Low temperature diseases caused by Microdochium nivale.

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Introduction
The fungus Microdochium nivale (Fr.) Samuels & Hallett is the most widespread snow mould fungus. It is distributed over a wide range of temperatures, but is regarded as a serious pathogen mainly in cold to temperate regions of the Northern Hemisphere. Microdochium nivale causes pink snow mould on turf and forage grasses, and winter cereals. At warmer temperatures, it causes stem rot, leaf blotch, and "Fusarium ear blight". This species is highly opportunistic due to its ability to attack plants over a wide range of environmental conditions.

Symptoms and disease cycle
The main hosts of M. nivale are wheat, rye, barley, oats, turf and forage grasses. Immediately after snowmelt, the fungus appears as pinkish-white mycelium in patches. After drying, the dead leaves form a compressed paper-like layer. In Nordic countries, serious injury on fodder grass and winter cereals due to infection by M. nivale occurs following two months or more of snow cover (Årsvoll 1973). Mortality of infected winter-sown cereals (seedling blight) is common, and may be so severe that re-seeding of
a spring crop is required (Jamalainen 1956, 1959). Lawn and golf course grasses may be attacked in autumn and spring during cold, wet weather, and even during cool wet summers. The disease, known as Fusarium patch, occurs as small patches, which first appear water soaked, later turning yellow-orange-brown and sometimes possess a fringe of pinkish white mycelium (Årsvoll and Smith 1979). Lesions with straw-coloured centres and dark margins may be found on leaves (Årsvoll and Smith 1979). In Ireland, the fungus also causes “leaf blotch” on oats (Diamond et al. 1995). It can also cause stem rot and head blight or the so called "Fusarium ear blight" of cereals. The disease cycle on an overwintering host is shown in Figure 1. The fungus is believed to be spread on infected seed, and inoculum (mycelia, conidia and ascospores) from infected plants or debris.

Figure 1: Disease cycle of Microdochium nivale

**Inoculum and soil survival**

The infective propagules of *M. nivale* are mycelia, conidia and ascospores. Pronczuk & Messyasz (1991) reported that inoculation of *Lolium perenne* with conidia did not give rise to any symptoms, whereas mycelial inoculum incited severe disease. Extensive genetic variation within populations from turf grasses, revealed by RAPD and RFLP analyses, suggests that ascospores are the major source of inoculum for snow mould patches on turf grass (Mahuku et al., 1998).

The relative importance of seed- versus soil-borne inoculum of *M. nivale* for initial infection of plants has not been clearly established (Booth and Taylor 1976). In both wheat and oats seeds either infested or infected by *M. nivale*, establishment and
yield were reduced (Cristani, 1992; Humphreys et al. 1995; 1998;). Infection also occurs from infested soil or plant debris. According to Domsch et al. (1980), *M. nivale* has relatively good saprophytic ability and can grow over soil surfaces and through soil, especially at low soil temperatures. It can survive for periods of between 13 and 52 weeks in naturally infected or artificially infected wheat straw, either buried or on the soil surface (Bruehl and Lai 1966, Snyder and Nash 1968).

Koizumi et al (1993) investigated survival of propagules of *M. nivale*. Perithecia that formed on wheat heads on the soil surface in the field remained viable for approximately three months. Conidia buried in soil and held at 5, 15 or 25°C remained viable for 150, 30 or 10 days, respectively. Non-viable conidia were lysed in soil and the lysis was severe at higher temperatures. These results suggested that *M. nivale* survives in soil as hyphae in straw or debris and support the conclusion by Booth & Taylor (1976) that inoculum from debris is the primary source of inoculum. Wind-dispersed ascospores originating from debris are likely the primary source of infection (Parry et al. 1995).

In Japan, the severity of pink snow mould disease varies among major soil types. Disease severity ranking in artificial inoculation tests was as follows: neutral volcanic ash soil (pH 5.8) > acidic volcanic soil (pH 5.2) > granite diluvial soil (pH 5.7) = alluvial soil (pH 5.0) (Nakajima and Naito 1995). Just prior to snow melt in the field, microflora in these soils were investigated. Antagonistic fluorescent pseudomonads were frequently isolated, but there were no clear differences in bacterial flora among soil types (Nakajima and Naito, 1995). The physical environment such as soil water potential and temperature may affect disease development because *M. nivale* can neither penetrate nor colonise frozen foliage (Nakajima 1998).

**Taxonomy of the organism.**

The fungus was first described as *Lanosa nivalis* by Fries in 1825 (Jamalainen 1943). The nomenclature has since then changed several times (Table 1). Before 1980, it was commonly called *Fusarium nivale*, and many workers still refer to it by this name. The lack of a conidial foot cell caused it to be transferred out of *Fusarium* into *Gerlachia* (Gams & Müller, 1980) and eventually to *Microdochium* (Samuels & Hallett, 1983). The teleomorph is *Monographella nivalis* (Schaffnit) Müll. (Tronmo 1986, Smith et al., 1989). See Table 1 for full details.

The mycelium varies from sparse to densely flocculated (or felt-like). The
colonies are white to pinkish white. Macroconidia are the only asexual spore, and they are curved, falcate, tapering towards each end, with a pointed apex and a wedge-shaped, rounded base. These conidia have 1 to 3 septa, predominantly 3 septa, and a maximum length of 30 um. The conidia are formed sparsely in aerial mycelium and more abundantly in pale orange, slimy sporodochia. Chlamydospores have not been reported (Booth 1971).

Table 1. Synonyms of Microdochium nivale

<table>
<thead>
<tr>
<th>Anamorphs</th>
<th>Teleomorphs</th>
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<tr>
<td>Lanosa nivalis Fries 1825</td>
<td>Calonectria graminicola (Berkeley &amp; Broome) sensu</td>
</tr>
<tr>
<td>Fusarium nivale (Fries) Sorauer 1901</td>
<td>Wollen-weber 1913 (non Nectria graminicola Berkeley &amp; Broome 1858) Sphaerulina divergens Rehm 1913</td>
</tr>
<tr>
<td>Fusarium nivale Cesati ex Berlese &amp; Voglino 1886</td>
<td>Monographella divergens (Rehm) Petrak 1924</td>
</tr>
<tr>
<td>Fusarium hibernans Lindau 1909</td>
<td>Calonectria nivalis Schaffnit 1913</td>
</tr>
<tr>
<td>Gerlachia nivalis (Cesati ex Sacc) Gams &amp; Müller 1980</td>
<td>Griphosperia nivalis (Schaffnit) Müller &amp; von Arx 1955</td>
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<tr>
<td>Microdochium nivale (Fries) Samuels &amp; Hallet 1983</td>
<td>Micronectriella nivalis (Schaffnit) Booth 1971</td>
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<td></td>
<td>Monographella nivalis (Schaffnit) Müller 1977</td>
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Perithecia have often been found in leaf sheaths near the base of the stem below the epidermis, and they appear as numerous small black dots. The perithecia are immersed in the leaf sheath, with a hyaline papilla protruding through the epidermis. The asci are clavate (or cylindrical) with 6-8 hyaline, fusiform and 1(-3) septate ascospores (Årsvoll 1975, Årsvoll and Smith 1979, Gerlach and Nirenberg 1982). The teleomorph (perithecial stage) is commonly observed on cereals, but has not yet been found on turf grasses (Smith et al. 1989).

Microdochium nivale has been divided into two varieties based on conidial morphology (Wollenweber, 1931; Gams & Müller, 1980). Var. majus differs from var. nivale in having larger and predominantly 3-septate cells; var. nivale has 1-3 septate cells (Gerlach & Nirenberg, 1982). The varieties may also exhibit some host specificity (Maurin et al., 1995) and even specialization to tissue types (Lees et al., 1995). Lees et al. (1995) found a continuum of values for conidial length and number of septa among isolates of the two varieties, but maintained that conidial width could distinguish the narrower var. nivale from var. majus. However, this distinction is not universally accepted and some researchers have questioned the validity of these morphological characteristics for taxonomic purposes (Nelson et al., 1983; Litschko & Burpee, 1987).

Molecular studies using restriction digests of the ITS (internal transcribed spacer)
region of the ribosomal DNA (Parry et al. 1995) and RAPD analysis (Lees et al. 1995) have separated isolates of *M. nivale* from wheat into two sub-groups which correspond broadly to the morphologically-defined varieties. There was very little genetic variation within one RAPD sub-group which was classified as var. *majus*. This low level of variation suggests that var. *majus* is homothallic. This is consistent with other studies that have demonstrated that single isolates of var. *majus* can produce perithecia *in vitro* (Lees et al., 1995, Parry et al., 1995). Var. *nivale* can also reproduce homothally (Lees et al., 1995), but the level of perithecial production *in vitro* is lower than that of var. *majus* (Parry et al., 1995). Lees et al. (1995) suggested that heterothallic reproduction by var. *nivale* may be common in nature. Smith (1983) and Mahuku et al. (1998) found only var. *nivale* from different turf grasses. RAPD and RFLP analyses of turf grass isolates revealed extensive genetic variation within populations. This supported the hypothesis that ascospores are the major source of inoculum for snow mould patches on turf grass (Mahuku et al., 1998).

**Effects of temperature on Microdochium nivale.**
The existence of distinct strains of *M. nivale* that differ in their optimum temperature on artificial media was reported more than 30 years ago (Booth 1971). Strains of *M. nivale* were able to grow from -6 to 28°C, with optimum growth between 18 to 21°C (Årsvoll 1975). An asporogenic strain of *Monographella nivalis*, grown in Czapek-Dox medium supplemented with vitamins and sucrose as energy sources, showed optimum growth rates between 9 and 12°C, while growth at 15°C was significantly depressed (Cairns et al., 1995a). Okuyama et al. (1998) reported that the optimum temperature for growth and cell yield was 15°C. The differences reported in the different studies are probably due to variation between the strains investigated. However, the optimum temperature for *in vitro* growth for the pink snow mould fungus is consistently higher than that for other major snow mould fungi such as *Typhula* species.

An asporogenic strain of *M. nivalis* utilised a range of simple and complex carbohydrates such as sucrose, maltose, mannose, glucose, fructose, raffinose, which are components of the tissues of grasses and cereals. This strain also grew well at 5°C, on cellulose, starch, inulin, and oligolacturonic acid. These results showed that this strain degrades these main classes of plant carbohydrate polymers, and demonstrated the activity of cold-active enzymes in polymer degradation (Cairns et al., 1995a).
Effects of temperature on morphology and physiology of *M. nivale*

In general, temperature affects morphology of psychrophilic micro-organisms (Ferroni and Inniss 1973, Takada et al. 1979, Ward 1968). However, no significant morphological changes have been observed in *M. nivale* hyphae grown at temperatures from 4 to 20°C; numerous lipid bodies mainly consisting of triacylglycerol were formed in hyphae at the stationary phase at all growth temperatures (Istokovics et al. 1998).

Istokovics et al. (1998) found that *M. nivale* hyphae grown in potato dextrose broth were very rich in neutral lipid. In the neutral lipid fraction, triacylglycerol was the sole major component. The neutral lipid fraction accounted for approximately 75% of the total lipid in hyphae grown at 15°C, and it increased to 90% in hyphae grown at 4°C (Okuyama et al. 1998). More than 90% of the neutral lipid was triacylglycerol at 4°C. Therefore *M. nivale* appears to preferentially accumulate triacylglycerol as storage lipid.

*M. nivale* responds to low temperatures in two ways with respect to fatty acid composition (Okuyama et al.1998). As temperatures decreased from 25 to 10°C, the level of linolenic acid (18:3) increased at the expense of linoleic (18:2) and oleic acid (18:1). This type of alteration in fatty acid saturation is common in many eukaryotic poikilotherms and prokaryotes and serves to modulate membrane fluidity (Harwood and Russell 1984). There were few notable differences in fatty acid composition between *M. nivale* hyphae grown at 4°C or 10°C. In place of the qualitative alterations in fatty acid, *M. nivale* accumulated fatty acids as triacylglycerol at 4°C, which seemed to bring about a decrease in growth rate and cell yield (Okuyama et al. 1998). Survival of *M. nivale* at low temperatures involves both qualitative and quantitative alterations in fatty acid components; the latter may be very crucial in the survival of this fungus at very low temperatures.

When grown on sucrose as the sole carbon source, the *M. nivalis* hyphae catalysed the extracellular hydrolysis of sucrose, releasing glucose and fructose together with a small amounts of fructan trisaccharides and traces of tetra- and penta-saccharides (Cairns et al. 1995a). Fructan accumulation was transient, corresponding to maximal rates of sucrose hydrolysis and was regarded as a side reaction of extracellular invertase. However, most biomass formation occurred in the absence of fructan in the culture, hence fructan was not considered necessary for growth at low temperature and did not appear to function as a cryoprotectant (Cairns et al. 1995a).

When the stationary-phase *M. nivale* hyphae which included lipid bodies were transferred to fresh medium, lipid bodies transiently disappeared at the early exponential
phase (Istokovics et al. 1998), suggesting that the induction or activation of lipase(s) might be involved in triacylglycerol degradation. Intracellular lipase activity was detected in *M. nivale* hyphae only during the early exponential phase (Ono and Okuyama, unpublished results). The activity - temperature profile of the lipase showed that the enzyme had mesophilic characteristics (Ono and Okuyama, unpublished results).

**Mycotoxin controversy**

*Microdochium nivale* does not produce trichothecene mycotoxins, but initial studies indicated that mycotoxins were produced. Nivalenol was first extracted from cultures of “*F. nivale*” isolate Fn-2B recovered from wheat head blight in Japan (Tatsuno et al. 1968). Fusarenon and fusarenon-X were also detected in the same isolate (Ueno et al. 1973). However, Marasas et al. (1985) re-examined toxigenic strains of *M. nivale* in the International Toxic Fusarium Reference Collection and reported that Fn-2B should be classified as *Fusarium sporotrichioides*. A study of many *M. nivale* isolates found that, except for one (NRRL3289), none of the strains produced trichothecene (Marasas et al. 1985). Furthermore, Logrieco et al. (1991) were unable to confirm deoxynivalenol production in the (NRRL3289) isolate. Nakajima and Naito (1995) surveyed mycotoxin production of *M. nivale* isolates in Japan, and failed to detect mycotoxins in any isolates. *F. sporotrichioides* strain Fn-2B was not found to be pathogenic to winter wheat in field trials. Classification of *M. nivale* isolate Fn-2B has been controversial because of mutations due to periodic transfers over 25 years. Recently, this isolate has been renamed *Fusarium* sp. strain Fn-2B.

**Plant resistance to *M. nivale***

Artificial inoculation and screening for resistance have revealed significant genetic variation in resistance to *M. nivale* in both forage grasses and winter rye (Miedaner et al., 1992; Tronsmo 1984, 1992, 1993). Improvement of resistance by selection should be feasible, since the broad sense heritability of resistance to *M. nivale* was reported to be 0.79 in winter rye and 0.49 in cocksfoot (Miedaner et al. 1992, Tronsmo 1993).

Maximal snow mould resistance only develops in cold-hardened plants. Cold hardening enhances both resistance to freezing and to snow mould injury (Tronsmo 1984, 1994). The ability to cold harden, however, varies among genotypes. There is also genetic variation in the ability to develop “cold-induced” snow mould resistance (Tronsmo 1994). Hömmö (1996) found that snow mould resistance in a detached leaf test
was unaffected or repressed by cold hardening, and proposed the existence of different types of snow mould resistance. There is also evidence for host specialisation and cultivar x isolate interactions in the wheat/M. nivale pathosystem (Diamond and Cooke 1997). Therefore, in the development of cultivars with resistance to M. nivale, it may be necessary to search for both specific genes for resistance to the fungus as well as genes encoding the ability to develop “cold-induced” snow mould resistance.

Future directions
Crop injury due to M. nivale is commonly controlled by fungicides applied to seeds and foliage. Increased focus on negative effects of fungicide use may restrict future use of disease management strategy. In addition to causing severe snow mold damage, M. nivale is also a serious pathogen on cool-season grasses and cereal crops in wet cool areas without snow cover. The loss of fungicide use will require development of new methods of disease control. Future research should therefore be directed towards the development of resistant varieties and biological control strategies. In order to attain these goals, we need to better understand this fungus. We know little about the effects of M. nivale on plants surviving the seedling blight or those that become infected at a later stage. How does the pathogen affect the quality of the final product? Can this pathogen affect the nutritional quality of grains or fodder grass? And even though several research groups have documented that some strains of M. nivale do not produce the trichothecene mycotoxins, it is possible that this fungus does produce other (myco)toxins in plant tissue. There is some evidence that isolates of M. nivale produce phytotoxins that are not necessary for pathogenicity (Hofgaard and Tronsmo, unpublished). There also appear to be considerable differences among different strains in both morphology and pathogenicity. In addition to its pathogenic aspects, M. nivale may also become useful as a model for studying the biology of low temperature organisms.

Literature cited
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