Each point is the average of several values, and the steady increase in relative survival rate when based on Kämpe II as the standard is apparent. The northern populations start at a high level and show a similar trend as the southern populations.

One variety from each group is examined separately in figure 2. The south Swedish variety Kämpe II is following the same curve as the southern populations in figure 1. In the north Norwegian variety Engmo, however, no progress at all has been achieved during the five selection cycles when comparing with the unselected variety itself.

The selected populations have been included in the Weibull yield testing system and the results are shown in tables 1 and 2. The selections in Kämpe II have been tested in south Sweden at Weibullsholm (56° N) and Bjertorp (58° N), whereas the Engmo selections have been included in tests in north Sweden at stations between 63 and 65° N. The number of trials is limited and the conclusions must not be too definite. The total yield level in the Kämpe II selections seems to be just below the original population. A higher proportion of the total yield is harvested in the first cut and less in the regrowth as compared to the unselected Kämpe II (table 1).

The unselected Engmo has not been included in all trials, and the comparisons in table 2 have to be made via the control variety Bottnia II. Engmo in itself is a very hardy, persistent variety and this character has been maintained up to the second generation of selection. The third and fourth generations have lost much of the yielding ability and the seasonal yield distribution in these selections is varying.

Meadow fescue (Festuca pratensis)

Like timothy meadow fescue is a fairly healthy grass species even if snow mould attacks may occur in both south and north Sweden. Widely different populations of meadow fescue have been chosen for selection, representing south Swedish and north Scandinavian material of the diploid type. The reasons for work in meadow fescue are the same as for timothy, i.e. an expansion of its area of adaptation.

The relative survival rates in the different population types of meadow fescue are shown in figure 3. Each point represents one or the average of several measurements. All survival rates are in percent of the standard variety Mimer. The southern and northern populations are on two separate levels of survival.

Figure 1

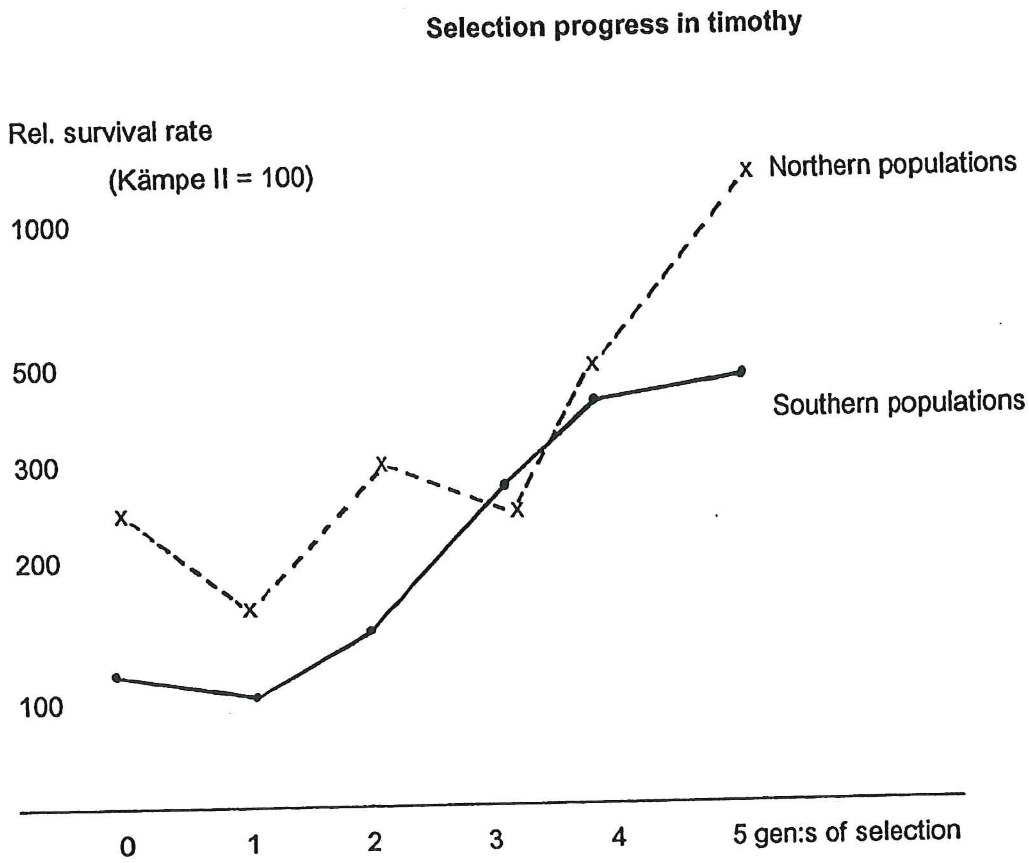
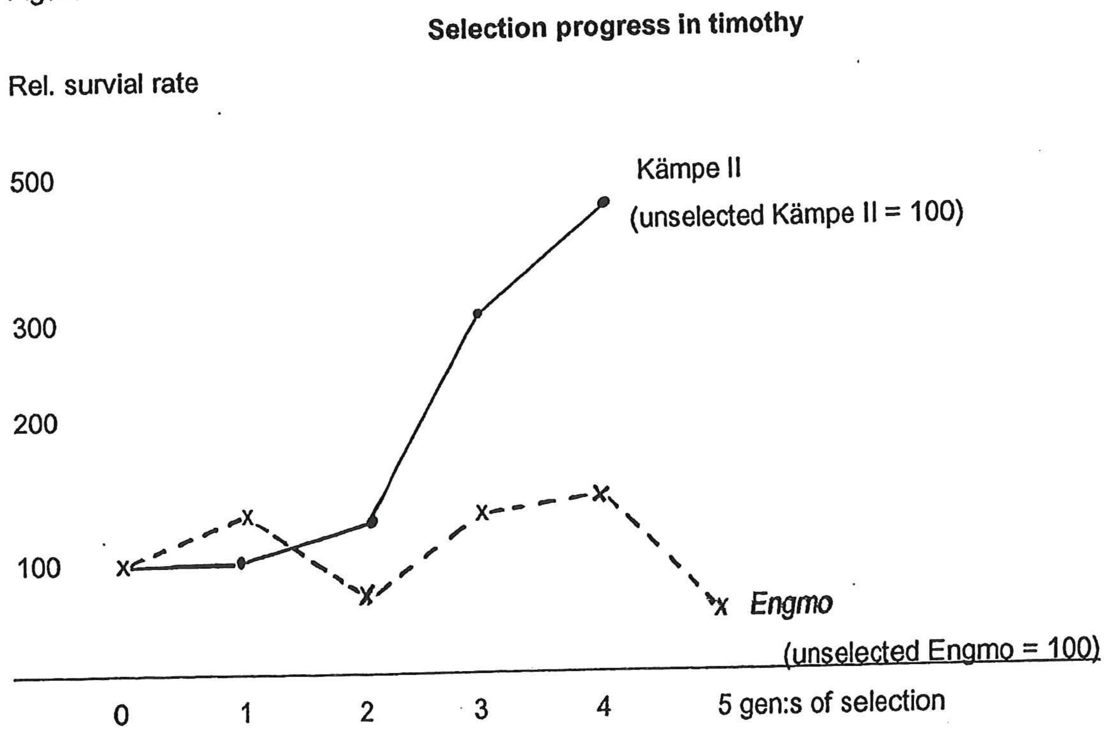


Figure 2



Apart from the fifth generation of selection which is based on just a few observations, both sets of populations show a fair degree of response to selection. The southern populations start from a low level but show a more rapid increase in survival rate.

In figure 4 the results of one variety from each of the two groups are shown separately. No selection has been performed in the control variety Mimer, and another south Swedish variety, Äs 74, is chosen from this group. Äs 74 is originally inferior to Mimer in survival but improves very rapidly with the selections. The unselected Äs 74 has not been included after the first generation. Instead, Mimer has been used as a control variety. The Norwegian variety Salten, which is representing the northern group, shows some improvement during the generations of selection but not at all as strong as that of Äs 74.

The meadow fescue selections have also been included in yield trials, Äs 74 in south Sweden at Weibullsholm and Bjertorp, and Salten at the north Swedish testing sites. Äs 74 has lost some of its yield potential with these selections and the seasonal yield pattern has changed dramatically. The later generations of selection have a much heavier first cut and less regrowth than the original Äs 74 (table 3). The selection in Salten has also led to some decrease in total yield but no change in yield distribution between the cuts (table 4).

Discussion

These results clearly show that the selections have had a distinct impact on the survival rates as measured in the laboratory. Timothy and meadow fescue are two fairly sound species and we have had no problems with their field survival during these years. Under more severe winter stress we might be able to show differences in survival between the selected generations.

The south Swedish populations of both timothy and meadow fescue show a stronger response to selection than the northern populations. This is clear from the survival rates (figures 1 and 3) but also from the seasonal yield distribution (tables 1 and 3 compared with tables 2 and 4). To clarify further the change in the seasonal yield pattern the first cut percentage is listed in table 5. The selections in the southern populations, Kämpe II and Äs 74, show an increasing proportion in the first cut yield, whereas the selections in the northern populations, Engmo and Salten, don't show any such clear tendency.

The reason to this different reaction in material from south and north is probably to find in natural selection. All our material has been multiplied in south Sweden (56° N) which is within the adaptation area of the southern populations but is too far to the south for the northern populations.

Our laboratory selection changes the growth type into a plant which produces a higher first cut yield but less regrowth. The plant goes into winter dormancy earlier in order to better withstand the stress caused by winter fungi.

The seed multiplication in south Sweden favours the type adapted to this area i.e. a type with good regrowth which can wait until late before going into winter dormancy. This type is a more prolific seed yielder and multiplies at the expense of the more northern type.

Figure 3

Selection progress in meadow fescue

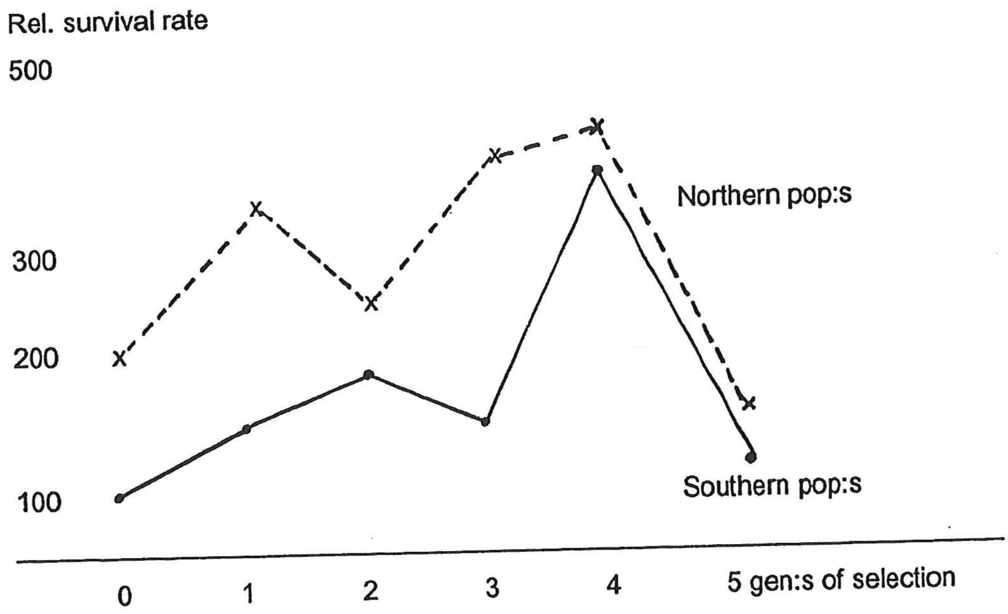
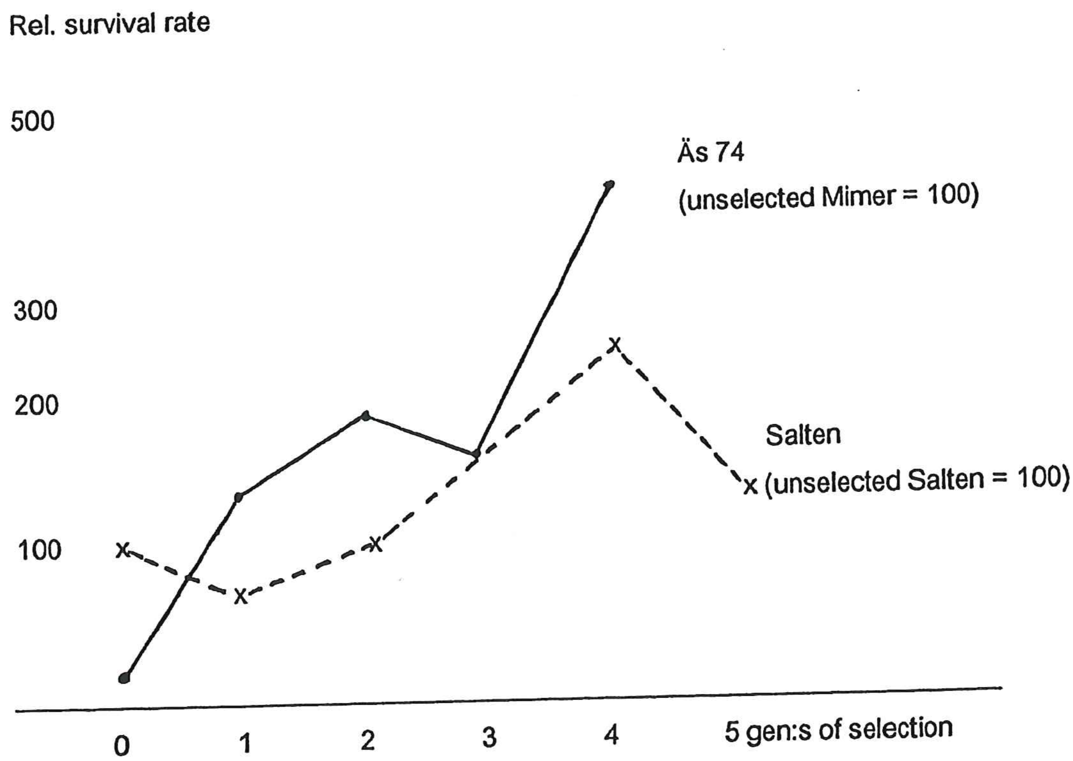


Figure 4

Selection progress in meadow fescue



These two forces, the laboratory selection and the natural selection, counteract. In the southern populations the laboratory selection is the dominating one and results in populations with a more northern adaption. In the northern populations the result is not so clearcut. The counteracting forces sometimes are equally strong, sometimes one or the other is stronger. During the years, however, the natural selection has been dominating, causing a genetic shift of the material into a more southern growth type. This tendency could be counterworked by harvesting one panicle only per plant for the further multiplications and selections. Another alternative is to transfer the selected plants to north Sweden for seed harvest.

This change in type in crossfertilizing species was clearly demonstrated by Sylvén (1937) in timothy, meadow fescue as well as in red and white clover. He reported that some plants of Bottnia meadow fescue were so depending on the long day that they didn't set any seed at all when grown in south Sweden. Myhre & Rognli (1991) observed a non-random mating in polycrosses of northern meadow fescue when grown in south Norway and therefore strongly recommended the testing site to be chosen in the area of cultivation.

Crossfertilizing species always contain plants of different types. The variability may, however, be stronger or weaker. Present-day varieties of timothy are considered to be quite variable, whereas varieties of perennial ryegrass are more uniform. Lucerne varieties are also comparatively uniform. As an example the Swedish variety Vertus has been grown for seed in latitudes down to 35° without a shift in type.

Conclusion

Selection in south Scandinavian populations of timothy and meadow fescue has improved the survival rate after artificial snow mould inoculation. The site of seed multiplication of the selected plants should be within the future cultivation area in order to avoid genetic drift.

Literature

Jönsson, H.A. & C.Nilsson, 1986. Plant breeding for resistance to snow moulds in grasses. NJF Seminar No 84, Jokioinen, June 4-6, 1986. Pp 145-148.

Jönsson, H.A. & C.Nilsson, 1992. Grass variety reaction to selection for resistance to Typhula ishikariensis. NJF Seminar No 188. Norw. J. Agric. Sci:s Suppl. No 7:25-34.

Myhre, A & O.A. Rognli, 1991. Non-random mating in polycrosses of Festuca pratensis in different environments. In "Fodder crops breeding: Achievements, novel stategies and biotechnology" Ed:s A.P.M. den Nijs & A.Elgersma - Pudoc, Wageningen, pp 195-196.

Sylvén, N., 1937. The influence of climatic conditions on type composition. Imperial Bureau of Plant Genetics, Herbage Publication Series, Bull.21:1-8.

Tronsma, A.M., 1992. Resistance to snow mould fungi in breeding material of grasses. NJF Seminar No 188. Norw. J. Agric. Sci:s, Suppl. No 7:35-38.

Table 1 Replicated yield trials in timothy at Weibullsholm and Bjertorp. No. of comparisons within parenthesis.

Total dry matter yield

	First	Second harvest year
Kämpe II unselected, kg/ha	8860	8550
Kämpe II, unselected, rel.	100	100
Kämpe II, 1 gen. of sel.,rel.	98 (2)	100 (2)
Kämpe II, 2 gen:s. of sel.,rel.	98 (4)	99 (4)
Kämpe II, 3 gen:s. of sel.,rel.	98 (4)	99 (2)

Seasonal yield distribution

	First cut	Regrowth cuts	Total
Kämpe II, unselected, kg/ha	5430	3290	8720
Kämpe II, unselected, rel.	100	100	100
Kämpe II, 1 gen. of sel.,rel.	101	95	99 (4)
Kämpe II, 2 gen:s of sel.,rel.	99	98	99 (8)
Kämpe II, 3 gen:s of sel.,rel.	100	94	98 (6)

Table 2 Replicated yield trials in timothy in North Sweden.
No. of comparisons within parenthesis.

Total dry matter yield

	First	Second	Third harvest year
Bottnia II, control, kg/ha	8730	7440	6900
Bottnia II, control, rel.	100	100	100
Engmo, unselected, rel.	96 (2)	102 (2)	118 (2)
Engmo, 1 gen.of sel.,rel.	98 (2)	104 (2)	120 (2)
Engmo, 2 gen:s of sel.,rel.	100 (3)	106 (3)	103 (3)
Engmo, 3 gen:s of sel.,rel.	82 (3)	89 (3)	103 (3)
Engmo, 4 gen:s of sel.,rel.	87 (1)	80 (1)	-

Seasonal yield distribution

	First cut	Second cut	Total
Bottnia II, control, kg/ha	5060	2660	7720
Bottnia II, control, rel.	100	100	100
Engmo, unselected, rel.	110	94	104 (6)
Engmo, 1 gen.of sel.,rel.	113	93	107 (6)
Engmo, 2 gen:s of sel.,rel.	111	83	103 (9)
Engmo, 3 gen:s of sel.,rel.	98	78	90 (9)
Engmo, 4 gen:s of sel.,rel.	96	62	83 (2)

Table 3 Replicated yield trials in meadow fescue at Weibullsholm and Bjertorp. No. of comparisons within parenthesis.

Total dry matter yield

	First	Second harvest year
Mimer, control, kg/ha	8620	8360
Mimer, control, rel.	100	100
Äs 74, unselected, rel.	97 (1)	99 (1)
Äs 74, 1 gen.of sel.,rel.	98 (1)	94 (1)
Äs 74, 2 gen:s of sel.,rel.	95 (2)	91 (2)
Äs 74, 3 gen:s of sel.,rel.	90 (2)	101 (2)
Äs 74, 4 gen:s of sel.,rel.	92 (2)	95 (1)

Seasonal yield distribution

	First cut	Regrowth cuts	Total
Mimer, control, kg/ha	4190	4310	8500
Mimer, control, rel.	100	100	100
Äs 74, unselected, rel.	88	104	98 (2)
Äs 74, 1 gen. of sel.,rel.	87	102	96 (2)
Äs 74, 2 gen:s of sel.,rel.	81	105	93 (4)
Äs 74, 3 gen:s of sel.,rel.	91	100	96 (4)
Äs 74, 4 gen:s of sel.,rel.	93	92	93 (3)

Table 4 Replicated yield trials in meadow fescue in North Sweden. No. of comparisons within parenthesis.

Total dry matter yield

	First	Second	Third harvest year
Salten, unselected, kg/ha	7720	7810	7660
Salten, unselected, rel.	100	100	100
Salten, 1 gen.of sel.,rel.	95 (10)	93 (11)	92 (10)
Salten, 2 gen:s of sel.,rel.	98 (8)	99 (9)	98 (8)
Salten, 3 gen:s of sel.,rel.	98 (5)	98 (5)	101 (3)
Salten, 4 gen:s of sel.,rel.	106 (1)	91 (1)	-

Seasonal yield distribution

	First cut	Second cut	Total
Salten, unselected, kg/ha	4810	2930	7740
Salten, unselected, rel.	100	100	100
Salten, 1 gen. of sel.,rel.	91	96	93 (31)
Salten, 2 gen:s of sel.,rel.	98	99	98 (25)
Salten, 3 gen:s of sel.,rel.	97	100	98 (13)
Salten, 4 gen:s of sel.,rel.	99	96	98 (2)

Table 5 Dry matter yield in first cut, percent of total.

	Southern		Northern types	
	Tim. Kämpe II	M.F. Ås 74	Tim. Engmo	M.F. Salten
Unselected	62	35	71	62
1 gen. of sel.	56	35	71	61
2 gen:s of sel.	64	41	76	63
3 gen:s of sel.	68	47	66	54
4 gen:s of sel.		*59*	*72*	*61*

Gösta Vestman
Dep. of North Swedish Plant Protection
Röbäcksdalen, Box 4097
S-904 03 UMEÅ

The susceptibility to different biotypes of *Sclerotinia trifoliorum* in red clover cultivars

INTRODUCTION

Clover rot has been known as a disease of forage legumes for more than a hundred years but there has been some confusion over the casual agent. Three Sclerotinia species can cause a similar rotting disease of forage legumes, namely *S. trifoliorum, S. sclerotiorum* and *S. minor.* Controversy has existed whether these should be regarded as synonymous but it is now agreed, that although closely related they are distinct species (Scott 1984). *S. trifoliorum* infects only forage legumes while the other two species has a very wide host range. The Plant Pathology Committee of the British Mycological Society (1944) recommended clover rot to be the name of a disease of herbage legumes, caused by *Sclerotinia trifoliorum.* In North America the name Sclerotinia Crown and Stem Rot (SCSR) is used.

Clover rot is a major disease on forage legumes in temperate and subarctic regions and causes considerable damage to red clover even in nothern Sweden. In field experiments on chemical control of winter killing fungi in clover-grass leys the effect of the treatment (10 kg/ha a.i. of quintozene) increased with increasing percentage of red clover (fig. 1) (Vestman 1971).

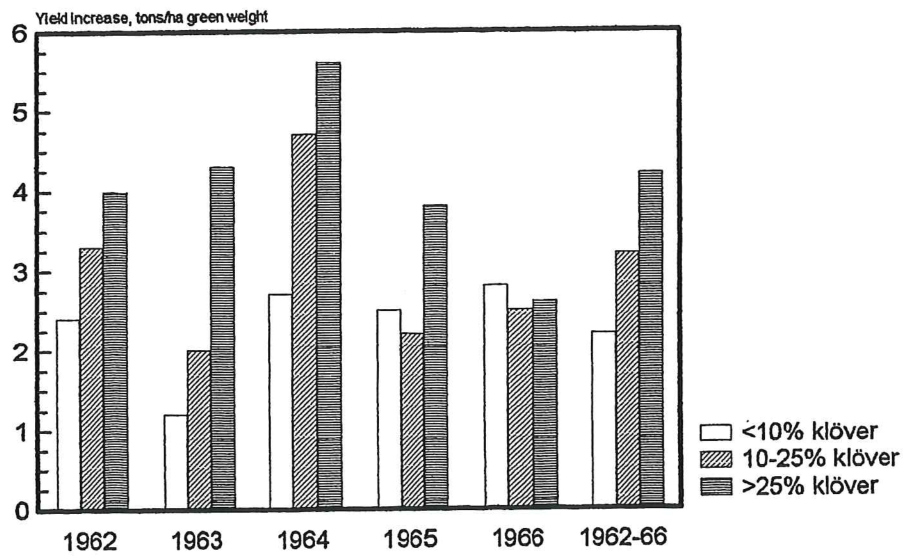


Fig. 1. Control of winter killing fungi in leys with 10 kg/ha a.i. quintozene.
Yield increase tons/ha green weight.

Control

Although chemical control of winterkilling fungi has been experimentally successful it has never been commonly used in northern Sweden, mainly because of the prohibitive cost of the treatment. At present chemical control is no alternativ because there is no fungicide registered for use in grasslands other than lawns and golfgreens etc.

The sclerotia can survive for many years in the soil. Halkilahti (1962) reported 4½ years survival in dry conditions and Pape (1937) up to 7-8 years. Consequently the effect of crop rotation in controlling diseases like clover rot is limited in regions with extensive ley farming.

Biological control by destruction of sclerotia has been demonstrated but has not yet been exploited.

Breeding cultivars resistant to clover rot (and indeed other diseases limiting the prevalence of clover in the leys) seems to be the ultimate way of control.

Breeding for resistance

Screening for resistance by planting material on naturally infested land or by using artificial inoculation techniques in the field or controlled enviroments has often been criticised for beeing unreliable. Resistance may, in part, be the result of subjectivity by the experimenter (Scott 1984).

The specialization and variability of the fungus is another factor that can explain some of the difficulties achieved in breeding for resistance. Significant differences in pathogenecity of fungal isolates has been reported (Frandsen, 1946, Raynal, 1981, Góral and Matusik, 1983). Cultivars may be resistant when grown in one area but susceptible in another (Nilsson-Leissner, 1942).

When screening for resistance the breeder evidently have to decide which isolate to use in order to achive the best differentation among cultivars.

EXPERIMENT WITH FUNGAL ISOLATES

Normally a mixture of fungal isolates is used in breeding for resistance to clover rot. In a preliminary experiment the pathogenecity of some isolates from northern Sweden and the interaction with common cultivars of red clover was investigated.

Materials and methods

Two cultivars of red clover, Bjursele (a local variety from northern Västerbotten) and Björn (a selection for resistance in the local variety Offer from Ångermanland) were raised in the greenhouse for 4 weeks, hardened in a cool chamber at +1°C - +5°C and c:a 8000 lux from HPLG-lamps with a photoperiod of 10 h for 2 weeks. Following inoculation the plant boxes were incubated at +5°C in plastic boxes with water on the bottom to insure high humidity. After 4 weeks the plants were moved to the greenhouse for regeneration.

Four isolates of Sclerotinia trifoliorum were propagated on sterilized barley kernels in 500 ml Erlenmeyerflasks and used as inoculum . When plenty of sclerotia had developed the material was airdried, grinded and sprinkled over the plants.

The origins of the isolates and the cultivars are shown on the map (fig 3).

The experiment was planned as a randomized block design with replications in time but due to unfortunate circumstances only one test period has been carried out so far.

Result

The result is presented as percent surviving plants (fig 2). The two northern isolates (AC1 and BD1) were more aggressive than the two southern ones (Y1 and Z1). Bjursele was more resistant than Björn to Y1 and Z1 while the opposite was the case for AC1 and BD1.

Discussion

The result, uncertain as it may be, seems a bit surprising. The cultivars reacted differently from what could be expected. Bjursele is a local variety from northern Västerbotten and is a result of natural selection in the field. Yet it seems to be less resistant than Björn to isolates from the same area. Björn on the other hand is selected from the local variety Offer after heavy infections in the fields of Svalöv-Weibulls breeding station in Lännäs near Offer. But it is less resistant than Bjursele to the isolate from Offer.

Even though any safe inferences can't be drawn from this one experiment screening of fungal isolates from the region and their interaction with cultivars of red clover seems to be indispensable for a successful breeding for resistance in red clover in the future.

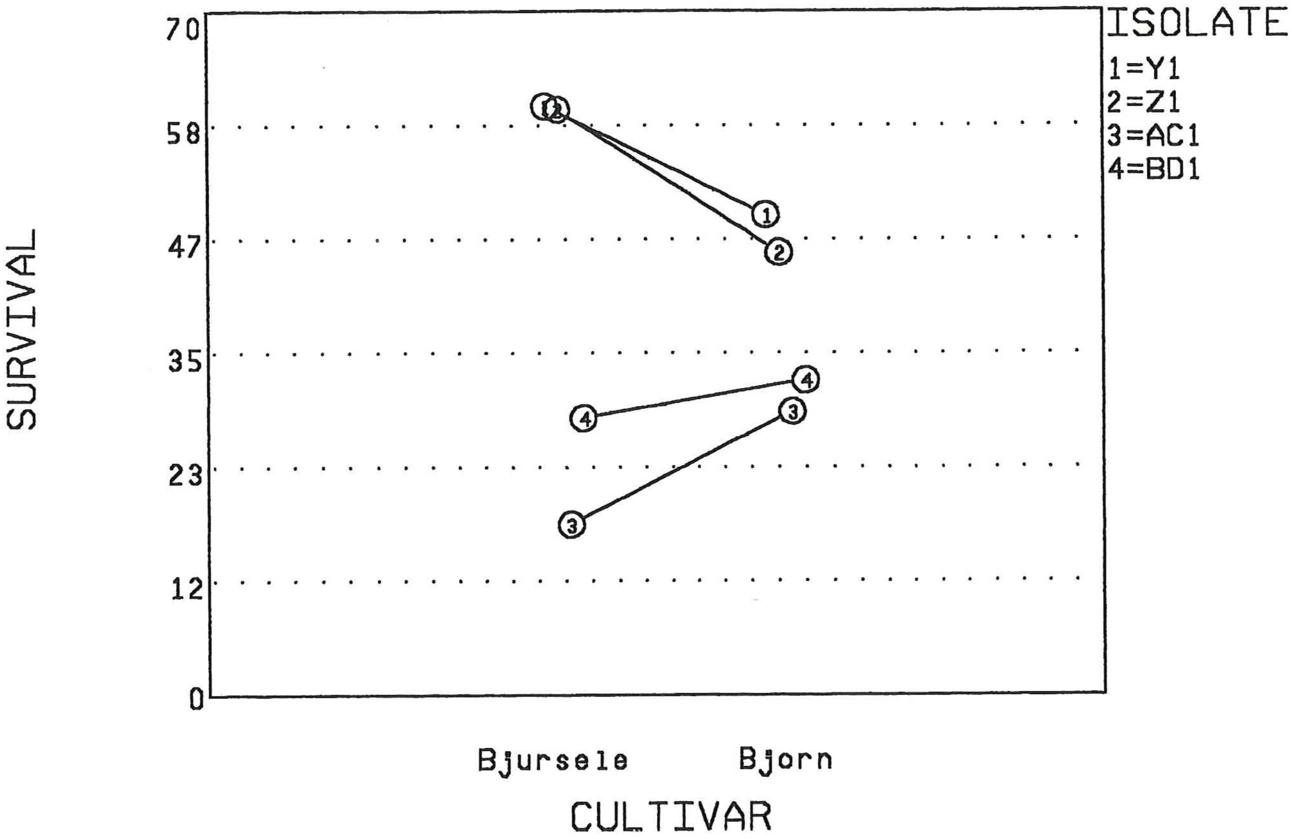


Fig. 2. Isolates of *Sclerotinia trifoliorum* on cultivars of red clover. Percent survival on the Y-axis.

LITERATURE

Frandsen, K,J. 1946. Studier over *Sclerotinia trifoliorum* Eriksson. Det Danske Forlag, Copenhagen.

Góral, S and Matusik, H. 1983. (The resistens of red clover to root and crown rot (*Sclerotinia trifoliorum* Erikss.)). Biuletyn Instytutu Hodowli i Aklimatyzacji. No 150, 93-99.

Halkilahti, A-M. 1962. The survival of sclerotia of Sclerotinia trifoliorum Erikss. in the soil and the occurence of clover rot in various years. Maataloust. Aikakausk. 34:154-161.

Nilsson-Leissner, G. 1942. Skriftligt diskussionsinlägg. Nord. Jordbrugsforsk. 24:24-32

Pape, H. 1937. Beiträge zur Biologie und Bekämpfung des Kleekrebses (*Sclerotinia trifoliorum* Erikss.). Arb. Biol. Anst. (Reichanst.) Berl. 22:159-247.

Plant Pathology Committee of the British Mycological Society. 1944. A list of common British plant diseases. Cambridge University Press, Cambridge.

Raynal, G. 1981. La sclérotinioise des trèfles et luzernes à *Sclerotinia trifoliorum* Eriks. II. Variabilitè du parasite, résistance des plantes en conditions contrôlées. Agronomie, 1(7):573-578.

Scott, S.,W. 1984. Clover Rot. The Botanical Review. 50:491-504.

Vestman, G. 1971. Försök med kemisk bekämpning av utvintringssvampar i vall i norra Sverige åren 1962-66. Medd. Statens växtskyddsanst. 14:135, 451-472.

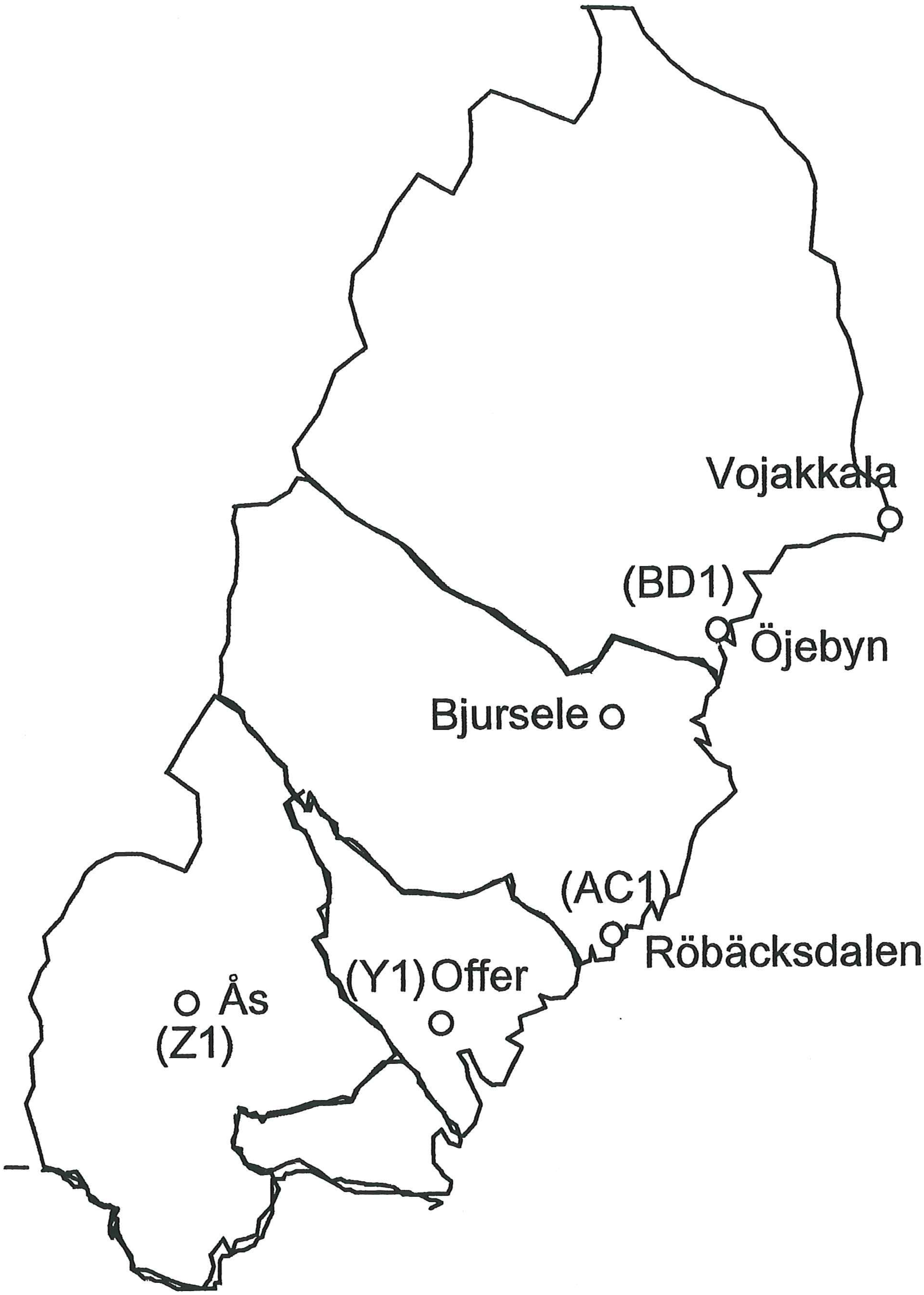


Fig. 3. Map of northern Sweden showing the field experiment stations.The places where the fungal isolates were collected, are marked Y1, Z1, AC1and BD1. The origin of the cultivar Bjursele is marked accordingly. The cultivar Björn originates from Svalöv-Weibulls breeding station near Offer.

Agronom Bengt Andersson
Svalöf Weibull AB
268 81 Svalöv

BREEDING FOR RESISTANCE TO ROOT ROT IN RED CLOVER

Introduction

The use of red clover is often restricted by poor persistence which is among other things caused by attacks of fungal diseases like clover crown rot and root rot.

Investigations have shown (Rufeldt 1986) that root rot is distributed in almost every field where red clover is grown and is one of the main reasons for the poor persistence. In the same investigation Fusarium roseum, Phoma medicaginis and Cylindrocarpon sp. are pointed out as the most important fungi in the root rot complex.

One way to control the root rot disease would be to produce resistant cultivars. This paper describes a laboratory method for selection for root rot resistance and the results obtained when this method was applied to two populations of early red clover.

Materials and methods

The basic red clover material used was two cultivars of early type, the diploid Essi II and the tetraploid Molly. This basic material is in the following refered to as Ro generation. Plants were grown in plastic pots, 8x8 cm, in a greenhouse soil containing 20 % clay, 20 % leca and 60 % peat. The work was carried out during the autumn/winter period and artificial light, 5000 lux, was addes with a photoperiod of 16 hours light. For each cycle of selection 500 plants were used.

An isolate of Fusarium avenaceum was used as inoculum. This isolate was originally received by the Department of Plant and Forest Protection at the Swedish University of Agricultural Sciences. The inoculum was prepared by growing the fungus for ten days on PDA. The agar plates with the growing fungus were then cut in pieces and mixed with water in a mixer.

After 3 months the plants were taken out of the plastic pots and the roots were cut about 2 cm below the crown. The inoculum was then added directly to the cut tap root. The plants were replanted and placed in the greenhouse. After another six weeks plants were removed from the pots, the roots were washed and the taproots split longitudinally. The plants were graded in 5 classes, 0-4, according to the amount of root-rot developed. The class 0 represented plants where no root-rot was found and the class 4 plants with completely decayed roots. Plants with little or no root rot were crossed under open pollination to produce the next generation. In both populations 3 generations of selection were produced, and these generations are in the following refered to as R1, R2 and R3.

The evaluation of the material was done by testing root rot resistance after artificial infection. In this test 200 plants from each generation were used. The root diameter was measured on about 25 plants grown in a greenhouse for 4 months. The green-matter yield was measured in a field trial with a plot size of 5 m² in 4 replications. In order to investigate possible morphological changes, 50 plants from each generation were planted in the field. On these plants the date of flowering and the stem length were measured.

Results and discussion

The 200 plants from each generation were after the artificial infection divided into the 5 classes according to the amount of root rot found. The results are shown in fig.1 and fig. 2. There has been a clear positive effect of the selection in both populations. In the basic material, Ro, or in the R1-generation only a small proportion of plants in the class 0 can be found, but in the R3 generation this proportion has raised to 15-28 %. The proportion of plants with severe root-rot damage, classes 3 and 4, is reduced from almost 50 % to 5-8 % in the R3 generation. This test shows that there has been a clear improvement in resistance under laboratory conditions.

The material has also been tested in a field trial (table 1). Results from this trial indicate a better survival in the field after artificial selection for root rot resistance. In the diploid Essi II-selections the improvement is much more obvious than in the tetraploid Molly-selections.

The effect of the selection on root size is shown in table 2. Root diameter has increased, there is a significant difference between the Ro and the R3 generation in both populations.

50 single plants from each generation were observed in order to discover morphological changes. Results from two characters, time of flowering and stem length, are shown in table 3. There is no clear change in flowering time in any of the varieties although there is a small tendency to earlier flowering in the Molly-selections. The stem length has not changed in the Molly selections, but Essi II R2- and Essi II R3-generations have a shorter stem than Essi II Ro. Other observed characters have not revealed any other morphlogical changes.

Conclusion

The recurrent selection for root rot resistance using laboratory infection has been sucessful and is possible to use in a practical breeding programme. After 3 cycles of selection the resistance is improved when tested in the greenhouse and results from a preliminary field trial are good. Laboratory selection for root rot resistance will be one important tool in the work to produce more resistant red clover varieties.

References

Rufeldt, S. (1986). Studies on Fusarium root rot of red clover (Trifolium pratense L.) and the potential for its control. Plant protection reports, Dissertation. Swedish University of Agricultural Science.

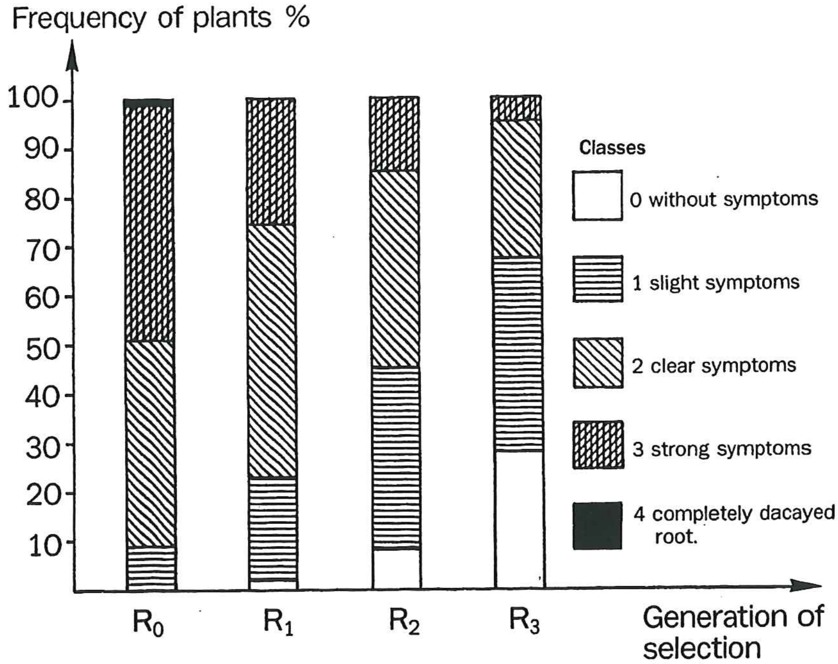


Fig 1. Effect of selection in tetraploid red clover Molly.

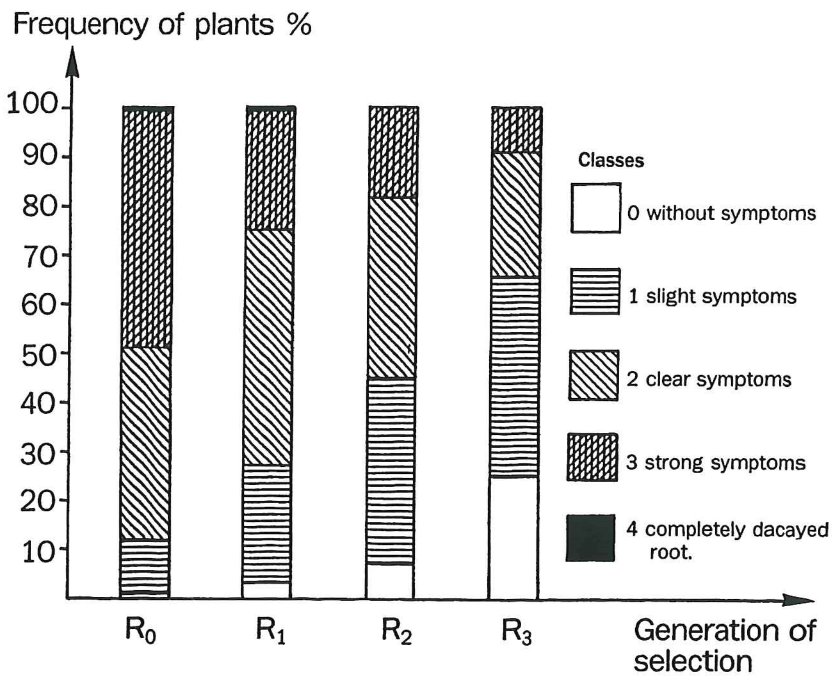


Fig 2. Effect of selection in diploid red clover Essi II.

Table 1 **Relative green matter yield of the different generations of selection**

	1:ST HARVEST YEAR				2:ND HARVEST YEAR			
Generation of selection	1:st cut	2:nd cut	3:rd cut	Total	1:st cut	2:nd cut	3:rd cut	Total
MOLLY RO	100	100	100	100	100	100	100	100
MOLLY R1	115	102	98	104	109	97	111	107
MOLLY R2	125	102	92	104	117	114	105	113
MOLLY R3	109	99	80	93	106	94	96	101
ESSI II RO	100	100	100	100	100	100	100	100
ESSI II R2	136	120	97	115	119	109	106	111
ESSI II R3	123	166	99	111	169	155	136	154

Table 2 **Root diameter on 4 month old plants**

* Indicates significant difference to the Ro generation at 5 % level of probability.

Generation of selection	Root diameter (mm) n	$\bar{x}$	s
MOLLY RO	24	6,08	1,25
MOLLY R1	23	6,22	1,24
MOLLY R2	23	6,26	1,01
MOLLY R3	24	6,96*	1,16
ESSI II RO	23	5,74	1,14
ESSI II R1	23	5,96	1,33
ESSI II R2	24	6,33	1,01
ESSI II R3	24	6,71*	0,81

Table 3 **Results from measurement of time of flowering and stem length**

Generation of selection	Date of flowering, june			Stem length		
	n	$\bar{x}$	s	n	$\bar{x}$	s
MOLLY RO	37	16,0	4,77	39	77,2	10,9
MOLLY R1	40	16,1	4,69	40	78,0	10,8
MOLLY R2	40	15,9	4,81	40	76,2	9,5
MOLLY R3	43	15,1	4,77	45	76,4	12,8
ESSI II RO	32	10,1	4,25	32	61,6	7,6
ESSI II R1	42	9,4	3,53	42	61,4	8,7
ESSI II R2	32	9,3	3,59	32	57,6*	8,1
ESSI II R3	37	10,5	2,77	37	57,3*	7,6

Vija Strazdina, Martins Roze
State Stende Plant Breeding Station
Talsi region, p/o Selekcija, LV-3258, Latvia

WINTER DAMAGE HAZARD OF WINTER WHEAT IN LATVIA

General information

Selection of winter wheat in Latvia can be divided into 3 different periods. The first period was from 1923 until 1940, the second from 1941 until 1986 and the third - after 1986.

The first trials with wheat were started at Plant Breeding station Stende in 1923. In this time the winter wheat varieties had not only been tested, but also crossed. The local material was winter hardy, but with very bad resistance to diseases, bad straw stiffness, and small thousand kernel weight. Therefore foreign winter wheat varieties from Sweden, Norway, Finland, Poland, Germany, France and other European countries were investigated too.

The selection work was very successful in 1923 to 1933 and Latvian farmers provided the nation with high quality wheat. The production of grain achieved 200 thousand tons in 1933 and Latvia stopped the import of wheat.

The Second World War hardly suppressed wheat and other crop breeding as well as all agriculture. For a long time the prevailing opinion was that Latvian agro-climatical conditions were not suitable for growing high quality bread wheat and it was necessary to import grain from other parts of the Soviet Union and other countries. As it was mentioned above the wheat breeding in Latvia was restricted for breeding only fodder wheat without testing baking quality.

Winter wheat breeding was interrupted in Lithuania and Estonia in late 1950 and remained only in Latvia at Stende. Now Latvia is an independent republic and the main task of agriculture is to provide our nation with bread. Breeders must develop new varieties suitable for Latvian agro-climatical conditions with high yield, good resistance to diseases and lodging, with high bread baking quality.

Agro-climatical conditions of Latvia

In spite of Latvia's small area, there are very large differences of climate in the various zones of Republic. There are 4 zones:

1. Northeastern
2. Southeastern
3. Central
4. Western

The largest sowing acreage of winter wheat crop is in the Western and Central zone because the agro-climatical conditions (soil, winter temperatures etc.) are more suitable for growing this crop.

In the Northeastern and Southeastern zones winter wheat is grown too, but the soil and weather conditions are more unfavorable here. The largest area is covered by winter rye in this zones, because it is more reliable crop.

The winter wheat varieties, which are grown in our republic must be very winter hardy. Winter stress has very negative influence on the crop density.

Winter hardiness is the plant ability to survive during winter and renew vegetation. The main factors damaging wheat during winter are:

1. frost
2. overflooding (drowning)
3. choking
4. ice crust.

The weakened plants can be attacked by snow mould (Fusarium nivale (Fr. Ces) (syn. Monographella n.), Typhula incarnata Lasch. ex Fr. (syn. T. itoana Im.) and T. idachoensis) and other fungi.

The observations in the meteorological station show, that the frost damage in our republic is repeating nearly after each 12 years, during the winter with little snow and with frost more than 6 days below -10°C.

Frequently (after 5-8 years) snow covers land, while the soil is not frozen. Under the thick snow, the plant continues vegetation and spends all nutritive substances. Many various fungi, such as Typhula, Fusarium etc., begin to attack the plants. These weather conditions are very dangerous for winter crop, if they have been sown very early with too high sowing rates, without seed treating.

The winter wheat is very sensitive to temperature fluctuations during the winter period, when the frost exchanges with thaw, and when snow melts.

Winter wheat frost resistance

The winter wheat frost resistance is very dependent from the plant hardening in the autumn. The plant hardening can be divided into the 2 stages. The first stage begins when the temperature is +10°, +15° in day, but 0°C in the night. If the weather is sunny, the photosynthesis produces sugar with further accumulation in the plants. This period continues for 3 or 4 weeks. If the weather is rainy and foggy, the sugar accumulatin is reduced.

The second stage begins, when temperature falls under -5°C. This stage is always connected with the first period of the hardening. At this time the plants are usually covered by snow, and the light is not necessary, good hardened plants can survive in the temperatures -20° - 25°C, but not hardened can be killed already at -14°C to -17°C. The temperature below 0°C or more very dangerous not only in winter, but also in the spring, when plants are very weak.

Winter wheat varieties earlier grown in Latvia were very winter hardy but with tall straw and with low yields. In our breeding work we use also many foreign varieties, which are high yielding, with good straw stiffness and resistant to diseases. Usually they have not good enough winter hardiness.

In order to breed new high yielding cultivars, resistant to frost we use various methods for testing our material in the fields and in the laboratory.

Materials and methods

Outside we use the following methods:

1. Evaluation of winter wheat varieties and lines 2 weeks after spring growth starts (1-5 points, 5 - good)
2. Evaluation of snow mould infection (1-9 points, 9 - high)
3. Counting of live and dead shoots (dig out plants from 1/6 m^2)
4. Removing of snow cover from test plots to expose plants to frost.

The survived plants we use in our breeding work. For screening our winter wheat material we use also laboratory methods. 25 kernels of each variety are sown in boxes (40x50x12), the distance from each row is 4 cm, in 3 replications. The boxes are placed out of doors. Varieties frost resistance are tested in January, February, in the period when plants have natural winter hardiness. We switch the same temperature in freezing chambers as it is out-doors before taking test boxes in. The decrease of temperature is 1°C per hour till the final temperature (-14°C; -16°C; -18°C) according to the method is reached. The freezing continues for 20 to 25 hours, then temperature is increased up to complete thaw. After 10 days and 28 days plants staying alive are counted which would be grown in the hothouse for harvesting, to use in further breeding work. As the winter hardiness standard we use variety Mironovskaya 808. Our winter wheat lines were tested in Ukraine at Mironovka Plant Breeding Institute too.

According to the data of Sozinov and Poperelya, some winter hardiness determination genes are connected with gliadins blocks detectable by polyacrylamide gel electrophoresis (PAGE). The presence of following allele blocks improves the winter hardiness: 1A1, 1A2, 1D5, 6A3, 6D2, where 1A, 1D, 6A, 6D are chromosomes, but 1, 2,.5, 3, 2 - numbers of blocks according to system of Sozinov-Poperelya.

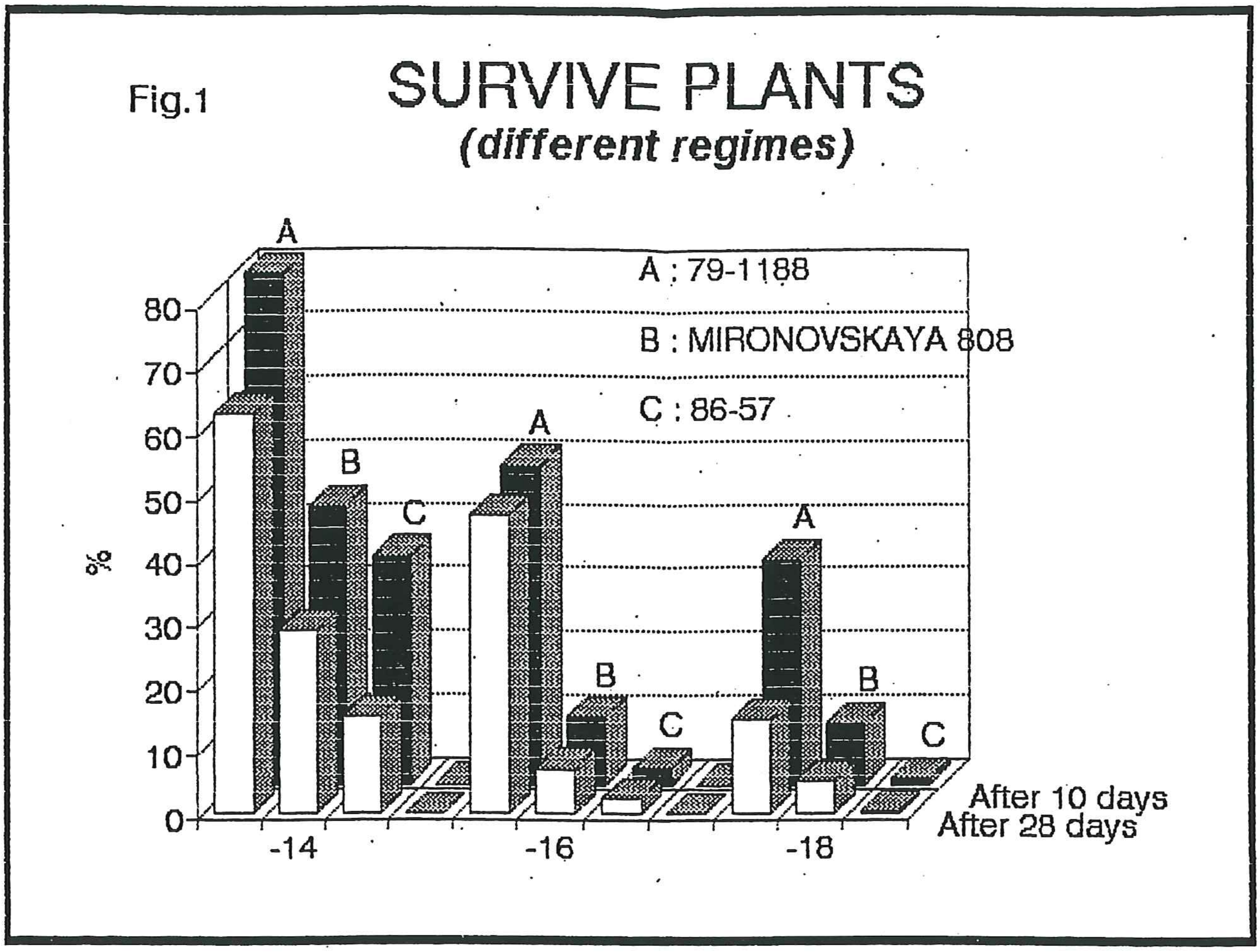
Conclusion

According to field trials results at State Stende Plant Breeding station, winter wheat breeding material (varieties and breeding lines) has a great variation in the winter hardiness.

Varieties from Latvia, Russia, Belourussia, partly Ukraine have a good winter hardiness, cultivars from Poland, Germany, Sweden, Finland, USA are medium frost resistant, but breeding lines from France, Holland, China, Italy have a very low frost resistance, and they are completely killed in the first winters in our conditions. When we use in our breeding work foreign varieties which are high yielding, with good straw stiffness, resistance to diseases, but with bad winter hardness, we must test the new breeding lines to frost resistance in the field and laboratory very carefully and pick out only suitable forms.

According to trial results more than half of our (55.5%) winter wheat lines have good frost resistance, but only few of them (21.0%) have the insufficient straw stiffness.

Observation in laboratory show that the plant frost resistance in chamber coinsides with field trials data. Results are shown in fig. No 1 and fig. No 2.



Gliadin blocks / Variety	1A1 or 1A2	1D5	6A3	6D3
Mironovskaya808	-	+	+	-
Donskaya poluk.	-	-	+	-
Krista	-	+	+	-
Kosack	-	-	-	-
Širvintas-1	-	+	-	-
78-872 (Sakta)	+	+	-	-
912 (89-912)	+	+	+	-

Fig. No. 2 Presence of different gliadin blocks coding winter hardiness in some winter wheat varieties.

Leena Hömmö
Agricultural Research Centre of Finland
Institute of Plant Breeding
SF-31600 Jokioinen, Finland

SNOW MOULD (*Microdochium nivale*) RESISTANCE OF WINTER RYE

Introduction

In Finland the weather during winter time is very unpredictable and in most years all the stress factors causing damage in overwintering plants are met at least in some parts of the country. To survive in these conditions plants must have resistance to both abiotic (frost, ice-or/and water cover, heaving) and biotic (e.g. snow moulds) factors. *Microdochium nivale* (Fr.) Samu & Hall, the most important causal agent of snow mould in Finland, is present throughout winter cereal (wheat and rye) growing area. Proper laboratory tests for screening the breeding materials under controlled conditions must be developed since the results of the field trials tend to characterise more the general winter survival abilities of varieties than their specific resistances to any particular stress factor, like snow mould.

Materials and Methods

The field trials including 13 winter rye and 24 winter wheat cultivars were carried out at five locations (Jokioinen, Anjalankoski, Pälkäne, Laukaa and Sotkamo) in years 1990-91 and 1991-92. In laboratory tests different kinds of methods were used:
1) plants growing either in plastic boxes filled with peat-soil-mixture or in beakers containing nutrition solution (HOAGLAND & ARNON 1938) were hardened and inoculated with *M. nivale*. The snow mould resistance of variety was estimated by rating the damage on the green parts of the plant and the final survival-%.
2) leaf pieces of hardened plants were soaked in solution consisting of cellulolytic and pectolytic enzymes extracted by *M.nivale*. The amount of clorophyll soluted in liquid and the amount left in leaf pieces were determined spectrophotometrically and used as a measure of snow mould resistance of a variety.
3) leaf pieces (2-3cm long) of plants were transferred onto benzimidazole-agarmedium and a drop of 10μl of inoculum (1milj. spores/ml) was placed in the middle of the leaf segment. The amount of leaf damage was taken as the measure of snow mould resistance.

Results and Discussion

In all the tests used there were differences in the snow mould resistance of the studied varieties. In most cases the results of field tests and laboratory tests of group 1 correlated positively, indicating that this kind of laboratory tests simulate quite well the wintering conditions on field. By using this kind of tests it is possible to find out the varieties which are doing well on the field conditions under quite a hard snow mould infection pressure. The differences in the amounts of snow mould damage between the varieties might, however, rather be due to the differences in the metabolism activity of varieties than the pure snow mould resistance of them.
To find out the real snow mould resistance of varieties to be used in breeding work the number 3 laboratory test method seems to be the best one.

Kurt Hjortsholm
Nørremarksvej 67
DK 8700 Horsens Denmark

Comparisons of different laboratory methods testing winterhardiness in cereals.

Introduction

The former Internordic Plant Breeding (SNP) now Nordic Gene Bank (NGB) has in 1989 initiated a project, with the aim to develope and compare laboratory methods for testing winterhardiness, in the different nordic countries. Cereals is a part of this project, and in this paper a preliminary report from this part will be given.

Methods

Differential sets of varieties have been established in winterbarley, winterwheat and winterrye.The intention with these differentials was to cover the greatest known variation concerning winterhardiness in general within these species. These differentials were sown out in fields in Iceland, Norway, Sweden, Finland and Denmark to measure the natural winterkilling.

Different laboratories have meanwhile tested the differentials with their own specific developed artificial laboratory testmethods.

At moment, 15 different laboratory tests have been run. 11 of these measured the frostresistance, two tests measured the resistance to icecovering and two tests measured the resistance against snow mould.

Results concerning field survival

The field observations have been made on 17 locations in 3 years. Unfortunately these winters have been rather mild, and the damage was not that severe as expected.

From the most southern part in Scandinavia there has been no winterkilling reported at all. But from the more northern part of Scandinavia the surviving ability of the genotypes could be measured every year. As an average from the winterdamaged locations, we got the surviving percentage in nature for the differential varieties. A great deal of variation was found in this trait,especially within winterbarley and winterwheat. Least variation was found in the rye material. The average survival percentage correlated well between the individual years.

An attempt has been further made to classify the locations in snow damaged locations and locations without snow cover. Concerning the snow/no snow classification, there was for wheat and barley survival found a high positive correlation. For rye the correlation for survival between snow and no snow was significantly lower. Both snow and no snow survival were important in determining the average survival for the varieties.

Artificial laboratory methods

The many different laboratory methods correlate different to eachother and to the surviving in field.
Generally the frosttests have shown good prediction of average surviving in wheat and barley. The highest found correlations were about 0,8-0,9. In rye the frosttests did not correlate that well with the average field surviving, but correlated better to the survival under no snow conditions.The different frost tests correlated reasonably well to eachother.

The tests, in which resistance to icecovering are measured, have not generally correlated too high to the average of field survival.But for wheat and rye, these test have shown better correlation to survival, under no snow conditions.

The specific tests, which measure the resistance against snow mould, show the best correlation to the field survival under snow conditions.

Discussion

Not all investigations in the cereal part of the project are finalised, and the results are still under calculation.New informations can therefore still come up, but as expected it seems that winterhardiness in cereals is a rather complex trait, which is difficult to explain in all details.

The last 3 years results in this project indicates that the frostresistance has been rather important for the wintersurvival in cereals.
It has also been demonstrated, that the developed artificial testmethods, can measure at least a great part of the frostresistance. Frosttests are not that expensive to run, and can be used routinely by breeders to screen lines in the breeding program of wintercereals.

The other laboratory methods than frostresistance, e.g. resistance against icecovering and fungi, are much more laborious to work with routinely in the breeding process, but give additional valuable informations about the wintersurviving ability. On locations where icecovering and fungi are important, these tests can be used in the breeding program in cereals, but it seems that frostresistance in many cases is the first trait to concentrate upon in developing more winterhardy cereal varieties.

The work with increasing winterhardiness is going on, and the coordinators in the different countries can give actual information.

Coordinators in winterhardiness:

Seppo Pulli
Landbrukets Forskningscentral, 31600 Jokioinen, Finland.
Bjarni Gudleifsson
Forsøgsgården Mödruvellir, 601 Akureyri, Island.
Arild Larsen
Vågønes Forskningsstation, 8000 Bodø, Norge
Bo Kristiansson
Svalöf Weibull AB, 26882 Svaløv, Sverige
Kurt Hjortsholm
Sejet Planteforædling, Nørremarksvej 67, 8700 Horsens, Danmark

Arne Wiberg
Eva Lindvall
Svalöf Weibull AB
Box 4097, Röbäcksdalen
S-904 03 UMEÅ, Sweden

PLANT BREEDING COOPERATION IN THE NORDIC COUNTRIES

(as exemplified by the projekt "Nordgrass")

The northern part of the Nordic countries (Norway, Sweden, Finland) has unique climatic conditions. The high latitude in combination with the nearness to the warm Atlantic Gulfstream creates a climate characterized by a long, often snow-rich, winter and a short intensive summer with long days and short bright nights.

In northern Sweden the length of the growing season is about 150 days or 60 days shorter than in the southern part of the country. As a result of the long days in the north the average temperature as well as the amount of light at midsummer is about the same all over Sweden. Already 45 days later the light, however, is 10–15% less in the north than in the south.

The agriculture situation

The rather favourable climatic conditions have permitted the development of an agriculture with relatively high production. The cultivated area north of the 62nd latitude is about 1.000000 ha in Finland, 230.000 ha in Sweden and 200.000 ha in Norway. Forage is the main crop, covering about 50 % in Finland, 60 % in Sweden and 80 % in Norway. On the second place early varieties of barley and oats will come.

At least in Sweden the cultivated area is slowly decreasing every year. There the groving of barley and oats had its maximum in 1978 with 80.000 hectares but right now it covers less than 40.000 hectares. Also the forage crops have decreased but improved growing and harvest technique has kept the total yield on a relatively high level.

As the growing of conventional crops gradually is getting less important there is a need for new ones if the fields continously shall be used for farming and the landscape remain open. Both in Finland and in Sweden there is an interest to develope reed canarygrass as a energy or fibre crop. Reed canarygrass is a perennial species and native to the region in question. The breeding work is just in the beginning but preliminary field observations and laboratory analyses are very promising for the future. A lot of work, however, has to be done concerning the growing technique, harvest, transportation and further processing.

Another crop of potential future interest is turnip rape grown for oil production. From an environmental standpoint vegetable oil has many advantages compared to the mineral oil. Turnip rape, however, has to be better adapted to a more northern climat. Varieties of spring turnip rape are normally too late and needs improvements in maturity. Winter turnip rape on the other hand would better fit the growing season and thus be an alternative to prefer. The winter hardiness, however, is not sufficient but too little is known whether damage is caused by low temperature, sensibility to ice encasement or other factors.

Seed production

Seeds of good quality are necessary for a high yield. Normally the commercial seed for the northern regions is produced under safer climatic conditions further south. For self pollinated crops this arrangement is without problems, but for cross pollinated ones a continous growing of many consecutive generations outside the area to which the variety is adopted may change the genotype in unwanted directions. Therefor a system now is practiced where the production of basic seed is performed in the north and then used for production of commercial seed at lower latitudes.

Summarizing the situation for the agriculture in the northern regions of the nordic countries the following can be stated:

A large geographical area with varying climatic conditions is covered. In some cases two or more varieties of each crop are needed to get a well adapted meterial for the whole region.

Except for northern Finland the cultivated area is small and the seed demand limited.

As the seed production often has to be done outside the region and for cross pollinated crops according to a special system in two steps the seed cost will be high and less commercially interesting.

To limit the costs and to get varieties with a wide adaptation cooperation in plant breeding for the Nordic countries is important.

Present cooperation in plant breeding between Svalöf, Hahkiala and Bjørke

Already in 1973 an agreement was made between the Swedish Seed Association later Svalöf AB, Sweden, and Hahkiala experimental farm (owned by Kesko), Finland, about mutual cooperation in breeding of all actual agricultural crops.

A few years later, in 1978, the same kind of cooperation was established by Bjørke experimental farm (owned by Felleskjøpet), Norway.

The agreement means that promising lines are tested simultaneously in the tree countries. The breeding material at Svalöv is open for Hahkiala and Bjørke to selections and further observations. Material of interest in only one country can be developed to special varieties just for that country.

There is also a direkt exchange of material between Hahkiala and Bjørke and between those stations and Svalöf department for Northern Sweden.

For a long period of time the breeders from the tree countries are meeting every year to discuss results and breeding policy, to exchange experiences and to plan for further work.

The main crops involved in this cooperation are barley, oats, spring weat, rape and turnip rape. As a result many varieties from Svalöf are grown in Finland and Norway. Selections and tests in Finland have resulted in special Finnish barley varieties as Kulta, Kymppi and Kalle

The "Nordgrass" project

The "Nordgrass" project is earlier described at the NJF–congress in Uppsala, Sweden, by Áslaug Helgadóttir (1991) and at the Circumpolar Congress in Yukon, Canada, by Arild Larsen (in press).

The project, started in 1981, is including the five countries Finland, Sweden, Norway, Iceland and Denmark. The goal is to coordinate the breeding of forage crops for the most northern areas of the region in question. The work is financed by the Nordic Council of Ministers and directed by Internordic Plant Breeding.

The project began with a common test of available varieties and breeding populations of different forage crops such as timothy (*Phleum pratense*), meadow grass (*Poa pratensis*), meadow fescue (*Festuca pratensis*), red fescue (*Festuca rubra*), common bentgrass (*Agrostis capillaris*) and red clover (*Trifolium pratense*). The first test series are finished and the results for most of them are reported. In total about 100 trials with more than 5000 plots have been included in the experiments.

During 1992 new trials were sown with timothy, red fescue and common bentgrass. For 1993 trials with meadow fescue and meadow grass are planned.

Based on the experiences of the first common trials the main work within "Nordgrass", however, is dealing with a special, rather large breeding program in timothy. It started in 1985 with the purpose to obtain a variety or varieties adapted to as many different climatic conditions as possible.

Genotypes from Finland, Sweden, Norway and Iceland, in all 60, were included in a polycross planted in Copenhagen, Denmark. The progenies from the polycross were tested for 2 – 3 years at all stations involved in the project. According to the results of the progeny test a number of 16 genotypes were selected and intercrossed to a synthetic variety. This is now under multiplication and as soon as enough seed is obtained it will be evaluated in comparative yield trials at all the participating stations. Hopefully this will finally result in the first market variety from a joint Nordic breeding work.

A second synthetic variety is further planned and under multiplication. This is derived from another set of polycross progenies.

Material from the polycross project will also be utilized in a special program for studies of the importance of ecological and physiological characters for adaptation to different growing conditions.

The "Nordgrass" project which now has lasted for 12 years ends during 1993. The remaining test work of the new syntetic varieties will be performed and financed by each station involved. Discussions are going on but no decisions are yet made concerning the ownership, maintaining and marketing of the potential new common timothy variety.

At the same time as "Nordgrass" is finished a new common project, called "Nordclover", is under development. "Nordclover" is sponsored in similar way as "Nordgrass" and also involving the same countries. The project will include work with diploid and tetraploid red clover as well as white clover.

Concerning red clover promising genotypes from all the countries will be intercrossed, followed by selections, multiplication and tests. New tetraploid populations are also developed from diploid material.

Conclusion

The common work between two or more Nordic countries has given possibilities to test breeding material under a wider range of climatic conditions and thus also opportunities to obtain new varieties with a broad adaptation. A more effective use and selection in available populations can also be expected.

The timothy program within "Nordgrass" is further a very illustrative model how national breeding material systematically can be used on a international level to produce new genotypes of common interest.

Litterature

Helgadóttir, Á, 1991: Nordisk Jordbrugsforskning. 73:235.

I *Röbäcksdalen Meddelar* nyligen utkomna nummer

1992:10 Växtodling	Lennart Lomakka. Odlingsförsök med rörflen till biobränsle och fiberråvara.
1992:11 Bioenergi	Jan Burvall Möjligheten att reducera svavelutsläpp genom sameldning av torv och energigräs. Proveldning vid Åsele energiverks 3.5 rosterpanna.
1992:12 Husdjur Växtodling Trädgård	7:e regionala lantbrukskonferensen för norra Sverige den 17-18 mars 1992, Umeå. Föredragen i sammandrag.
1992:13 Trädgård	Erland Svensson†, Elsa Viklund, Elisabeth Öberg. Förökning och distribution av träd och buskar utvalda för norrländska förhållanden.
1992:14 Allmänt	Martin Wik. Sammanställning av utgivna nummer 1992 samt Publicerat från Röbäcksdalens verksamhetsområde 1992.
1993:1 Växtodling	Anne-Maj Gustavsson och Sven Andersson. Sortförsök med korn, havre och ärter i norra jordbruksförsöksdistriktet 1983-1992.
1993:2 Husdjur	Gun Bernes. Grovfoder till getter - en litteraturöversikt.
1993:3 Husdjur	Gun Bernes. Vinterlamm - hö eller ensilage till slutuppfödningen.
1993:4 Husdjur	Gun Bernes. Hö och tallris som grovfoder i intensiv getmjölksproduktion.
1993:5 Växtskydd	Susanne Hoffman. Biologisk bekämpning i växthus.
1993:6 SLL Husdjur	Carina Jonsson, Robert Samuelsson och Jerker Öhman. Torbjörn Pettersson. Jämförelse av metoder för energianvändning av norrländskt korn.
1993:7 Växtodling	Lennart Lomakka. Skördetidsförsök i norra Sverige i korn och ärtblandsäd för våtkonservering.
1993:8 Bioenergi	Jan Burvall, Karin Segerud. Pulverbränsle från rörflen.
1993:9 Bioenergi	Jan Burvall Tillverkning och proveldning av rörflenpulver - ett fullskaleförsök.
1993:10 Husdjur	Gun Bernes och Bodil Cornell. Ensilage eller hö i en KRAV-anpassad foderstat för getter.
1993:11 Växtodling	Sven Andersson (red.). Lantbruksväxternas övervintring, teorier och testmetoder.

Institutionen för norrländsk jordbruksvetenskap
Swedish University of Agricultural Sciences
Dept. of Agricultural Research for Northern Sweden

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