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Plant and Microbe Adaptations to Winter Environments in Northern Areas

Abstracts

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How will climate change affect terrestrial ecosystems?

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The paper will summarise some of our understanding of the effects of climate change on populations, processes and systems in the natural environment. Winter warming has been identified as a particularly important factor, but it does not act in isolation. Responses will be influenced by associated changes in precipitation and from other environmental factors such as UV-B, CO_2 enhancement and N deposition. There is also the potential for cooler conditions to develop in some areas.

Plants, animals and microbes have been subject to considerable variations in climate in the past and may be 'pre-adapted' to respond to future changes. Past observations, recent changes, experiments, theory and models help to assess potential impacts of environmental change. The impacts are expected to be spatially variable, depending on, for example, the soil conditions, the margins of species range, the controls on populations and on soil processes.

The paper will draw heavily on a three year international programme which has developed Impact Scenarios, based on a wide range of research. It may raise more questions than answers, but it highlights the value of exchange of information and experience across disciplines.

Climate variations, grass growth and winter kill in Iceland

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The relationship between temperature and hay yield from the beginning of this century is considered, and the changes that have occurred in the usage of artificial fertilizer have been weighed in. It is conclusively shown that a deviation in winter temperature has a somewhat greater effect on the hay production than the same deviation in summer temperature, in addition to the fact that year to year variations in temperature are usually much greater in winter than they are in the summer. This makes it possible to predict plant growth in hay fields with resonable certainty by the end of April, while this prediction may then be used to to control the amount of fertilizer used inorder to reduce the variation in yield from one year to the next and mostly eliminate the deviation in hay yield between decades. The forecast gives the total hay yield of the whole country. A fourteen year experiment which has been conducted at Hvanneyri in order to verify this is outlined. It is inferred that winter kill in hay fields is closely connected with an index computed on the basis of the combined summer temperature and the temperature of the preceding winter which however is much more important. While local conditions are also important for the winter kill this will ensure the availability of hay for farmers badly hit by the damage of their hay fields.

An important fact in this connection is that it is possible to a certain degree to forecast the average climatic conditions of several coming years in Iceland, based on the extreme variations in temperature and sea ice in the ocean to the north of the country. These variations in the sea are reflected by for example the climatic variations at Spitzbergen. Due to the extreme persistence of the ocean temperature and the long time it takes for the ocean currents to travel towards Iceland from the north this has a predictive value of the climate of several years in Iceland. Spreading of these effects by the global winds will furthermore make possible a forecast of average temperature conditions in the whole northern hemisphere for a period of many years if at the same time the additional expected greenhouse effect is taken into consideration.

Cold-regulated genes and cold signal transduction pathways

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Cold stress causes two major responses of microorganisms; a decrease in fluidity of membrane lipids and a block of protein synthesis. The former can be overcome by increasing unsaturated fatty acids in membrane lipids by the cold induction of fatty acid desaturases [1]. The latter can be overcome by transient induction of a specific set of proteins. In *Escherichia coli* and *Bacillus subtitis*, these proteins are termed cold-shock proteins (Csps). Csps are supposed to activate translation by stabilizing or defolding mRNAs. In cyanobacteria, cold stress induces another set of proteins, termed RNA-binding proteins (Rbps), and RNA helicases, which are both supposed to defold mRNAs and activate translation [1].

Regardless intensive studies on the cold induction of a number of proteins involved in protein synthesis, how the organisms sense and transduce the cold signal remained unanswered for many years. We addressed this question by systematic mutagenesis of all the 43 putative genes for histidine kinases and by random mutagenesis of almost all the genes in the genome in *Synechocystis* sp. PCC 6803, and identified two histidine kinases (Hik33 and Hik19) and a response regulator as components of the pathway for perception and transduction of the cold signal [2]. His33 is likely to span the membrane twice and is a strong candidate for the cold sensor. Hik19 is hydrophilic and of hybrid type, and is likely to be a transducer of the cold signal.

A recent achievement of the genome research has provided us with a great advantage for understanding the mechanism of cold response of microorganisms. The total genome sequences have been determined in a number of eubacteria, archaebacteria and cyanobacteria. The DNA microarray analysis based on the genome information of *Synechocystis* has allowed us to identify the cold-inducible genes which are under control of the Hik33.

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Chilling resistance among isolates of *Pythium* from Polar and Temperate Zones

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Moss vegetation plays an important role as a producer in early stages of primary succession in the Arctic and Antarctic. Moss colonies also offer living space for terrestrial invertebrates such as insects, ticks and nematodes. Therefore, moss communities play an important key role in the terrestrial ecosystems in Polar regions. Microorganisms, especially fungi in Polar regions, are well known for their formation of mycorrhizae, basidiolichens and decomposer associations. In addition, some fungi have been reported to attack actively mosses growing in the Arctic. However, there have been very few ecological and physiological studies on phytopathogenic fungi in Polar regions.

In the moss carpets of *Sanionia uncinata* in Svalbard (79 N, 12 E) in Barents Sea and King George Island (62 S, 58 W) in maritime Antarctic, many dying moss colonies were found after the snow bed had melted. We isolated see several *Pythium* spp. from dying moss. Our collected *Pythium* isolates were *P. ultimum* var. *ultimum* (Svalbard) and *Pythium* group HS (Svalbard and King George Is.). *Pythium* group HS was frequently isolated in both Polar regions. Isolates of *P. ultimum* var. *ultimum* and *Pythium* group HS killed moss shoots of *S. uncinata* in artificial inoculation, and physiological characteristics of isolates showed that they were cold tolerant. The Polar isolates could grow and survive at 0 C (same conditions as under the snow cover and in spring ground temperature in Polar regions). The Temperate Zone isolates of the same fungus did not grow at 0 C and were destroyed by storage at 0 C for 2 weeks. The optimum growth temperature of Polar isolates was 20 - 25 C. These results suggest that Polar isolates originally developed from Temperate Zone isolates and adapted to growth under polar conditions.

We have following hypothesis of ecological role of moss pathogenic fungi in Polar regions. Pathogenic fungi have invaded in a moss carpet and after several years formed patches. The host moss shoots died in the center part of patches. Some higher plants and other moss species in the dying moss patches were often found. Thus, the invasion of phytopathogenic fungi in a moss carpet of *S. uncinata* is thought to lead to the formation of dead patches which are open spaces where other plants easily can colonize. The formation of these patches might be the first step reward changes in plant communities in Polar regions.

Reference

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Variation between *Microdochium nivale* isolates. Is pathogenicity correlated with growth rate and molecular markers?

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Microdochium nivale is causing pink snow mould on winter cereals and grasses in the Nordic countries. At warmer temperatures it causes leaf blotch, stem rot and head blight. Based on morphological traits, the species is divided in two varieties, var. *nivale* and var. *majus*. In spite of recent molecular characterisation, it is still unclear whether these two groups also represent host specialisation.

In the effort to identify host variation in resistance to pink snow mould in breeding material of winter cereals and grasses, the pathogen must also be characterised. Any host specialisation and variation in aggressiveness of the strains employed, affects the outcome of the screening for resistance. Goals for our studies are to reveal whether there is any host-specialisation among strains of *M. nivale*, and if variation in pathogenicity or aggressiveness between the strains are correlated with molecular markers or physiological parameters.

M. nivale strains, several of var. *nivale* and a few of var. *majus*, isolated from different grass and cereal hosts have been inoculated on different cereal and grass species and incubated under artificial snow cover. The strains show great variation in aggressiveness and growth rate, but the variation in cardinal temperatures limited. Preliminary results indicate some degree of host specialisation. According to the estrease profile, the Norwegian isolates tested so far falls in two groups, one of them assumed to be the var majus group. Several isolates do however show "private" band patterns. We are currently also studying the AFLP pattern of the isolates.

Selection of cold tolerant *Trichoderma* species for biological control

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To be able to control plant diseases caused by fungi in cold weather and during cold storage, antagonists are needed which can grow and control the pathogen at low temperature.

Trichoderma isolates have been used for control of a range of fungal diseases on many plant species. Because of the large genetic variation in *Trichoderma*, it was possible to select cold tolerant isolates with antagonistic activities at low temperature. 54 isolates from 7 *Trichoderma* species were tested. Of these, 32 were able to grow at 2°C and most of them were antagonistic (produced inhibitors and showed hyphal interaction) at 5°C (Tronsmo and Dennis 1978). Five of these isolates were tested in field trials against *Botrytis cinerea* and *Mucor mucedo* rot on strawberries. Two isolates showed as good control as the standard fungicide (Euparen). One of these isolates, *Trichoderma harzianum* P1, has since been extensively used to control fungal diseases at low temperature on field grown grasses, strawberries, cherries and apples, and some successes have been obtained. Fungal diseases on cold stored carrots (Tronsmo 1989), apples (Tronsmo 1996) and strawberries (Tronsmo and Dennis 1977) have also been controlled by *T. harzianum* P1, whereas a mutant of this fungus that had lost the ability to grow at low temperature had no disease reducing activity. Work is in progress to understand the physiology of *T. harzianum*, with the aim to improve biocontrol control under environmental conditions unfavorable for this antagonist.

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Growth of rhizobia at low temperatures

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The significance of rhizobia will probably increase with growing interest in the use of legumes in agriculture and land reclamation. Rhizobial strains which are commercially available as inoculum on the market might not be suitable for use in cold areas like Iceland because they originate from areas with higher soil temperatures than prevail in subarctic soils. The rhizobia on roots of native legumes in Iceland are probably adapted to low soil temperatures. Mean soil temperatures in the beginning of the growing season may range from 3 to 6 degrees in 5cm depth and reaching only 10 to 12 degrees in the warmest month. Research on Icelandic lupine rhizobia (lupine is not a native genus in Iceland) has shown variation in nitrogen fixing efficiency between different strains. Selected strains are now produced commercially by Soil Conservation Service in Iceland for inoculation of lupine seed for land reclamation work.

In a Nordic research project the ability of strains of *Rhizobium loti* and several other strains to grow *in vitro* at low temperatures has been measured, and the results showed that one of the strains of *R. loti* could grow in vitro down to 2,7 degrees centigrade while one other strain of *R. loti* needed 6,6 degrees. One Icelandic strain of *R. leguminosarum* biovar. *viceae* needed minimum temperature as high as 7,8 degrees. A Nordic project work has also been done on testing three Norwegian strains of *Rhizobium leguminosarum* biovar. *trifolii* for their effectiveness on white clover in Iceland and the results showed that a mixture of the strains gave better yield than any single strain. Similiar work with red clover and *Galega orientalis* is beeing done. Three strains, originating from Iceland, Norway and Finland, are beeing tested on red clover and the first results show significant variation in efficiency of the strains, with the Finnish strain beeing the most effective.

How do plants know it’s cold out there?

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Being immobile, higher plants are frequently confronted by many stressful conditions such as freezing temperatures. Many plants succumb to freezing and those that survive the freezing winter do so through a series of adaptive actions. First, of course, the plant has to sense the approaching winter. Fortunately, winter approaches gradually. Chilling temperatures (generally below 10°C) persist for a long time before it starts freezing. This talk will focus on the perception of the low temperature signal and its transduction into biochemical events that lead to the development of freezing tolerance, a process called cold acclimation (CA). We shall provide evidence for the following sequence of events during cold sensing: the temperature signal is perceived by plasma membrane through decreased fluidity, followed by reorganization of cytoskeleton, activation of calcium channels, calcium influx, activation of specific MAPKs (Mitogen-Activated Protein Kinases), cold-activated SAMK and heat-activated HAMK

In our studies of low temperature signal transduction, we have used the expression of cas genes or activation of a specific mitogen-activated protein kinase of alfalfa as end-point markers. Triggering of cold acclimation requires an influx of calcium, largely from the cell wall, through the plasma membrane-located mechanosensitive channels into the cytosol. Prevention of this calcium influx by either calcium chelators or by calcium channel blockers prevents cold-induced gene expression and CA. Conversely, experimental increase in cytosolic calcium induces the *cas* genes at 25°C. How cold triggers the opening of calcium channels has not been clear. We are investigating the nature and temporal order of early events during cold sensing leading to cold-specific gene expression. Using alfalfa cells as experimental system, we have examined the role of membrane fluidity, cytoskeleton stability and calcium influx in cold activation of SAMK, an alfalfa MAPK activated by cold, drought and touch.

Temperature is known to directly modulate membrane fluidity, cold rigidifies and heat fluidizes cellular membranes. Low temperature is known to activate a specific MAPK, called SAMK. High temperature, on the other hand, activates another MAPK which we have named HAMK (Heat shock-Activated MAPK). Both these MAPKs have the same electrophoretic mobility but can be distinguished immunochemically. Rigidification of cell membranes with DMSO activates the SAMK at 25°C but fluidization of the membranes with benzyl alcohol (BA) at 4°C prevents SAMK activation and at 25°C activates HAMK. How opposite changes in membrane fluidity activate SAMK or HAMK? Activation of both of these MAPKs requires reorganization of actin cytoskeleton , because it is prevented by jasplakinolide but both MAPKs are activated by cytochalasin D at 25°C. The activation of both these MAPKs by either temperature, chemically modulated membrane fluidity or by cytochalsin D is prevented by calcium chelators or calcium channel blockers. We show that cytoskeleton reorganization is downstream of membrane fluidity changes but upstream of calcium influx. MAPKs are activated by MAPKKs and the latter by the upstream MAPKKKs. What activates the MAPKKK involved in cold signaling is not yet clear but we have preliminary evidence that calcium-dependent protein kinases may be involved. The relationship of these MAPKs with freezing tolerance is still unclear. It is possible that a plant may be competent in signaling but either does not have or fails to express genes the products of which are needed to develop freezing tolerance. Such appears to be the case with *Medicago truncatula*. Recently, it has been shown that the constitutive expression of a MAPKKK, NPK1 confers tolerance to multiple stresses, including freezing, in tobacco.

Early signaling events in cold acclimation-specific gene expression in alfalfa

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Cold acclimation in plants is associated with specific gene expression triggered by a transient calcium influx into the cytosol. The nature and temporal sequence of events leading to the calcium influx are not known. We have shown that the expression of a cold acclimation-specific (*cas*) gene of alfalfa, *cas*30, calcium influx and the development of frost tolerance at 4°C are all prevented by cell membrane "fluidizers" but, conversely, induced at 25°C by membrane "rigidifiers". *Cas*30 expression and calcium influx at 4°C are also prevented by an actin microfilament stabilizer but induced at 25°C by an actin microfilament destabilizer. Furthermore, membrane rigidifier-induced *cas*30 expression at 25°C is inhibited by actin microfilament stabilizer. Biochemical evidence demonstrates dynamic reorganization of actin microfilaments as an early response to cold stress. Chemicals modulating actin microfilament organization and membrane fluidity dramatically affect these changes. Our results suggest that actin microfilament reorganization provide an essential link between the cold-induced membrane rigidification and calcium influx leading to *cas* gene expression and cold acclimation.

Genetic bases of barley physiological response to cold environments

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Acclimation to low temperatures in plants is an adaptive response that prevents damage due to winter freezing. The ability of plants to withstand winter temperatures is mediated by specific sets of genes which modify cell metabolism making cells able to cope with adverse conditions. Molecular dissection of the cold acclimation process has shown that many cold regulated (cor) genes are involved.. The accumulation of cor mRNAs depends primarily on the low temperature, but it can also be modulated by other factors such as application of ABA, drought stress or light. For example, we have found that expression of the barley cor14b occurs only at low temperatures, but it is enhanced after even brief exposure of the plants to light. We have seen that the cold induced expression of a number of cor genes such as cor14b, cor tmc-ap3 and of the blt14-gene family was strongly reduced in plants carrying the albino mutation an. Notably, these plants were not able to harden when exposed to low temperature providing a direct evidence of the relationship between expression of cold-regulated genes and the development of cold hardening.

Genetic studies proved that in cereals only few chromosome regions, particularly on the long arm of the homeologous group 5, carry loci that play an important role in frost tolerance. While most of the cold-regulated genes known so far map outside the homeologous group 5, we have shown, by using wheat chromosome substitution lines, that the chromosome 5A carries few regulatory genes which control the expression of known cold-regulated sequences, demonstrating a genetic linkage between the frost resistant locus/i and the expression of cold regulated genes.

A putative function can be assigned to several cor genes on the basis of their sequence similarities. We have found that the barley gene cor tmc-ap3 is highly similar with oep16, a pea gene that codes for a putative amino acid-selective channel protein of the chloroplast outer membrane. A homologue cold-induced Arabidopsis EST sequence suggests the existence of a common mechanism in mono and dicotyledonous plants. According with the putative function of cor tmc-ap3, the chloroplast ability to exchange amino acids was assayed by an in vitro test performed using radioactive-labelled methionine in both barley and Arabidopsis intact chloroplasts. In both species chloroplasts extracted from cold-acclimated plants had higher ability to import amino acids than those extracted from control plants.

The magnetizing dehydrins

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The dehydrin protein family is one of the most abundant group of novel proteins accumulating in plants in response to environmental stresses such as drought, salinity and low temperature and in the drying seed during embryo development. They have been found to be widely distributed within the plant kingdom. The dehydrins fail to show homology to any other known proteins and assigning a physiological function to the dehydrins has proven an elusive task.

We have recently found biochemical evidence for a strong metal ion affinity for several of the dehydrins. Some dehydrins show strong affinity for several divalent metal ions, however, the specificity of the metal binding of the dehydrins varies somewhat. Structural features of the dehydrins that may be important for their metal affinity will be adressed.

Oxidative stress is an important aspect of many abiotic stresses, such as cold and drought, particularly when in combination with high light conditions. Divalent metal ions play a role in oxidative stress in plants, where they catalyze the formation of the highly reactive and damaging hydroxyl radical. Chelation of metals is a means to neutralize their catalytic activity and may provide the plant with protection by preventing or attenuating the formation of hydroxyl radicals and the subsequent, highly damaging chain reactions.

Ectopic expression of dehydrins in transgenic plants provides an opportunity to test the relevance for stress tolerance of our finding that dehydrins are efficient metal binders. We suggest that the metal binding nature of several of the dehydrins may prevent or attenuate damage due to oxidative stress during exposure to abiotic stress.

Desiccation injury in winter cereals: The role of dehydrins, heat stable proteins and carbohydrates.

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Winter injury to winter cereals is a complex phenomena with little or no progress made in the last 100 years. The type of injury sustained is a function of winter condition, e.g. low temperature injury versus ice encasement. A high correlation was observed between duration of low temperature exposure and winterkill for cultivars selected for continental climates. Winter injury under these conditions appears to be related to freeze-induced injury.

Temperatures approaching 0°C initiate cold acclimation, however, it is not known how low temperatures transduce this signal. Our results suggest a change in water potential may be the initial signal. During cold acclimation of winter hardy continental adapted cultivars, there is an increase in both freezing tolerance and freeze desiccation tolerance. Cultivars may attain the same level of freezing tolerance as determined by a 2°C ha^{-1} freeze-test (LT_{50}), however they can differ dramatically in their freeze-induced desiccation tolerance (LT_{50}). This suggests there are different genes associated with freezing tolerance and freeze desiccation tolerance. Dehydrins are prime candidates to be involved in the development of desiccation tolerance.

Southern analysis revealed that spring and tender winter cultivars possess similar stress genes as the winter hardy cultivars. However, these genes are either not, or only transiently, expressed during cold acclimation according to results from northern and western analysis. Boiling stable proteins and dehydrins were investigated for their role in freeze-induced desiccation tolerance. These proteins are thought to maintain native protein structure during freeze-induced dehydration and consequently maintain the capacity to recover the biological function of the protein upon rehydration. A strong correlation between winter survival and these proteins was observed. However, these proteins alone did not account for the increase in freezing tolerance. Sugar(s) in combination with these proteins appear to confirm freeze-induced desiccation tolerance.

Water and ABA content in fully-expanded leaves of cold-hardened barley

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Intact mature leaves provide a suitable model of non-expanding green tissues (where changes connected with growth and cell differentiation are completed) and we used them for studying physiological changes during cold hardening of winter barley (*Hordeum vulgare* L.). After cultivating plants at 16 ^{o}C (light period 16 hours, 400 ìmol/m^{2}/s) in Hoagland 3 nutrient solution till the second leaves fully expanded, we exposed the plants to cold hardening at 3 ^{o}C and under the same light conditions. We sampled the second mature leaves at the end of the dark period, at the stage of full water saturation. In the second mature leaf the ratio of water weight to dry weight decreased significantly under hardening conditions. When we expressed both leaf water weight and dry weight per leaf area (the leaf area remains constant) we observed increase only in dry weight. Hence, the decrease of the ratio of water weight to dry weight during hardening was due to an increase in dry weight rather than a decrease in water content. The accumulation of dry weight was accompanied with decrease of osmotic potential and increase of freezing tolerance during cold hardening. The content of ABA did not change significantly in the second mature leaf of cold hardened plants. Further, we compared the changes in dry weight, ABA content, osmotic potential and freezing tolerance of cold hardened plants with those in plants exposed to drought treatment (20% of PEG 8000 solution) at the same temperature of 16 ^{o}C and light conditions as in foregoing cultivation. This drought treatment induced only negligible increase of freezing tolerance and of dry weight accumulation in the second mature leaf. The ABA content transiently increased at the onset of drought hardening. We concluded that cold hardening resulting in increase of dry weight content and decrease of osmotic potential is prerequisite for the development of full freezing tolerance of plants. The role of ABA in induction of freezing tolerance could be mediated via different mechanisms in cold or drought hardened plants.

Frost tolerance among provenances and families from the Picea complex in Alaska

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Sitka spruce (*Picea sitchensis (Bong.) Carr*.) is the most promising tree species for production forestry in the cool, oceanic regions of southern and western Iceland. Experience suggests, however, that the species may be susceptible to frost damage during late spring and early autumn. To reduce this risk it was nessesary to examine the genetic variation in growth rhythm and frost hardiness in spring and autumn among those provenances of Sitka spruce that can be successfully cultivated under Icelandic conditions. Freeze-testing under controlled conditions was used on a total of 8000 Sitka spruce (*P. sitchensis (Bong.) Carr*.) and Lutz spruce (*P. x lutzii*) seedlings, from among 10 families from each of 20 provenances. Differences in frost tolerance during spring & autumn were significantly different among provenances and among families within provenances. Provenances of White spruce (*P.glauca (Moench) Voss*) and Lutz spruce were more susceptible to damage from spring frosts than those of Sitka spruce. The converse was however true for autumn frost damage, where damage was greatest in Sitka spruce. Correlations between frost tolerance and latitude, longitude and elevation at orgin, as well as seedling height were insignificant. There was however a strong and significant relationship between damages observed among provenances and families in the nursery one year earlier, attributed to autumn frosts, and damages observed after controlled freezing conditions in the fallowing autumn. These results suggests the opportunity for using freeze-testing for early selection for frost hardiness in the nursery and in the field.

Factors involved in ice nucleation and propagation in plants: An overview based on new insights gained from the use of infrared thermography

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The majority of data reported on how plants freeze has been obtained through the use of thermocouples. Although the use of this technology has provided great insight into the freezing process, numerous questions still exist about the role of intrinsic and extrinsic nucleating agents in the freezing process. This is partially due to the limits in the information that can be gained using thermocouple technology. Several years ago, the ability to use high-resolution infrared thermography to directly observe the freezing process in plants was demonstrated and since then

has been used to characterize ice nucleation and propagation in several herbaceous and woody plant species. The present report will provide an overview of these various studies and detail the factors that apparently play a significant role in determining when a plant will freeze and how ice will propagate through a plant.

Role of Moisture and Extrinisic Ice Nucleating Agents. - One of the critical factors in determining when a plant will freeze is the presence or absence of surface moisture. Dry plants will always supercool to a lower temperature than wet plants. Secondly, if ice nucleating agents, such as INA bacteria, are present, they will induce plants to freeze at a warmer temperature than just the moisture alone. The presence of nucleators on the surface without moisture is not effective because nucleators are only active in aqueous solutions.

Role of the Cuticle and Stomates in External Induction of Freezing. In order for the presence of external ice (frozen moisture on a leaf surface) to induce ice formation in a plant, the ice must physically grow through a break in the surface of the cuticle (eg. crack or broken hair cells) or through a stomatal opening. A thick cuticle, such as found on evergreen leaves (eg. azalea, cranberry) serves as an effective barrier to external nucleation. Water can freeze on the upper surface of these plants and the plant will continue to supercool. When external ice does induce the plant to freeze it is through the growth of ice through a stomatal opening on the abaxial surface. In herbaceous plants, the cuticle is not an effective barrier, or there are sufficient avenues of ingress that allow ice to readily propagate from either the upper or lower surface. Providing a barrier of silicone grease sufficiently prevents external ice from inducing herbaceous plants to freeze. To test the hypothesis that a hydrophobic barrier can prevent plants from freezing, an emulsion of hydrophobic kaolin (Englehard, Inc.) was applied to the surface of 4-6 week old tomato plants prior to application of an extrinsic nucleating agent (Cit7 strain of Ina^{+} *Pseudomonas syringae*). Results indicated that dry, young tomato plants can supercool to as low as -6 C whereas plants having a single droplet of Cit7 would freeze at -1.5 to -2.5 C. Application of the hydrophobic barrier blocked the effect of Cit7 and allowed the plants to supercool to -6 C, despite the presence of frozen droplets on leaf surfaces.

The Initial Freezing Process and Subsequent Ice Propagation. Ice nucleation, when it occurs at warm temperatures in herbaceous plants, is a two step process. In the first step, only water that is present along the surface of cells is induced to freeze. This can be seen as a small exothermic response after which the plant tissue quickly cools back down to ambient temperatures. In the second stage, which can often be distinctly separated from the first step, the extracellular ice induces a much more substantial freezing event where bulk water in the xylem conducting elements freezes, water is drawn out of cells and freezes extracellulary. This is seen as a substantial exothermic event that persists for an extended period of time. This second freezing event is easily propagated throughout the rest of the plant. Ice does not, however, move down through a stem into a below-ground portion of a plant and then back up into another above-ground portion of a plant. For example, ice does not propagate down a potato stem into a tuber and then back up another stem. If ice formation occurs at a warm temperature, appendages attached to the organ that initially froze may escape freezing for a period of time, indicating that some barriers to ice propagation do exist. The two steps of the freezing process become superimposed on each other if freezing is initiated after a significant amount of supercooling has occurred.

Freezing of Woody Stems and Barriers to Ice Propagation. - As previously documented, the presence of effective, intrinsic nucleators, appears to be common in woody plants. These nucleators appear to be as effective as external ice nucleators, such INA bacteria, and induce the stems to freeze at warm, subzero temperatures. Barriers appear to exist, however, that prevent ice propagation into lateral appendages such as buds, or newly extended primary tissues (flowers, inflorescences, etc.). These barriers are most effective if the initial freezing event occurs at a relatively warm temperature. These barriers have been observed in the propagation of ice into the strigs of Ribes and grapevines, the pedicel of cranberry fruits, and flowers of peach indicating that the ability of buds, flowers, and inflorescences to supercool in the presence of frozen stem material may be an active mechanism of freeze avoidance.

Influence of Cold Acclimation and Antifreeze Proteins on Ice Nucleation. - When plants are cold acclimated, they develop a greater ability to supercool. This has been demonstrated in canola and barley, and rye plants. When cellular extracts of canola were placed on filter discs, similar responses to those of intact plants were made, indicating that sugars and proteins present in acclimated plants may play a role in enhancing supercooling. Transgenic Arabidopsis plants expressing an insect antifreeze protein also exhibited an enhanced ability to supercool. Interestingly, when acclimated canola plants were allowed to supercool to low temperatures (-12 to -15 C) and then frozen, they exhibited no injury despite the rapid rate of ice formation and propagation. This indicates that the acclimated plants have the ability to rapidly lose water in order to prevent intracellular ice formation. Distinct differences between acclimated and non-acclimated rye plants have also been observed.

Summary - The use of infrared thermography has revealed new details about the freezing process in every plant species in which it has been used. Thus far, it has indicated several new possibilities for enhancing frost protection. Development of thicker cuticles, or providing hydrophobic barriers, may provide a method of blocking extrinsic ice propagation. Selecting for the presence of barriers to ice propagation in woody plants that allow flowers and inflorescences to supercool despite the presence of ice in woody stems may also provide a method for enhacing cold hardiness during spring frosts. Finally, evidence has been obtained supporting the role of antifreeze proteins in enhancing supercooling in plants.

Glycine betaine increases chilling tolerance and reduces chilling-induced lipid peroxidation in herbaceous plants.

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Low temperature stress to crop plants has long plagued agronomists, horticulturists and biological scientists. It lowers crop quality, reduces yield, delays harvest, or causes crop failure. There are two major types of crop species in terms of low temperature sensitivity. They are chilling sensitive species such as *Zea mays,* and chilling insensitive ones such as *Triticum aertivum.* Chilling sensitive species define as those that can be injured or even killed by exposure to temperatures ranging from 15 to 0°C. The impact of chilling on crop production may be as great as freezing, but the event of damage may not be as dramatic as the freeze damage. We report herein some information of the effectiveness of glycine betaine (GB) in improving maize (*Zea mays* L.) chilling tolerance.

When the suspension-cultured cells and the seedlings of cv `Black Mexican Sweet' maize were grown in GB-contained media, their chilling tolerance increased. Both triphenyl tetrazolium chloride (TTC) reduction test and cell re-growth assay indicated that cells treated with 1 mM GB at 26°C for 1 day had a survival rate twice as high as that of the untreated control. Accumulation of GB in the cells was proportionate to the GB concentration in the medium and saturated at a concentration of 240 μmol (g DW)$^{-1}$. The degree of increased tolerance was positively correlated with the level of GB accumulated in the cells. The increased tolerance was time-dependent, *i. e.*, 1st observed 3 h after treatment and reaching a plateau after 14 h. Feeding seedlings with 2.5 mM GB through roots also improved the tolerance as evidenced by the prevention of chlorosis after 3 d chilling at $4^{o}/2^{o}C$ (D/N). Lipid peroxidation, as expressed by the final product of malondialdehyde, was significantly reduced in the GB-treated cells compared with the control during chilling. The increased tolerance may be due, in part, to the reduction of membrane lipid peroxidation in the presence of GB. When maize was chilled, active oxygen species (AOS) accumulate in the cells. AOS accumulation may lead to lipid peroxidation of the plasma lemma and organelles, resulting in chilling injury.

It has been reported that GB increases freezing tolerance in plants. Our finding seems to be the first dealing with the effectiveness of GB application in improving chilling tolerance of plants such as maize. We found that `Black Mexican Sweet' was unable to accumulate detectable amounts of GB. In some plant species, GB is synthesized by a 2-step oxidation choline, catalyzed by choline monooxigenase (CMO) first and then by betaine aldehyde dehydrogenase (BAD). These two enzymes and their corresponding genes have been well characterized. Stresses such as salinity, drought, and low temperature can induce the expression of BAD and/or CMO genes. An attempt to obtain transgenic maize, which expresses the choline oxidase gene from bacteria, is in progress.

Winter hardiness of white clover (*Trifolium repens*)

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In northern Scandinavia and Iceland the use of white clover is limited by the lack of varieties that combine winter hardiness and high biomass production. Physiological characters in relation to winter survival were studied on plants grown in pots and hardened naturally outside (lat.66°N). Samples were collected in September, January and April. For our research we used the population HoKv9238 that is used in breeding programs in northern Norway, and the cultivar AberHerald from Wales, both as an original population (AberHerald original) and a selection surviving one winter in southern Iceland (AberHerald selected). Those three populations were tested for **frost tolerance** and **ice encasement tolerance** in the laboratory using meristematic stolon cuttings. After the treatment the regrowth was estimated visually and the result presented as LT_{50} or LD_{50}. The Norwegian population HoKv9238 was much more winter-hardy than the others. In September AberHerald selected was significantly more frost hardy than the original, but at the same time there was not a significant difference in ice encasement tolerance. Samples were also taken for **fatty acid** and **carbohydrate** measurements. Higher amount of unsaturated fatty acids correlated with increased frost tolerance. HoKv9238 had higher amount of fatty acids per dry matter than AberHerald, especially the18:2 fatty acid. AberHerald selected had significantly higher amount of 18:2 fatty acid than AberHerald original. In September the starch content was significantly higher in stolons of HoKv9238 (55 mg/g dm) than in AberHerald selected (35 mg/g dm) and AberHerald original (25 mg/g dm). In January the starch content of HoKv9238 was drastically decreased and the amount of sucrose and glucose was much higher in HoKv9238 than in AberHerald populations.

Relationship between carbohydrate concentrations and winter hardiness in red raspberry (*Rubus idaeus* L.)

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Frequent winter injury to raspberry (*Rubus idaeus* L.) causes economic losses and is a primary limiting factor for expanding raspberry production in Finland. The objectives of this study were to analyse the relationships between carbohydrates and cold hardiness in raspberry cultivars and to search for specific biochemical markers for cold hardiness. Raspberry cultivars with different levels of cold hardiness were grown either in the field, in containers in a greenhouse, or *in vitro* and cold acclimated under either natural or controlled conditions. Cold hardiness (LT_{50}) and carbohydrate concentrations were determined. Furthermore, the effect of exogenously applied sucrose on cold hardiness was studied.

A decrease in starch and an increase in soluble carbohydrates, especially sucrose, but also accumulation of glucose, fructose, raffinose and stachyose, coincided with cold acclimation. A positive correlation between LT_{50} and starch, and negative correlations between LT_{50} and the amounts of soluble carbohydrates were established. Changes in carbohydrate concentrations due to cold acclimation were also expressed *in vitro*, except for decrease in starch. However, both container-grown and *in vitro* plants acclimated under artificial light and temperature regimes failed to reflect exactly the reaction of the raspberry plants acclimating under field conditions. High concentrations of total soluble carbohydrates and a high ratio of sucrose to glucose plus fructose were characteristic of a hardy cultivar under field conditions and *in vitro*, and low concentrations of starch in container-grown plants.

Exogenously applied sucrose was taken up by plants and amplified the effect of cold acclimation on raspberry plants *in vitro*, 5 % sucrose in the culture medium being optimal for maximum cold hardening. Shoot moisture content was not simply correlated with cold hardiness *in vitro*. Rather, low moisture content allowed a plant to become hardier under appropriate conditions. After cold acclimation, osmolality predicted hardiness better than shoot moisture content. The results demonstrated that sugars have more than a purely osmotic effect in protecting acclimated raspberry plants from cold.

This study provided further evidence of the importance of carbohydrate reserves, especially sucrose, on winter survival of raspberry. Cultural practices that enhance accumulation of carbohydrates in raspberry plants should be developed and tested.

Re-analyzing historical records of winter injury in Finish apple orchards

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In Finland, apple trees are grown on the fringe of their northern growing limits. Commercial production is restricted to the south-west of the country. Even there, however, noticeable winter injury occurs every 10 years, on an average. - The degree and nature of winter injury in Finnish apple orchards has been thoroughly surveyed after nine severe winters since 1928/29. The most common type of damage found in apple trees is killing of annual shoots and a general dieback of branches. Damaged trees are often completely killed during the following season.

It is generally assumed that the main causes of apple winter injury in Finland are exceptionally low temperatures between December and March as well as unfavourable weather conditions during the preceding summer and autumn. In addition, mild spells during early winter have been suggested as a major factor affecting the occurrence and severity of winter injury induced by subsequent low temperatures.

In the current study, the long-term climatic data of 1927 to 1998 were explored in the context of past winter-injury occurences in south-western Finland. The objective was to identify the weather conditions leading to apple winter-injury by applying statistical multivariate techniques. Verification of the most critical weather patterns would provide a sound basis for breeding and selection of cultivars hardy in northern conditions.

The analyses revealed low mid-winter temperatures as the dominant factor controlling winter injury of apple trees under the growing conditions of south-western Finland. However, even unfavourable weather conditions during growing season or during the fall hardening-off process often coincided recorded occurences of winter-injury. The results are compared with those obtained in New Brunswick, Canada.

The effect of climate on freezing injury in strawberries

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The study was conducted in four experiments, one examining the effects of alternating freezing and thawing at –12/+6 °C, the second the effects of permanent long term freezing at –12 °C both under controlled conditions after a growth period at a coastal or at a continental location; and the third and fourth in two continental and two coastal experiments on field grown strawberries on high beds. The freezing injury increased the higher the stress, indicated by reduced growth, fruit yield and fruit size. It was shown that 'Korona' was the most hardy cv at one day of permanent freezing, while 'Senga Sengana' was the most freezing tolerant cv at long term permanent freezing. However, 'Senga Sengana' was least adapted to alternated freezing and thawing. In general the results indicate that the plants induce freezing tolerance according to some factor of origin, since plants grown in the continental climate better survived long term permanent freezing than plants grown in a coastal climate and vice versa. The field experiments showed that use of winter cover reduced freezing injury in plants without snow cover during winter. Cover positively affected development of flower stalks. Also, it reduced injury in flower primordia and thus the number of misshapen fruits, and made the plants produce larger fruits. As a sum these effects increased the yields up to 9% in the continental and 45 % in the coastal fields. The yield differences of the coastal vs the continental experiments were due to more freezing and thawing at the coastal than at the continental fields, and a snow roof that was used to keep the snow off the plants in the continental experiments. Generally, covering with artificial winter covers gave a better result than straw. When straw was used it was essential to remove it in early spring. Using artifical winter covers there was no difference in yield by removal of the cover first week of April or May for the cvs 'Bounty' and 'Senga Sengana', but cv. 'Korona' reacted generally positively of prolonging the covering period. Covering from 1 September to 1 November only, had a negative effect on yield compared with no cover, while covering in April yielded equal to no covering.

Physiological and biochemical responses of overwintering forage crops to anoxia

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The formation of an impermeable ice sheet over perennial plants during winter induces modifications of the atmosphere around the plants. The resulting anaerobic conditions can affect the winter survival of economically important forage crops. The differential sensitivity of alfalfa, timothy, orchardgrass and red clover exposed to a progressively developing anoxic stress was assessed in the field and under simulated winter conditions. The study took place in eastern Canada, near Quebec city (46°34' N, 71°13' W).

In the field experiments, an impermeable ice cover was simulated by covering plots ($3m^2$) of alfalfa, red clover, orchardgrass and timothy with gas-tight plastic sheets at the end of November, just before the onset of a permanent snow cover. The control plots were left uncovered. Gas sampling systems and thermocouples were installed in each plot. The concentrations of O_2 and CO_2 at the soil-atmosphere interface were measured on six occasions during the winter while soil and air temperatures were monitored hourly. Throughout winter, the O_2 concentration decreased progressively from ~21 to 2-5 % whereas CO_2 increased from ~0.03 to 4-8 % under impermeable covers. For all species, forage yield measured twice during the following summer was unaffected by the hypoxic conditions reached at the end of winter. There are indications that changes in the atmospheric composition under gas-tight covers may vary with the soil organic matter content. For that reason, a field experiment was undertaken in which the soil C content of alfalfa plots was raised by applying an organic amendment (54t/ha, pig slurry and chopped alfalfa) one week prior to installing the impermeable covers. The amendment increased the rate of CO_2 accumulation as compared to the control alfalfa plots without C amendment.

In a separate experiment, the four species were exposed to a progressively developing anoxic stress by enclosing potted plants in gas-tight plastic bags in late fall and exposing them to simulated winter conditions in an unheated greenhouse. Furthermore, four pots filled only with soil were used to assess the contribution of the microflora to atmospheric changes. Gas composition in bags, regrowth potential and biochemical components related to cold acclimation and anoxic metabolism were measured at different intervals after the initiation of treatments. Anoxic conditions (0% O_2) were reached after 60 d of enclosure for orchardgrass, alfalfa and red clover, 80 d for timothy and 106 d for bare soil. A large proportion of the O_2 consumption was attributable to the soil microflora activity. Based on plant regrowth, the sensitivity to anoxic conditions of the species was: red clover and orchardgrass > alfalfa > timothy. Concentrations of cryoprotective sugars were modified by anoxia with responses differing among species. Sucrose concentration declined in anoxia-stressed orchardgrass, alfalfa and red clover but increased in timothy. Ethanol, an end product of anaerobic metabolism, increased under anoxia and reached the highest level in the very anoxia-sensitive red clover whereas its accumulation was the lowest in timothy. Expression of the alcohol dehydrogenase gene was also markedly lower in timothy than in the other three species.

We conclude that the presence of a lasting impermeable cover in winter may affect winter survival and yield of major forage crops if extreme concentrations of 0% for O_2 and 15% for CO_2 are reached. The development of anoxic conditions under impermeable covers such as ice depends on many factors including soil C content, microflora activity as well as plant metabolism.

Towards an understanding of the nature of snow mould resistance.

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Winter wheat infrequently survives winter stresses in the deep snow regions of the central and northern Canadian prairies due to the extensive damage caused by activity of several different species snow mould damage. During the autumn and early winter, the gradual decrease in average ambient temperatures induces a hardening process in plants, which is essential for the development of snow mould resistance. Resistance to snow moulds is also clearly linked to developmental stage in winter cereals, legumes, and grasses; early seeded plants are more resistant to snow moulds than later seeded plants. Hardening temperatures induce the accumulation of soluble carbohydrates in plant tissues during the autumn and early winter which is an important mechanism by which plants develop snow mould resistance and freezing tolerance. A positive association between the total amount of carbohydrates, especially fructans, remaining in wheat crowns in the early spring and the level of snow mould resistance among cultivars has been demonstrated. However, the nature of snow mould resistance is still unknown, as is the role of simple and complex carbohydrates in the expression of snow mould resistance. Resistant winter wheat cultivars possess higher levels, and more highly polymerized fructans than susceptible cultivars. Furthermore, early seeding promotes the development of high levels of fructan accumulation. We have also demonstrated that the Pathogenesis Related (PR) -protein transcripts for chitinase, -1,3-glucanase, peroxidase, gamma-thionin, and a lipid transfer protein increase in winter wheat plants during the autumn in the field, and in response to low temperature hardening conditions under controlled environment conditions; thus, PR-proteins may be involved in a generalized resistance response to snow moulds in winter wheat. Snow mould infection has been reported to increase the expression of PR-proteins in winter wheat. Snow mould fungi also appear to be less capable of utilizing highly polymerized carbohydrates for growth compared with simple sugars. Our results, and those published in the literature, suggest that snow mould resistance that accumulates in plants during the autumn and early winter may be attributed to a number of factors: 1) the form and quantity of carbohydrates may directly affect fungal development within the plant; and, 2) low temperature hardening conditions and snow mould infection induce the expression of PR-proteins; and, 3) an increase in osmotic potential during hardening.

Studies of *Sclerotinia trifoliorum* Erikss. and red clover cultivar interactions in Sweden.

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Sclerotinia crown and stem rot (SCSR) caused by the fungus *Sclerotinia trifoliorum* Erikss. is known to attack red clover (*Trifolium pratense* L.) during winter dormancy. The symptoms are often mistaken for physical winter damages. Therefore, the role of *S. trifoliorum* in over-winter plant death is not completely known and its actual prevalence is uncertain. Despite long time of selection for resistance in red clover, major outbreaks of SCSR still occur.

Over 60 years ago Björling (1939) showed that local cultivars of red clover had adapted to local isolates of *S. trifoliorum* leading to less severe damages than did isolates from other areas. We wanted to test the cultivar – isolate interactions of today in a greenhouse experiment as a complement to ongoing studies of practical field resistance against SCSR. Twenty different cultivars of red clover, adapted to different regions of Sweden and diploid as well as tetraploid, were inoculated with two isolates of *S. trifoliorum* originating from clover fields in SW and NE Sweden, respectively. When grown on agar the two isolates look somewhat different and they show mycelial incompatibility.

Seedlings of red clover were grown for two months in commercial potting mixture in 40 x 60 x 10 or 28 x 50 x 10cm boxes, 128 or 98 plants in each. After a hardening period, the plants were inoculated with dry ground sclerotia of *S. trifoliorum* and incubated for two months in the dark at low temperatures to imitate winter conditions. After one month of recovering in the greenhouse the number of surviving plants was recorded. There were five equally handled control boxes with a random set of non-inoculated cultivars. The experiment was done in six replicates over time, each containing 40 boxes i. e. one box of each red clover cultivar inoculated with one isolate of *S. trifoliorum* per replicate.

In this study, cultivars bred for northern Sweden were generally better to resist and survive from a mycelial infection of *S. trifoliorum*, 23 to33% mean surviving plants, compared with southern cvs, 0 – 19% surviving plants. Controls had 100% surviving plants, demonstrating that the pathogen was the cause of plant death. There was no significant cv. x isolate interaction and hence only small differences in the ranking order of red clover cvs between the two isolates. Of these, the one originating from NE Sweden. caused more plant death than the other used in this study. The results show that breeding region rather than diploid or tetraploid state was important for surviving rate.

In future greenhouse experiments two red clover cultivars will be tested against twenty isolates of *S. trifoliorum* to further study the cultivar – isolate interactions.

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Screening winter wheat for resistance to speckled snow mold under controlled environment conditions.

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Speckled snow mold (Gray snow mold), caused by *Typhula* spp., is the most important snow mold disease on winter wheat in north central Washington State. Early seeding of resistant cultivars is the primary control method for speckled snow mold in this region. Snow mold development in the field is affected by weather conditions and, on average, severe disease occurs in about half of the growing seasons. As a consequence, progress in screening for snow mold resistance in field plots is slow. A more effective and reliable method to screen for snow mold resistance is desirable to hasten development of resistant cultivars. This study was conducted to develop a consistent method of screening winter wheat for resistance to speckled snow mold under controlled environment conditions.

The specific objectives were to determine the effects of duration of pre-hardening growth, cold-hardening temperature and duration, inoculum density, inoculation time, and soil type on the expression of resistance to speckled snow mold under simulated snow cover. 'Daws' (susceptible) and 'Sprague' (resistant) winter wheats were grown at 20°C day/15°C night for 2, 4 or 6 weeks, cold-hardened at 2° or 4°C for 1, 2 or 4 weeks, inoculated with 0.2, 0.4, 0.6 or 0.8 ml of sclerotia of *Typhula ishikariensis* 4, 2 or 0 weeks before simulated snow cover was applied, and then incubated at 0°C in the dark for up to 105 days.

Mycelium colonized all above ground tissues and abundant sclerotia were formed. The number of tillers per plant was a more useful indicator of recovery after snow mold than number of recovered plants, percentage of plants, or dry weight of recovered plants. Duration of pre-hardening growth, cold-hardening temperature, and duration of cold-hardening all had significant effects on recovery of wheat following snow mold. The greatest differences in recovery between the resistant and susceptible cultivars occurred when plants were pre-hardened for 2 weeks, cold-hardened at 2°C for 2 to 4 weeks, inoculated 2 weeks before applying the artificial snow cover, and then incubated at 0°C in the dark for 75 days or more. No differences in recovery were found among the inoculum densities tested.

Verification of these conditions using a larger set of cultivars and breeding lines is still needed. However, development of a controlled environment method of screening winter wheat for resistance to speckled snow mold will accelerate the development of new, snow mold-resistant cultivars and allow genetic studies to be conducted under uniform environmental conditions.

Field resistance of winter wheat to *Typhula ishikariensis* and *Microdochium nivale*

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During 1997/1998, 6 wheat varieties varying resistance to snow molds were inoculated with 6 isolates of *T. ishikariensis* biotype A or B by sprinkling sclerotia in autumn. In ANOVA for disease severity caused by *T. ishikariensis*, variance due to isolate, variety, interaction between isolate and variety, and interaction between biotype and variety were significant. However, interactions between isolate and variety within each biotype were not significant. The significant interaction between isolate and variety was considered to be due to higher pathogenicity of biotype B. During 1998/1999, 7 winter wheat varieties were inoculated with 10 isolates of *T. ishikariensis* biotype A or B by sprinkling dried inoculum infested with mycelium. The increase of disease severity of each wheat variety with the increase of pathogenicity of inoculum was varied among snow mold resistant varieties. The increase was high in Niederndorferberg and Haunsberg, followed by PI 173438, and the increase was low in Münstertaler. This may suggest that snow mold resistance in Münstertaler is higher than that in other snow mold resistant varieties.

During 1996/1997, 1997/1998, and 1998/1999, seeds of 4 winter wheat varieties were inoculated with conidia of *M. nivale* and grown at Sapporo and Memuro. At Sapporo, in ANOVA for disease severity, variance due to variety was significant, and interaction between inoculation and variety was not significant in each ear. In 1998/1999 trial, disease severity caused by *T. ishikariensis* and *M. nivale* was compared among 31 varieties. The disease severities was positively correlated ($r=0.90$, $P<0.001$). At Memuro, where is located in colder region with minimum snow fall in Hokkaido, variance due to variety was significant, and interaction between inoculation and variety was also significant. In 1996/1997 and 1998/1999, wheat plants inoculated with *M. nivale* suffered from pink snow mold and also sclerotinia snow mold, whereas wheats without inoculation suffered only sclerotinia snow mold. In 1997/1998, the coldest year during three years, all plots suffered from severe sclerotinia snow mold damage even in plots inoculated with *M. nivale*.

Climate change and survival of perennial herbaceous crops in Eastern Canada

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Meteorological models predict an increase in average winter temperature of 2 to 6°C by 2050 in Eastern Canada. This warming is likely to affect the fall and winter factors responsible for winter survival of perennial crops. We identified causes of winter damage to perennial herbaceous crops in Eastern Canada during fall and winter. We then assessed the impact of climate change on the risk of winter injury by calculating specific agro-climatic indices related to each cause of damage. Fall indices describe the temperature and precipitation conditions leading to the acquisition of cold hardiness. Winter indices integrate the impact of cold intensity and duration with the protective action of a snow cover, assess the loss of cold hardiness due to warm temperatures, and estimate potential damages to the root system by soil heaving and ice encasement.

Twenty climate stations were selected within agricultural regions of Eastern Canada (latitude: 45°8' N to 49°24'N, and longitude: 65°41'W to 82°26'W). Predicted temperatures and precipitations for the periods 2010-2039 and 2040-2069 were obtained for each station by using climate change data from the First Generation Coupled General Circulation Model to adjust daily weather data from the 1961-1990 period. Estimated mean indices of the two future periods were compared to values under the current climate conditions.

Preliminary results indicate that the length of the cold period, that is the period of exposure to extreme sub-freezing temperatures equal or below -15°C, will decrease from the current length of 96-148 to 70-143 days. The number of days with snow cover, that is a layer of snow of at least 0.1 m on the ground surface, will decrease from 81-148 to 35-123 days. The current annual minimum temperature, ranging from-43 to -27°C, will increase in the future to temperatures of -37 to -22°C. Even though winters will be warmer in the future, the risk of winter injury to perennial crops is likely to increase. This increased risk will result from sub-optimal cold acclimatation conditions during fall, but mostly from a predicted lack of a sufficient protective snow cover during the cold period. This study will allow us to identify and recommend agricultural practices which would minimize the negative impact of climate change on agricultural production in Eastern Canada.

Frost resistance of sclerotia of *Typhula ishikariensis*

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Snow mold fungi are psychrophilic fungal pathogens that infect winter cereals grown countries in the Northern hemisphere. Among theses snow molds, *Typhula ishikariensis* has evolved several infraspecific taxa adapted to different winter climates. We reported that mycelial frost resistance of *T. ishikariensis* in Norway was one of the important factors for geological distribution pattern[1]. Recently, we found *T. ishikariensis* from central Siberia (Novosibirsk region) and Svalbard (high Arctic in Norway: 79 N, 12 E). The isolates from Siberia and Svalbard isolates showed similar genetic background as group III isolates[2] (Finnmark in Northern Norway), according to a mating experiment with tester monocaryons.

Effects of culture temperature on frost resistance of mycelia and sclerotia were determined in Norwegian and Russian isolates. The group III isolates and Svalbard isolate showed irregular growth, caused by inhibition of hyphal extensions at 10 C. However, the Siberian isolates had the same optimum growth temperature (10 C) as group I and Moscow isolates.

When mycelia of were frozen rapidly, the regrowth of group I strains were delayed at their optimal growth temperature (10 C) and the isolates from Moscow were destroyed in this freezing stress. The regrowth of group III and Siberian isolates were not affected when grown their optimal temperature (5 or 10 C). Rapid freezing stress destroyed sclerotia of the isolates from group I and Moscow. However, sclerotia of isolates from group III and Siberia survived this freezing stress.

These results suggested that group III and Siberian isolates are more resistant to rapid freezing than the isolates from group I and Moscow. Group III and Siberian isolates are well adapted to climatic conditions in the northernmost area of Norway and central Siberia. These result indicate that the ecological location of group III under the natural climate may be explained by freezing resistance and environmental adaptation.

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Heterothallic *Pythium* sp. from dying moss on Svalbard

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In the course of studies on moss pathogenic fungi in the Arctic Zone, heterothallic *Pythium* sp. was isolated. In the present study we describe morphology and growth temperature of the *Pythium* sp.

Roots of dying moss (*Sanionia uncinata*) were collected from Barentsburg, Svalbard (78 41'N, 14 14'E), in Jun. 18 and Aug. 3, 1999. The roots were immediately placed on VP_3 medium (Ali-Shtayeh *et al*. 1986) and incubated at temperature between 6 and 18 C. Mycelia growing on the medium were transferred to water agar and single hyphal isolates were maintained on corn meal agar plates at 15 C. The isolates were transferred to the grass leaf culture (Martin, 1992) and identified according to the keys of van der Plaats-Niterink (1981).

Two fungal isolates (OPU 445 and 446) showing rapid growth on VP_3 medium were obtained from the dying moss. Oogonia were rarely developed in single cultures but formed abundantly in dual cultures of the two isolates on potato-carrot agar (PCA). This species therefore behaved as a heterothallic species. Morphology and growth temperature of the isolates resemble those of *Pythium macrosporum* Vaartaja & Van der Plaats-Niterink. Compatibility of the present isolates to the type strains of *P. macrosporum* was not determined because the latter lost their compatibility during over 20 years preservation. Molecular tools such as the sequence of the rDNA ITS regions should be used for species identification of the isolates.

Pythium spp. has previously been isolated from the Arctic Zone (Gaertner, 1954; Höhnk, 1960; Kobayashi *et al.*, 1967; Booth and Barrett, 1976; Hoshino *et al.*, 1999) but the occurrence of the heterothallic species has not been reported. From the literature consulted, this is the first report of isolation of heterothallic *Pythium* species in the Arctic Zone.

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Winter resistance scale of wheat varieties of different geographical origin

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Sufficient winter resistance of winter wheat for the conditions of a particular country is necessary for its successful overwintering there. These conditions, of course, vary in different locations of Europe and the whole world. Since the seventies we have tested winter resistance of 1200 wheat varieties from different countries by a provocation method in our institute (Prášil and Rogalewicz 1989). The varieties were sown and grown in wooden boxes placed both on the surface and 50 cm above the ground, which enables better differentiation of their winter resistance under varying winter conditions. Frost is the main factor, which influences winter survival of plants in this method. Obtained winter survival data were processed after inverse logistic transformation by weighted two-way analysis of variance for unbalanced series of trials (here called classification method, Palovský et al. 1992). Winter survival index, representing a variety's survival under average conditions, was calculated as a percentage for each variety, regardless of the year when it was tested. The varieties were sorted according to the WSI values into nine degrees of the winter resistance scale (1 = the most sensitive to 9 = the most resistant).

We obtained the distributions of winter resistance degrees and the mean winter resistance degree of the varieties of a respective country. For example, the mean winter resistance of wheat was 2.6 for the varieties from France, 3.2 from the United Kingdom, 4.8 from the Netherlands, 5.0 from Germany, 5.7 from the Czech Republic, 5.9 from Sweden, 6.8 from Russia and Ukraine.

Further, we investigated how the winter resistance changed in 147 Czech and Slovak wheat varieties registered in different years of this century. We found out that the winter resistance distribution and the mean winter resistance were quite similar in these varieties, regardless of the decades of their registration from 1920 to 1999. From those results we infer that the obtained mean winter resistance degree of the varieties of a respective country can represent the optimum winter (frost) resistance degree for that country.

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Adaptation of alfalfa for growing on light acid soil of South-Estonia

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Alfalfa *(Medicago sp.)* as a forage crop was introduced in Estonia more than 200 years ago. Biological nitrogen fixation, excellent quality, good yielding ability and longevity made it one of the most popular forage legumes among local farmers. Today the growing of alfalfa is mainly restricted to calcareous well-drained soil type in North- and Central-Estonia. Since the last decades it has slowly been spreading to the areas with light acid Podzoluvisol soils (pH_{KCl} = 5.8-6.5) in South-Estonia. Besides acid soils, unstable weather conditions during wintering, especially in the second half of winter are typical to this region. These additional physical stress factors are connecting the growing of alfalfa strongly with harvesting schedule and using variety characteristics. Therefore two field experiments, supported by Estonian Science Foundation (grant No. 3688), were carried out during 1979 –1999 in Experimental Station of Department of Grassland Science and Botany, EAU:

Experiment 1 was conducted for the examination of dinitrogen fixation ability of the local Jõgeva 118 alfalfa by the difference method based on the crude protein yield. For inoculation of seeds *Rhizobium meliloti* stam No. 302 worked out by Laboratory of Microbiology of Estonian Research Institute of Agriculture has been used.

Experiment 2 was established to clarify persistence and yielding ability of alfalfa depending on cutting intensity (2-4 cuts per season), the date of last cut (28 August - 02 October) and variety characteristics (very winter hardy local Karlu and winter hardy Swedish Pondus were compared).

As a result of the investigation we found that even in such difficult soil conditions by using 3-cut harvesting schedule Jõgeva 118 alfalfa could fix dinitrogen up to 350-400 kg ha^{-1} yr^{-1}. The factors unfavorable for plant growth and development like aging of stand, lack of warmth in summer time, winter damages, etc. reduce N_2 fixation considerably and usually the numbers have stayed between 100-200 kg ha^{-1} yr^{-1}.

The highest yielding ability (in the second productive year 9.84-11.85 Mg ha^{-1} DM) and persistence of alfalfa growing on light acid Podzoluvisol soil were obtained in using 2-cut harvesting schedule. However, in aspect of forage quality 3-cut regime would be preferable. In that case the total DM yield decreased on an average 16% and the date of the last cut, and variety characteristics became more important. The “critical autumn period” was clearly observed which limited with the end of August and the first ten days period of September (growing period prior the last cut 25-40 days). Different varieties reacted for the date of the last cut differently. Less sensitive for the factor was very winter hardy local-bred cultivar Karlu with the absolute yield depression 1.30 Mg ha^{-1}. Compared with Pondus alfalfa Karlu stopped intensive growth and started to prepare for winter earlier (already from the middle of August). The significant difference ($P<0.05$) was also observed in the fall growing ability of tillers (6.7 cm Karlu compared with 9.6 cm Pondus alfalfa; $LSD_{05} = 1.1$) if the last cut was made during the “critical period”. Although Swedish variety seems more sensitive for mistakes in determining the cutting schedule, its advantage was good recovery ability after harvest and high annual productivity. Cutting alfalfa more intensively (4 cuts per season) a sharp deterioration in alfalfa persistence and DM yield took place.

Problems of winter wheat cold tolerance in Lithuania and their solution in breeding programmes

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Lithuania is situated in the Southeastern part of the Baltic, between 54^0 and 56^0 North. The annual mean temperature of the country is $6{,}2^0$ C. January is the coldest month and July the warmest. Mean temperatures are -5,1 and +16,7 respectively.

The weather conditions during the winter- spring season are very changeable. The air temperature in January and February over the period 1990- 1999 fluctuated between +8,7 and -26,8^0 C. The most severe problem for over winter survival of winter wheat is snow layer in winter time. Quite often short periods of warm weather are changed by cold and the temperature -15…16^0 C without snow is dangerous for winter wheat plants.

Winter wheat breeding in Lithuania was initiated in 1922. The first varieties 'Akuotuotieji ', ' Dotnuva 458 ' were developed on the basis of landraces. Later the initial material for wheat breeding in Lithuania were varieties from Russia and the Ukraine. Those varieties have in general a good winter hardiness. The problems of those varieties were insufficient disease- resistance and love grain yield. For the solution of these problems in winter wheat breeding programmes we included germplasm from West Europe and other regions. Winterhardiness test became very important.

More than 3000 winter wheat varieties from West and South Europe, CIMMYT's Winter wheat Improvement Programme u.a. were investigated in the natural and artificial conditions at the Lithuanian Institute of Agriculture in period 1990- 1999.

The results shoved that 20- 30 % of West European and 50- 60% of varieties and lines from CIMMYT's programmes demonstrated the same resistance to Lithuanian winter conditions as local varieties. The most suitable varieties for hybridization were 'Yuna', 'Dakha', 'Yuogtina', 'Nadia', 'Ukrainka odesskaja', 'Flair ', 'Zentos ','Kosack'.

The influence of *Fusarium* infection and low temperatures on the activity of soybean esterase and PR-proteins

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The mechanism of soybean resistance to Fusarium oxysporum and low temperatures (LT) and the cause of varietal differences in resistance are not known.

Since enzymes are principal regulatory components of the cell, the changes in the enzymic system induced by stress factors seem to play an important part in the adaptation processes occurring in plants under stress conditions.

PR-2 proteins (1,3-β-glucanase) have been shown to catalyse the hydrolysis of 1,3- β-glycan polymers. This indicates the possibility that PR-2 proteins play a role in plant defence, targeted against fungal pathogens with 1,3-β-glycan containing cell walls.

We investigated the expression of sevral resistance related genes in resistant (R) and sensitive (S) cultivars of soybean during infections with Fusarium oxysporum (F.o.) at optimal (24°C) and LT (8°C). Rootlets infected with F.o. culture medium and untreated rootlets (check) were studied for each cultivar. Esterase and PR-2 proteins were extracted from rootlets. Polyacrilamide gel electrophoresis for analysis, esterase spectrum was performed in vertical plates on two layers.

We detected that F.o. induced or enhanced the expression of esterase and PR-2 proteins in both R and S plants when incubated at LT, but the response was stronger and more rapid in R plants. A specific reaction of the esterase at the level of the cultivar resistance, to the thermostresogene factor and biotic stress was revealed.

The reaction of the isoenzymes with Rf=0.35-0.40, 0.50-0.60, 0.80-0.90 was more specific for the action of F.o. under LT conditions. Hence, these isoforms might serve as a molecular-biochemical marker in revealing the soybean types resistant to F.o. and LT.

Quantitative analysis of the activity of 1,3-β-glucanase (G) showed the major expression of the G under the effects of F.o. and LT destinquishing the R cultivars are most pronounced. The fact that the R cultivars have more evident G expression than the S ones shows that these genes play a very important role in developing of the resistant trait to F.o. in cultivars. Contributions of LT to the most evident G expression explains the complex mechanism of soybean plants resistance to F.o. and LT wich was established by us on the resistance testing backround.

Consequently LT induce resistance of cultivars to F.o. which has the ability of maximal plant infection under LT (8°C). It is quite possible that a certain linkage exist between genes that requlate resistance of cultivars to F.o. and LT.

Snow mold of herbaceous dicots and non-gramineous monocots caused by *Sclerotinia nivalis* in Russia.

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In the spring of 1998 and 1999, *Sclerotinia* snow mold was collected from weeds and cultivated plants which had decayed under the snow in St Petersburg, Moscow and Novosibirsk. The plants consisted of 5 species of Compositae, 3 of Cruciferae, one each of Campanulaceae, Legminosae, Caryophyllaceae, Iridaceae (German iris), Liliaceae (tulip), and several unidentified species of Crassulaceae. The nineteen *Sclerotinia* isolates obtained from the sclerotia on different hosts equally produced intermediate-sized sclerotia, smaller than those of *S. sclerotiorum* and larger than *S. minor*, in PDA at 20 °C. In these isolates, S1 from *Arabis alpina* L.(Moscow), S7 from *Iris germanica* L. (Moscow), and N2 from *Chrysanthemum cinerariaefolium* Bocc. (Novosibirsk) produced apothecia (teleomorph) on the sclerotia which were obtained from artificial cultures on autoclaved carrot root. The measurements of the asci and ascospores are approximately in the range of those of *Sclerotinia nivalis* I. Saito; i. e. 114-144 × 6.7-9.2 µm for asci and 9.2-11.7 × 3.8-5.0 µm for ascospores in the type specimen. Based on this data, and in addition to the number of nuclei in the ascospore (two) and sclerotial size, we identified isolates S1, S7 and N2 as *S. nivalis*. Furthermore, data from SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins in sclerotia confirmed our identification; i.e. three major protein bands which are characteristically seen in sclerotial extracts of *S. nivalis* were also detected for S1, S7 and N2. Based on these species-specific patterns of protein bands, we identified another 16 isolates (sclerotial anamorph) as *S. nivalis*. In addition, in SDS-PAGE of a fraction of sclerotial proteins that were obtained by a 50-70% saturation of (NH4)2SO4, a different band pattern for each isolate could be detected and the isolates could then be separated into two groups.

When different isolates of *S. nivalis* were co-inoculated in a PDA plate, compatible or incompatible responses were observed. The incompatible response appeared as light to dark brown zones in the contacting front of mycelia or as clear zones without significant mycelial growth separating one colony from the other. This is noteworthy since such intensive incompatibility often occurs between different taxa in *Sclerotinia*. Significant "intraspecific" variations are likely to be involved in *S. nivalis*. The host range of this fungus is very wide, while non-gramineous monocots hosts have never been found in Japan. Of these hosts, cultivated tulips are of particular interest, as a *Sclerotinia* rot of tulips in winter was once attributed to the infection of *S. sativa* Drayton et Groves (Drayton & Groves, 1943) in North America or to *S. bulborum* (Wakk.) Rehm in Russia (Procenko, 1968). *S. sativa* was later synonymized with *S. minor* by Kohn (1979), though this raises the question whether *S. minor* could be pathogenic during winter. Detailed systematic studies on *S. bulborum* have never been published since Wakker's description in Holand in 1889. The evidence of infection on tulip bulbs by *S. nivalis* suggests that it would be *S. bulborum*, considering the season of disease incidence and the symptoms. However, the only taxonomic features described for *S. bulborum* are measurements of asci and ascospores which are not sufficient for accurate, up to date identification. Further investigation is needed to reveal whether *S. nivalis* ranges not only in Europe but also in North America where *Sclerotinia* rot of spring-flowering bulbs, including tulips, used to be prevalent.

Low temperature- and winter environment-induced antifungal compounds in wheat: 1. Hydroxycinnamic acid amides

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In northern regions, low temperature and snow mold infection during winter can severely limit yields and quality of biennial or perennial crops. Therefore, elucidation of the mechanisms underlying plant resistance to biotic and abiotic stresses occurring at low temperatures would contribute significantly to the stabilizing the relationship between supply and demand of food in northern regions. Wintering crops such as wheat, barley and forage grasses acquire their freezing tolerance and snow mold resistance during cold acclimation. It has been reported that pathogenesis-related proteins are induced in freezing-tolerant crops by a low temperature. These indicate that wintering plants possess a cross-adaptation ability for a winter environment. The objective of this study was to isolate cold- and winter environment - induced secondary metabolites and to examine their relation to snow mold resistance.

A low temperature-induced compound was isolated from the crowns of winter wheat (*Triticum aestivum* L. cv Chihokukomugi) grown under artificial conditions. Its structure was identified as feruloylagmatine [1-(*trans*-4'-hydroxy-3'-methoxycinnamoylamino)-4-guanidinobutane] by NMR, MS and UV spectral analyses. It was also revealed that *p*-coumaroylagmatine [1-(*trans*-4'-hydroxycinnamoylamino)-4-guanidinobutane], which has a similar chemical structure to that of feruloylagmatine, accumulated in winter wheat at the end of a long-lasting snow cover. Both feruloylagmatine and *p*-coumaroylagmatine showed antifungal activity against snow mold, *Microdochium nivale*.

Lignification of Scots pine from the Arctic Circle up to northern timberline

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Lignification is a physiological process influenced by genetic and environmental factors. The variations in the lignin content are therefore a proper parameter indicating changes or differences in the living conditions of trees caused by external factors.

Scots pine trees of a five-stand transect from the Arctic Circle to northern timberline in northern Finland were investigated tree-ring by tree-ring for lignin content. Diffuse reflectance infrared Fourier transform-spectroscopy (DRIFT) was used. For the calculation of the lignin content the ratio of the aromatic skeletal vibration around 1510 cm^{-1} and the band around 896 cm^{-1}, which derives from the C1-H vibration in cellulose and hemicellulose, was used after baseline correction.

The measurements brought up three major tendences:

1. The lignin variation pattern through the stem cross section follow the same pattern in all trees, indicating "special" years or events influencing the lignification process
2. The variations are much more significant in the northern trees than in the southern ones. This indicates that the more northern the tree's location, the more intense is the stress response and the more lignin is produced.
3. The average lignin content of the trees is higher in the northermost trees than in the ones growing at the Arctic Circle.