The activity of low-temperature fungi under the snow cover in Iceland

HÖRDBUR KRISTINSSON and BJARNI E. GUBLEIFSSON

Náttúrugripasafnið á Akureyri, P.O. Box 580
Tilraunastöðin á Möðruvöllum, P.O. Box 151
Akureyri, Iceland

ABSTRACT: Samples of living and dead grass leaves collected in late winter and spring at the border of melting snow layers in North Iceland, were investigated for fungi. *Typhula incarnata* Lasch ex Fr., *Typhula ishikariensis* Imai, *Fusarium nivale* (Fr.) Ces., and some other species were isolated and cultured on sterilized medium. Their pathogenicity to timothy plants, *Phleum pratense* L., in axenic cultures under low temperature conditions was tested.

*Fusarium nivale* and *Typhula incarnata* are widely distributed in the hayfields of northern Iceland, and cause some minor damage to the overwintering grasses. *Typhula ishikariensis* is less frequent, but does apparently damage the grasslands more seriously than the other two species.

*Typhula ishikariensis* and *Truncatella truncata* (Lev.) Steyaert are recorded as new to the Icelandic flora.

It is well known, that certain fungi are active at low temperatures under the snow cover in many northern countries. Some species of *Fusarium*, *Typhula* and *Sclerotinia* cause heavy damage to winter cereals (LEHMANN 1965, JAMALAINEN 1969) or even to perennial grass fields (ARSVOLL 1973, 1975).

The severe damage caused in Icelandic hayfields in 1968 and 1969 did revive interest for research in this field. Even though the main cause of winter damage in Iceland apparently is of physical and not of biotic nature (BJARNI E. GUBLEIFSSON 1971, C. & H. ELLENBERG 1969), it is nevertheless of interest to know whether the low-temperature plant pathogenic fungi do exist in Iceland or not.

Spray experiments with fungicides in the fall 1968 did not influence the damage in the following winter (BJARNI E. GUBLEIFSSON...
Soil samples collected 1968 and 1972 in hayfields in many localities in Iceland were investigated for *Fusarium*. These samples revealed several species of that genus (KOMMEDAHL 1972, KOMMEDAHL and E. SIGGEIRSSON 1973), but the low-temperature plant pathogen, *Fusarium nivale*, was not among the species found. This is understandable, since only soil and root samples were investigated. *Fusarium nivale* was first isolated from a sample collected in Stardalur, Mosfellssveit, 1975 (EINAR SIGGEIRSSON 1976).

The results presented in this paper are based on isolations made from living and dead tissues of grass leaves and stems, collected in 32 localities of the Eyjafjörður District (Fig. 1A) from April to June 1975, and a few in January to March 1974. A few extra samples were collected whenever these fungi were seen by the authors in other regions of Iceland.

**METHODS**

The collections were made where fungal activity was directly visualized in the field, at the border of, and underneath melting snow layers. In the laboratory the collected samples were investigated under stereomicroscope, and microscopic preparations were made directly from the collected material for identification.

Isolations were generally made either by transferring conidia or diseased plant tissues onto malt-extract-peptone agar. The *Typhula* species were isolated by cutting small pieces of tissue from the inside of sclerotia with sterilized razor blade under stereomicroscope. The cultures were incubated at 4°C, thus selecting fungi with rapid growth at this low temperature. Some cultures were later transferred to potato-dextrose agar, or incubated at higher temperatures, in order to study their development and sporulation under these conditions.

For the pathogenicity tests the method previously described by Aarsvoll (1975) was used. Plants of *Phleum pratense* L., the Norwegian strain Engmo, were cultivated from surface sterilized seeds on modified Hoagland-agar. The seeds were treated with 70% ethanol for 30 seconds, and then placed in 50% Chlorox (a commercial Natriumhypochlorit-solution) in water for 90 seconds, and at last rinsed with pure water. Two seeds were placed on the surface of 5 ml nutrient agar in test tubes. The nutrient agar was made as follows:

<table>
<thead>
<tr>
<th>Macro-elements</th>
<th>NH₄NO₃</th>
<th>0.01 molar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca(NO₃)₂</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>KH₂PO₄</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>MgSO₄·7H₂O</td>
<td>0.002</td>
</tr>
</tbody>
</table>

| Iron-solution | Na–EDTA | 3000 mg/100 ml |
|               | FeSO₄·7H₂O | 2780 |

1971, 1972), a fact which did not indicate any damage made by fungi in the experimental plots.
<table>
<thead>
<tr>
<th>Micro-element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_3\text{BO}_4$</td>
<td>286 mg/100 ml</td>
</tr>
<tr>
<td>$\text{MnCl}_2+4\text{H}_2\text{O}$</td>
<td>181 mg/100 ml</td>
</tr>
<tr>
<td>$\text{CuSO}_4+5\text{H}_2\text{O}$</td>
<td>8 mg/100 ml</td>
</tr>
<tr>
<td>$\text{ZnSO}_4+7\text{H}_2\text{O}$</td>
<td>22 mg/100 ml</td>
</tr>
<tr>
<td>$\text{Na}_2\text{MoO}_4+2\text{H}_2\text{O}$</td>
<td>3 mg/100 ml</td>
</tr>
</tbody>
</table>

1 ml each of the micro-elements solution and the Fe-solution was added to every liter used of the macro-elements solution, which was solidified with agar.

The plants were cultivated at room temperature for 14 days, with 16 hours light per day. After this period the plants were hardened in a refrigerator with a light built in (Philips TLD 15W/32, 5F), at the temperature of 1-2.5°C and 16 hours light at the distance of about 25 cm from the lamp. The plants were kept under these conditions for 10 days. After this treatment the plants were infected by inserting a piece of agar with the fungus culture at the base of the plants.

After four weeks the condition of the infected plants was rated into one of the following five classes:

I. No damage  
II. The fungus growing around the plant, but no damage seen. 
III. Moderate damage. 
IV. Severe damage. 
V. Plants killed.

The rating was repeated periodically until 15 weeks had passed from infection. The most significant infection results were recorded on the first 6-8 weeks from infection, and after that the symptoms remained almost unchanged. The results of these tests as recorded 8 weeks after infection are presented in fig. 3.

**FREQUENCY AND DISTRIBUTION**

The most common fungi found on the overwintered grasses were *Typhula inoarnata* and *Fusarium nivale*. The highest frequency of *T. inoarnata* was recorded in Ólafsþjörður and Svarfaðardalur, where it was present in all visited localities. These districts have very much heavier and longer lasting snow cover during winter and spring, than the other visited areas, which had *T. inoarnata* only in 20% of the localities (Fig. 1B).

No such correlation between distribution and long lasting snow cover was found for *Fusarium nivale*, which appeared to be rather common in all areas. Mycelium, which could be recognized as belonging to *Fusarium*, was recorded in 44% of the visited localities in the Eyjafjörður District. *F. nivale* is probably responsible for the greater part of these records, even though successful isolation of that species was only performed in samples from 27% of the localities (Fig. 1C).

Another fungus, which in the field behaved in a similar way as *Fusarium* was isolated in samples from six localities (16%), all in the snow rich part of the district. It did not sporulate in our cultures, but some of the isolates were identified by Arsvoll (Norwegian Plant Protection Institute, Ås) as *Coniothyrium* spec.
Typhula ishikariensis and an unidentified Typhula were collected each in a few localities, and were within the Eyjafjörður District present in 6 and 16% of the localities, respectively. Trunca-tella truncata was only isolated in a sample from one locality.

OBSERVATIONS IN THE FIELD

Field observations indicated, that both Typhula incarnata, T. ishikariensis and Fusarium nivale are pathogenic to grasses. T. incarnata did infect and kill some leaves of the grasses without destroying the whole plant. The grass plants apparently recovered soon as the growth conditions improved during the spring after the snow had melted. In no case was a total damage to the grass cover observed by this fungus. The most severe attack by Typhula incarnata was seen in July outside the area of investigation, in a large hay-field at Stóra Fellsöxl near Akranes (SW-Iceland). This field was first noticed because of its strange appearance. Several cm of the leaf tips of the grasses in this field were dead, even though the rest of the plants were normally green. Closer investigation showed the presence of the sclerotia of Typhula incarnata everywhere in the dead parts. The total field was uniformly affected by the fungus, and even though the life of the plants apparently was not endangered, the harvest and the quality of the hay must have been reduced to some extent by the fungus.
Fig. 2. A. Sclerotia of *Typhula inoarmata*. — B. Sclerotia of *Typhula cfr. variabilis*. — C. Sclerotia of *Typhula ishikariensis* on grass leaves. — D. Mycelium of *Fusarium* on overwintering grasses in the field.

*Fusarium nivale* was usually found in the field as mycelium growing on leaves, and around the sheath of grasses, either underneath the snow cover, on grasses extending through holes in the last thin sheet of ice at the border of the snow layers, or on grasses already free from snow (Fig. 2D). It formed white to faint rosa colored mycelium, sometimes with reddish sporodochia. Like *Typhula inoarmata*, it apparently destroyed some of the first leaves formed under the snow, without destroying the whole plants.

*Typhula ishikariensis* was also found on living grass leaves, forming the small, black, round sclerotia in abundance (Fig. 2C). At one locality, near Másvatn, this fungus had totally killed the grasses in a rather large spot in a field of *Phleum pratense*. This locality has a very long lasting snow cover, and in June the snow was still melting at the winterkilled spot. This part of the field became green later in the summer, and was then dominated by *Poa pratensis* and *Festuca rubra*, but the plants of *Phleum pratense* had disappeared.

A *Typhula* species not identified (cfr. *T. variabilis*) was found
several times on grass leaves, stumps of oats from the previous year, or on cow dung. This species is probably not a pathogen, and did not affect timothy plants in the pathogenicity test.

A *Coniothyrium* spec. was often isolated from green grass plants growing through a hole in the last ice sheet bordering the melting snow layers. This habit of growth appears similar to that of *Fusarium nivale*. *Coniothyrium* was only isolated from samples collected late in the spring, in the area of Svarfaðardalur and Ólafsfjörður.

We have no observations on the activity in nature of *Fusarium avenaceum* and *Truncateilla truncata*.

Some myxomycetes are also frequent in these habitats, especially a species of *Lamproderma*), which evidently makes part of its development under the snow. It grows on dead straw, forming dense cluster of tiny sporangia with violet, metallic iridescence. Less frequent is *Physarum cinereum* (Batsch)Pers., which also was found near snow layers. Both these myxomycetes are harmless to living grass.

THE PATHOGENICITY TEST AND THE GROWTH MEASUREMENTS

Three species, *Typhula incarnata*, *T. ishikariensis* and *Fusarium nivale* were in most cases lethal to young timothy plants in axenic cultures under low temperature conditions. Three cultures of *T. incarnata* got contaminated during inoculation, and the infection was for that reason unsuccessful. Two isolates of *Fusarium nivale* were not infectious. The reason may be, that the cultures were too old, since the *Fusarium* species are known to be sensitive against prolonged culture (Booth 1971). Otherwise these fungi expressed vigorous growth on the test plants, and *Typhula incarnata* and *T. ishikariensis* formed sclerotia on them. Like in the field *Fusarium nivale* and *Coniothyrium* had similar growth habit on the test plants. A thick hyphal mat was formed, spreading around the plants like a conical tent, with the top attached to the stem and the base on the agar surface.

Fig. 3. Average size (diameter) of several fungus colonies after 10 days cultivation at 4-5°C on malt-peptone agar (top) and the pathogenicity of the same species (below). The number of lines represents the number of isolates tested for each species. The length of lines indicates the pathogenic effects on the host plants, rated into 5 classes (p. 46).

*) Recorded as *Lamproderma violaceum* (Fr.) Rost. by Larsen 1932.
In most cases the fungi finally killed the host plants during the test period. This does, however, not necessarily mean that they do so under natural conditions. The plants are apparently less resistant against these fungi under the experimental conditions than in nature. But it is a proof for the potential pathogenic ability of these fungi, which may come into action whenever the resistance of the host plants is suppressed by environmental conditions, such as the low temperature and the moist air under the snow cover.

The isolates of *Coniothyrium* and the single isolate of *Truncatella truncata* did also come out positive in the pathogenicity test, though not as lethal as the other three, while *Typhula* cfr. *variabilis* did not. Measurements were made on the diameter of the colonies formed on malt-extract-peptone agar at 4–5°C in 10 days and 18 days. The results of average growth per 10 days are presented in fig. 3.

It is apparent, that *Typhula incarnata*, *Fusarium nivale*, *Truncatella truncata* and *Coniothyrium* sp. all grow relatively fast at these low temperatures, even though their optimum is higher. According to Årsvoll (1975) all of these species (with the exception of *T. variabilis* for which no information is available) are able to grow around and below 0°C, especially *T. incarnata* and *T. ishi-kariensis*.

**DESCRIPTIONS OF SPECIES**

**TYPHULA INCARNATA** Lasch ex Fr.

*Syn.: Typhula graminum* Karst. Previous records from Iceland in ROSTRUP 1885, 221; ROSTRUP 1903, 294; LARSEN 1932, 520.

The mycelium of this fungus grows on overwintering grass leaves of different species. It produces light to medium brown, oval to kidney-shaped sclerotia (fig. 2A) of the size of 1.5 – 2.5 x 1 – 1.5 mm. The sclerotia may be formed on the outside of the leaves, or within them and braking through the epidermis. They are loosely attached to the grass by the hyphae, and fall easily off while being handled or dried. They can easily be seen from March to June at the margin of the melting snow, the suitable time depending on the duration of the snow cover. The sporocarps should be expected to appear from the sclerotia in the fall, but they were not looked for.

In agar cultures the fungus grows relatively fast compared to other *Typhula* species, forming colonies of the maximal size of 6–6.5 cm in diameter within 18 days at 4–5°C (fig. 3). It produces great number of sclerotia in the agar cultures, of similar type and color as under natural conditions, but often larger and more coalesced.


*) Where no other information is given, the specimens were collected by the authors and are kept in the herbarium of the Akureyri Museum of Natural History (AEY).
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**TYPHULA ISHIKARIENSIS Imai**

New to Iceland.

The mycelium grows on living, overwintering grass leaves. It produces large number of dark brown to black, globular sclerotia, only 0.6 - 1.0 mm in diameter. The sclerotia are typically crowded, or on narrow leaves densely packed in one row (fig. 2C). They usually start growing more or less inside the leaves, and break out as they mature. The species is apparently rather rare, but often present in great quantities where it is found. The mature sclerotia were seen in April to June. No observations were made on sporophores.

The fungus colonies reach on agar about 3.0 - 3.5 cm diameter in 18 days at 4-5°C, which is a considerably slower growth under these conditions, than by *T. inaernata*. It produces large number of tiny sclerotia on the agar plate, of the same type as in nature.


**TYPHULA spec.**

The specimens collected by us are identical to an unidenti­fied specimen found in the herbaria in Reykjavik and Copenh­agen (leg.: Ólíafur Davíðsson, 1897).

The fungus forms larger sclerotia than the preceding species, on dead grass leaves or stems, on overwintered oat stumps, or on old cow dung. The sclerotia are dark brown, to almost black, usually shining, with slightly pitted surface, flattened, one end generally broader than the other. The sclerotia are attached to the sub­strate by the narrower end, usually by a minute stalk (fig. 2B). Their size is 2-4 x 1.5-3 mm. Sterile fruit bodies were obtained late summer 1974 on sclerotia, that had been placed on moist sand, kept outdoors during the winter and at room temperature in the spring, and through summer. In agar cultures this fungus often grows quite slowly, both at low temperatures and at room temperatures.

The pathogenicity test turned out negative, and the occurrence of this species on cow dung does also indicate, that the fungus is not parasitic.

This *Typhula* did not readily form sclerotia in agar cultures, except in one case on very old, outdried agar plate. The sclerotia were large, of the same color and shape as in nature. In an attempt to identify the fungus, the Icelandic specimens were compared with *Typhula phacorrhiza* borrowed from CPH, and proved not to belong to that species, but they could possibly belong to *Typhula variabilis*. The structure of the cuticle and the epidermis of the sclero­tia (fig. 4A) support that view, but this needs to be verified.

![Image](image_url)

Fig. 4. Section through the surface of the sclerotia of A: Typhula cfr. variabilis; B: Typhula incarnata; and C: Typhula šikikariensis. Conidia of D: Truncatella truncata; and E: Fusarium niveale. The illustrated specimens are from A: Ýtra Hvarf, B: Helgafell, C: Baldurshelmur, D: Ýtra Fell, and E: Ýtra Gil.

**Fusarium niveale** (Fr.) Ces.

Previous record from Iceland in E. SIGGEIRSSON 1976.

The fungus was found growing on the leaves and shoots of green grass plants, sometimes when they were growing through the last sheet of ice at the margin of thick snow layers. It was also often isolated from dead or living leaves with visible masses of hyphae around them, usually with a rosa tint, and sometimes reddish spots of sporodochia.

Microscopic examinations of the collected samples indicated often poor sporulation in nature, except in the sporodochia, which sporulated in abundance. Most of the spores were two-celled, 11-15 μ long (Fig. 4E). Spores were usually not produced in the agar culture under the conditions used, until in old cultures, that light red sporodochia with rich spore production were developed. The colonies of freshly isolated strains reached up to 6 cm diameter in 10 days at 4-5°C, and had overgrown the whole plate within 18 days (Fig. 3).

Specimens were isolated and identified from: EYJAFJÁRBARSÍSLA: Ólafsfjörður, Hlíð, 24 May 1975. Uppsaströnd, Karlsá, 24 May 1975. Eyjafjörður, Ýtra Gil, 23
Some additional species were isolated from the overwintering grasses, most of which have not been identified. A few of these shall be shortly mentioned here.

A species of *Coniothyrium* has been repeatedly isolated, usually directly from green grasses growing through a hole in the last ice sheet of the melting snow. It was only found late in the spring, in Ólafsfjörður and Svarfaðardalur, those regions of Eyjafjörður which have the heaviest snow falls. In culture this fungus had limited radial growth compared to *Fusarium*. It produces yellowish brown or rosa tinted pigments, visible from the bottom of the plate. It did not sporulate in our cultures, but sporulation was induced in some of these cultures by Ársvoll (Norwegian Plant Protection Institute, Ås), who also identified them to this genus.

*Truncatella truncata* (Lev.) Steyaert was isolated from a sample collected in Ytra Fell, Eyjafjörður, on dead *Agrostis* and *Anthoxanthum* in a pasture. This species has characteristic septate spores with two dark center cells and appendages on the one end (fig. 4D). It was found to be moderately pathogenic to the test plants. The fungus is well known as saprophytic soil fungus (DOMSCH & GAMS 1970) and as a weak parasite on many plant species (ÁRSVOLL 1975). It has not been recorded from Iceland before.

In a few places fungi of the *Fusarium roseum* group were isolated from the samples. The cultures behaved uniformly, and one isolate was sent to Ársvoll and identified as *Fusarium avenaceum*. The same species had previously been isolated from soil in Eyjafjörður by KOMMEDAL & SIGGEIRSSON (1973).

An unidentified species of *Mortierella* was isolated from two samples. Both isolates were not pathogenic to the test plants.

**DISCUSSION**

It has been demonstrated in the present study, that three of the four well known fungi that attack overwintering grasses in northern Europe, do occur in Iceland. Ársvoll (1973) provides data on the correlation between the duration of snow cover in Norway, and the presence or damage caused by each of these fungi.

He found, that *Fusarium nivale* was relatively indifferent in respect of its requirements for snow cover, and occurred throughout the country. *Typhula incarnata* was also found in most parts of Norway, but rarely in localities with less than about 90 days of snow cover. *Typhula ishikariensis* on the other hand, did rarely occur in areas with less than 120 days of snow cover, and did first cause heavy attacks in areas with over 180 snow cover days.

The last sixteen years average number of days with total snow cover in Akureyri per year is 97 according to the records of the Icelandic Meteorological Institute. The corresponding figure for Siglunes is 102. Since storms do very much affect the snow layers in Iceland, these figures do not give information on the duration of snow layers at the sample localities, where snow accumulations remain until June in some cases. Neither do these figures give any idea about the significant difference in the amount of snow cover.
covering the ground in different parts of the district.

It seems more realistic for this purpose also to include the days with 1/4 to 3/4 snow cover together with the days of total snow cover. By doing so the average number in the last 16 years becomes 150 in Akureyri with variation from 82-187, and for Siglunes 187 with variation from 147-240. These are the only stations in the area, which have continuous information of snow cover for that long period.

The winter 1974-75, when most of the samples were collected, the district of Eyjafjörður had heavy snowfalls in December and January, and the snow drifts remained until end of April in the valley south of Akureyri, and until late June in the region of Ólafsfjörður. This means that the sample spots in the first area did have about 150 days of continuous snow cover before the collection was made, and those in Ólafsfjörður about 210 days.

For the three years period 1971-1973 continuous observation data are available from five stations in the area, and these are represented by numbers in fig. 5. Even though this short period may not give a good long-range average, it should inform on relative differences between localities. The number 218 for Skelðsfoss is probably representative for the snow rich Ólafsfjörður area, which itself has no station. The station Siglunes with 181 is less representative, since it is situated directly out to the open coast.

Useful information on the snow layers in the area can be obtained by mapping the lower limits of the vascular plant Gnaphali um supinum L. (Asteraceae) in the area. This plant is dependent on long snow cover, and is found all the way down to sea level, where snow accumulations persist until late in the spring in the lowlands. In localities with poor snow falls, like in the valley south of Akureyri, these conditions are not found until at an elevation of 360 m up in the mountainside (H. Hallgrimsson & H. Kristinsson 1965).

This information is shown on the map in fig. 5, and is based on unpublished data from about 25 localities, where the lower limit of Gnaphali um supinum has been measured. It is useful for relative comparison between different localities, but no data are available so far to relate these limits to certain duration of the snow cover.

There seems to be a fairly good correlation between the frequency of the low-temperature fungi in different parts of the Eyjafjörður area, and the results from Arsvoll about their requirements for snow cover. Fusarium nivale seems to be relatively indifferent to snow layers within the area, and Typhula incarnata, though found throughout the whole district, is most frequent where the snow cover surpasses 180-200 days. Typhula ishikariensis is too rare to make any comments on its snow requirements.

Sclerotinia borealis Bub. & Vleug., which in Norway did not cause much damage in areas with less than 180 snow cover days, could hardly be expected in the Eyjafjörður District, except in Svarfaðardalur, Ólafsfjörður and Höfðahverfi. This species has, however, not been found in Iceland at all.

Several characteristic forms of fungal infection were recognized in the field, which could not be correlated to any of the identified fungi. Among these were round, whitish, sharply delimited
spots of about 3-6 cm diameter on dead masses of *Deschampsia caespitosa* (L.) PB., observed frequently both in pasture and in cultivated hay-fields. These may be caused by some saprophytic, but definitely by low temperature tolerant fungi, since their activity must find place under the snow cover. Several isolations were made from these spots, but the results were not uniform.

After the snow has melted, a delicate network of hyphae may be seen hanging over the grass tips like a cobweb. Various fungi

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Fig. 5. Division of the Eyjafjörður District in zones with different lower limits of *Gnaphalium supinum* L. (Asteraceae) in the mountains. The map reflects in the same time the persistence of snow accumulations in the lowlands. This plant is dependent on continuous snow cover during winter and spring, and extends only so far down to the lowlands, that sufficient snow cover is ensured. The map on the right locates several geographical names used in the text.
were isolated from this, among those were *Fusarium*. We presume, that this web is a rest of different fungi, probably mainly *Fusarium*, active under the snow cover on the grass tips extending into the snow. The hyphae, which are partly attached to the snow itself, are left in the form of more or less continuous web after the snow has melted, with a great number of dust particles adhering to it.

Even though we now know, that three of the most common fungi causing winter damage to grasslands are present in Iceland, and two of them rather frequent, we have seen only one case, where such fungi have caused severe damage to a field. Their contribution to the damage, which in Iceland is known as "kal", is apparently quite small under normal circumstances, compared to the abiotic factors. The situation may however change when the snow covers the unfrozen ground longer than normal, as may happen in those areas generally exposed to heavy snow falls. Such conditions do very much increase the probability of attack by the more aggressive fungi like *Typhula ishikariensis*.

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