Occurrence of certain microfungi on reindeer pastures in northern Finland during winter 1996-97

Jouko Kumpula[1], Päivi Parikka[2] & Mauri Nieminen[1]

[1] Finnish Game and Fisheries Research Institute, Reindeer Research Station, FIN-99910 Kaamanen, Finland (jouko.kumpula@rktl.fi).

[2] Agricultural Research Centre, Plant Production Research, FIN-31600 Jokioinen, Finland.

Abstract: Thick snow covering on warm and unfrozen soil in late autumn is believed to promote mould growth on the winter pastures of reindeer. Natural feed containing potential mycotoxins is suggested to affect the condition and health of the reindeer. During this kind of winter and spring 1996-97 we collected 30 samples from winter forage plants on three winter ranges in nothern Finland. We identified altogethet 12 different species or species groups of fungi in plant samples. Most microfungi were found when the soil temperatute under the snow in winter was above 0 °C and when the snow was just melted in spring. Abundant fungi wete *Mortierella* spp., *Penicillium* spp. and *Trichoderma viride.* Without exception *T. viride* was, the most abundant when the temperature under the snow was above 0 °C and the soil was unfrozen, and *Penicillium* spp. when temperature was below zero and the soil was frozen. *Mortierella* spp. was abundant in both circumstances. These three fungi or genera were also abundant in samples just after snow melting in spring. Reindeer seemed to avoid digging in the places where fungi were the most abundant. Several *Penicillium* species and *T. viride* are known to be able to produce mycotoxins. Many symptoms observed among reindeer grazing on natural pastures were quite similar to those caused by mycotoxins. Potential mycotoxins on reindeer pastures and their effects on teindeer, should be studied in mote detail.

Key words: *Rangifer tarandus,* grazing, mould, mycotoxins, *Trichoderma, Penicillium.*

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Introduction

There is a wide variety of different microfungi species living and causing mould formation on soil, vegetation and other organic material. Several species can produce mycotoxins, i.e. fungal secondary metabolites that in small concentrations are toxic to vertebrates and other animals. The most known mycotoxins are produced by species in the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* (Frisvad & Thrane, 1995; Lautraite *et al.,* 1995). Mycotoxin producing fungi often invade agricultural products. Consumption of mouldy cereals as food and feeds gives serious intoxications of humans and animals (Ueno, 1977; WHO, 1990). Mould proliferation is favoured by temperature, humidity, and oxygen/carbon dioxide ratio (Ueno, 1977; Ciegler, 1978; Lautraite *et al.,* 1995).

For free ranging semi-domesticated reindeer (*Rangifer tarandus tarandus*) the availability of feed during winter is much affected by the weather and

snow conditions during early winter. With changing temperatures around 0 °C, snow or rainfall can form a thick layer of snow or ice on vegetation in early winter. On the other hand, the fotmation of moulds on winter ranges during early winter is also feared by reindeer herders, especially aftet warm and rainy autumns when the soil has no time to freeze before deep snow covers it. The yellowish dying of the muzzle hairs of free ranging reindeer during late autumn and early winter is regarded as a sign of mould formation in the pastures (Helle, 1980).

Autumn 1996 was warm, rainy and long lasting in northern Finland. The mean temperature in October was 1.5 to 2.0 °C higher and the precipitation 120-200% higher than the long term means (Ilmatieteen laitos, 1996a). A petmanent layer of snow fell suddenly in the last days of October and there was 40-70 cm of snow in mid December (Ilmatieteen laitos, 1996b; c). Several problems appeared soon in many reindeer herds which were grazed on natural pastures in northern Finland. Reindeer lost body weight fast and there were also some signs of starvation. Besides causes as poor wintet range quality (see Kumpula *et al.,* 1998) and hard snow conditions, the possibility of mycotoxication in reindeer herds was discussed.

As a pilot project we collected samples from the vegetation grazed by reindeer on three winter range areas in order to study the growth and occurence of different microfungi species in those conditions prevailed on reindeer pastures during winter 1996-97. Of the identified microfungi the species or genera which are able to produce mycotoxins were discussed.

Material and methods

During winter and spring 1996-97 we collected 30 plant samples from three reindeer winter range areas in nothern Finland (Fig. 1). Twenty-four of the samples were collected from dry or very dry lichen (*Cladonia* spp.) range, four from submesic dwarf shrub (*Empetrum nigrum, Vaccinium vitis-idaea* and *V. myrtillus*) and lichen dominated range and two from dwarf shrub and lichen dominated hummocks on bog. All winter food of reindeer (lichens, dwarf shrubs and vascular plants) were collected from holes dug in the snow. The snow depth was measured in each sample hole as well as the temperature of the soil surface by pushing a 35 cm long, thin bulb through the snow to the soil surface. The state of the soil and vegetation was observed and the

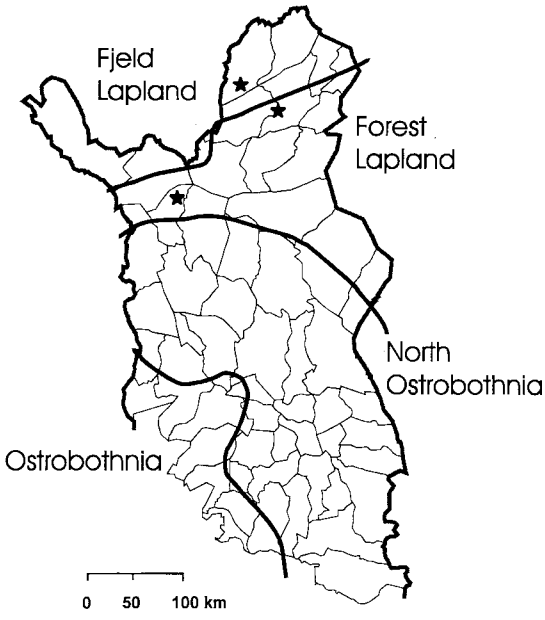

Fig 1. Three sampling areas in reindeer winter ranges, northern Finland. The Finnish vegetation zones and reindeer management districts are also depicted.

amount of reindeer cratets near the sampling site. Eleven samples were collected from December to January, eleven from March to April and eight samples were taken just after the melting of the snow in spring 1997. Different microfungi genera and species, and the amount of each cathegory were identified in laboratory.

Ten grams of each sample were weighed, ground to a homogenous mass and mixed with 100 ml of distilled water. Ten milliliters of this suspension was diluted with 100 ml of water and the dilution was repeated. From the final dilute 0.75 ml was pipetted on four different nutrient media (cornmeal agar with steptomycin sulphate (0.2 g/l) (CMA), potato dekstrose agar (PDA), Martin agar (Martin) and peptone PCNB agar (PCNB) (Singleton *et al.,* 1992). The agar plates were incubated at +25 °C for 5 days and thereafter one week at room temperature (+22 to +24 °C) and four weeks in a refrigerator (+5 °C). The developing fungal colonies were calculated on the agar plates, and the fungi identified to genera or species. For identification, the fungal colonies were isolated on PDA-medium. *Penicillium*- and *Mortierella*-isolates were also grown on malt extract agar to separate the species. Domsch *et al.* (1980) was used as a guide to other fungal species. The first assessments and counts of fungal colonies were

Table 1. Microfungi identified in samples to species and genera. Frequency (f) and proportion (%) of occurrences in samples (n=30).

Microfungus	f	(%)
Acremonium sp. Link ex Fr.	8	(26.7)
Gliocladium sp. Corda	1	(3.3)
Leptothyrium sp. Kunze	11	(36.7)
Mortierella ramanniana var. *angulispora* (Naumov) Linnem	11	(36.7)
Mortierella spp. Coemans	26	(86.7)
Mucor hiemalis Wehmer	11	(36.7)
Paecilomyces farinosus (Holmes ex S.F. Gray) Brown & Smirh	6	(20.0)
Papularia sphaerosperma (Pers. ex Gray) Höhn	1	(3.3)
Penicillium thomii Maire	3	(10.0)
Penicillium spp. Link ex. Fr.	21	(70.0)
Rhizopus nigricans Ehrenb. ex Corda	1	(3.3)
Trichoderma viride Pers. ex Gray	15	(50.0)

made after one week of incubation, the isolations and identifications after thtee and five weeks of incubation. Identification of *Penicillium*-species was made at the Centtaalbureau voor Schimmelcultures (CBS, The Netherlands) by the methods described by Pitt (1989) and Samson *et al.* (1995).

The occuttence of fungal colonies in each sample group were recorded as a frequency (f) and as a percent of occurrence (%). The average number of fungal colonies and the snow depth in sample groups are given as medians (Md). The average temperature on soil surface in sample groups are given as means (Mn). The statistical diffetences in the number of colonies, temperatures and snow depth between different sample groups were tested by Kruskall-Wallis' or Mann-Whitney's test. The probability of certain fungal colony occuring most abundant in a certain sample group was tested using Cochran's test (Ranta *et al.*, 1989).

Results

We identified 12 different fungi species or species groups (Table 1). There were a lot of microfungi colonies in each vegetation sample, most when the soil surface temperature beneath the snow in winter was above 0 °C and also just after the melting of the snow in spring (Table 2). The most common and the most abundant species were *Mortierella* spp., *Penicillium* spp. and *Trichoderma viride* Pers. ex Gray (Table 3).

M. elongata Linnem. was the main species among the genus *Mortierella*. *M. ramanniana* vat. *angulispora* (Naumov) Linnem. was identified separately. The *Penicillium*-gtoup contained several types of species in this genus. *P spinulosum* Thom was the most common species. The othets were *P. lividum* Westling, *P. expansum* Link ex Gray, *P cf. paxilli* Bain and *P. thomii* Maire.

Without exception *T. viride* was most abundant when the soil was unfrozen and *Penicillium* spp. when the soil was frozen (Table 4). *Mortierella* spp. seemed to be abundant in both circumstances. All these three species or genera were also abundant just aftet the melting of the snow in spring. Reindeer seemed to avoid digging the places where the amount of microfungi density was highest (Table 5).

Discussion

Of the observed fungi, *Trichoderma, Mortierella* and *Penicillium* are all abundant in cool and temperate regions. *Trichoderma* spp. grow well even at +6 °C

Table 2. The average number (median) of germinated fungal colonies in plant sample groups. A and B groups were collected from ground vegetation with different temperatures on soil surface under the snow in winter and C gtoup just after snow-melting in spring. Microfungi were grown in four different nutrient media. The probabilities of differences between the groups are prestented using Kruskall-Wallis' test.

Nutrient media	A Plus degrees n=13	B Minus degrees n=9	C Snow-melting n=8	A = B = C Kruskall-Wallis' test P
PDA[a]	66 983	28 659	36 823	0.591
CMA[b]	77 314	81 971	72 815	0.822
Martin[c]	28 992	4 332	833	0.031
PCNB[d]	1 666	666	2 999	0.034

[a] potato deksttose agar, [b] streptomycin sulphate (0.2 g/l), [c] Martin agar, [d] peptone PCNB agar.

Table 3. Frequency (f) and the proportion (%, in parenthesis) of the cases in which the occurrence of rhe fungi were rhe most abundant in each sample group. A, B and C groups are similar as in table 2. The probabilities of differences between the sample groups are presented using Cochran's test.

Microfungus	A Plus degrees *n*=13	B Minus degrees *n*=9	C Snow-melting *n*=8	A+B+C All samples *n*=30	A=B=C Cochran's test *P*
Mortierella spp.	8 (61.5)	5 (55.6)	4 (50.0)	17 (56.7)	0.600
Paecilomyces	1 (7.7)	0	0	1 (3.3)	0.301
Penicillium spp.	0	4 (44.4)	3 (37.5)	7 (23.3)	0.028
Leptothyrium sp.	0	1 (11.1)	1 (12.5)	2 (6.7)	0.234
Trichoderma viride	6 (46.2)	0	1 (12.5)	7 (23.3)	0.044

Table 4. The average (mean) temperature (°C) on the soil surface under the snow layer and the average (median) snow depth (cm) in the sample places divided into two groups according to fungal abundance. The probabilities of differences between the groups are presented using Mann-Whitney's test.

Microfungus	Occurence	*n*	Temperature on soil °C Mean	Mann-Whitney's test *P*	Snow depth (cm) Median	Mann-Whitney's test *P*
Mortierella spp.	non-abundant	9	- 0.4		80	
	abundant	13	+ 0.1	0.920	78	0.763
Penicillium spp.	non-abundant	18	+ 0.2		81	
	abundant	4	- 1.6	0.024	76	0.268
Trichoderma viride	non-abundant	16	- 0.5		83	
	abundant	6	+ 0.8	0.006	78	0.605

(Domsch *et al.*, 1980), e.g. cold-tolerant *T. viride* strains are widespread in Alaskan soils (Johnson & Bernard, 1987). Some *Penicillium*-species can grow in very low temperatures. *P. expansum* is known to have types adapted to grow at -3 °C. The identified species are also common inhabitants of acid forest soils and peatlands in cool temperate regions. *M. ramanniana* var. *angulispora* is one of the most widespread *Mortierella* species and has a clear preference to cold and temperate regions (Domsch *et al.*, 1980).

Table 5. The average (median) number of fungal colonies in vegetation samples divided into two groups according to the number of craters made by reindeer near the sample place. The probabilities of differences between the groups in each nutrient media are presented using Mann-Whitney's test.

Nutrient media	no/some craters *n*=16	a lot of craters *n*=6	Mann-Whitney's test *P*
PDA	66 317	21 494	0.043
CMA	90 487	64 484	0.065
Martin	10 164	2 999	0.197
PCNB	1 333	333	0.048

We found viable spores of *T. viride* most abundantly in plant material under the snow cover in winter if the soil was unfrozen but the growth of *T. viride* was suppressed when temperatures were below 0 °C. On the contrary, the best growth of *Penicillium* species was observed in samples from frozen soil and also just after melting of the snow in spring. *Mortierella* spp. favoured all these circumstances. We assume that the formation and occurrence of grey mycelial cover on lichen pastures just after the thaw in spring 1997 was formed by *Mortierella* spp.. However, those *Mortierella* species isolated from pasture samples are not known to produce mycotoxins (Domsch *et al.*, 1980). On the contrary, several strains belonging to *Rhizopus* and *Mucor* species (Ftisvad & Thrane, 1995) and also *Paecilomyces farinosus* (Vanninen, 1999) are known to produce toxic compounds which are, however, toxic in most cases only towards non-vertebrates.

Trichoderma species produce a range of biological active compounds, only few of these are mycotoxins in the strict sense. However, the mycotoxins alamethicins, emodin, suzukacillin, trichotoxin A and

thrichodermin have been isolated from *T. viride*. Several *Penicillium*-species can also produce mycotoxins (Frisvad & Thrane 1995). Some of these mycotoxins are produced at as a low temperature as +4 °C (WHO, 1990). *Penicillium expansum* can produce several toxic compounds, like roquefortine C, patulin, citrinin, communesins and chaetoglobosin C (Frisvad & Thrane, 1995). *Penicillium cf. paxilli* belongs to *P. braevicompactum*-group which can produce cytotoxins like botryodiploidin and mycophenolic acid and also antibiotic active compounds (Domsch *et al.,* 1980; Frisvad & Thrane, 1995). These fungi can grow even at -2 °C (Domsch *et al.,* 1980). Also many othet *Penicillium* species, like *P. lividum* and *P. thomii* can produce antibiotic active compounds suppressing the growth of other fungi and bacteria (Domsch *et al.,* 1980).

Mycotoxins are known to cause several symptoms to animals. Some mycotoxins induce dermal toxicity; non-specific acute dermal inflammation reaction, charactetised by hyperaemia, oedema and neuthrophil exudation with varying degrees of necrosis of the epidermis (Lattaute *et al.,* 1995). Mycotoxins can also decrease the number of circulating white blood cells or cause the disease which is characterised by pancytopenia, haemorrhagic diatheses, bone marrow aplasia, diminished haemostasis, severe lymphatic tissue alterations and histopathologic changes in ptolifetative tissue (Ueno, 1977; Lattaute *et al.,* 1995). It has been also shown that toxins could have attributed to a disturbance of the central nervous system (Latraute *et al.,* 1995) developing nervous disorders such as convulsions, cyclic movements and retarded reflexes (Ueno, 1977).

Mycotoxins are also known to reduce the reproductivity of animals by increasing fetus loss (WHO, 1990; Lautraite *et al.,* 1995) for instance some *Mortierella*- and *Mucor* species cause abortions. The immunosupressive effects of mycotoxins on animals have resulted in a decreased resistance to secondary infection by bacteria, yeasts and viruses in several studies (WHO, 1990). Many mycotoxins have antimicrobial effects, hence it is possible that in ruminants the toxins may influence the growth of specific micro-organisms or metabolic processes in the rumen. Several researchers have reported mycotoxins inhibiting fibre digestion and volatile fatty acid formation in the rumen (Mertens, 1979; Dawson & Allison, 1988). Yet some mycotoxins is known to be cleaved in forestomachs by protozoan and bacterial enzymes (WHO, 1990), nothing is known about their effects on digestion.

Many behavioural and physiological symptoms observed among live and dead reindeer grazed on natural pastures in northern Finland during wintet 1996-97 seemed to be quite similar to those known to be caused by mycotoxins. Reindeer lost body weight exceptionally fast. Refusal to feed was observed among reindeer taken from natural pastures to emergency feeding. A lot of plant material was observed in the rumen and plenty of undigested food remains were also found in the forestomachs when some post mortem examinations were performed at the Finnish Reindeer Research Station, Kaamanen. In these examinations it was also observed that many reindeer had edematic lungs and bloody foam inside the ttaceas and the bronchus. In one case, on December 1996, an adult female had an edema around the heart. Some reindeer herders reported also disturbances in the central nervous system among reindeer including convulsions, cyclic movements and retarded reflexes.

All of these symptoms may be caused by mycotoxins (Ueno, 1977; WHO, 1990; Lautraite *et al*, 1995) but can also be connected to long-term undernutrition, and a tapid change in nutrition especially when giving reindeer feed of bad or unsuitable quality. It is also known from reindeer that disturbances in the central nervous system may be connected to serious deficiencies of minerals, especially when there is a considerable decrease of magnesium and calcium level in blood (Hyvärinen *et al*, 1976; Hoff *et al*, 1993).

Notable losses of reindeer during winter and the subsequent collapse of calf production in spring were earlier reported once or twice in a ten yeat period (Helle, 1980). Although present day reindeer owners in Finland are more prepared to feed reindeer as earlier, the winter 1996-97 demonstrated that serious losses can still happen. We suppose that the weather and snow conditions in late autumn and early winter may have a considerable effect both on the digging conditions of reindeer and the quality of natural food utilized by reindeer during winter. It is obvious that the growth of certain microfungi species and their potential mycotoxin production on reindeer pastures as well as their toxic effects on reindeer health and digestion have to be studied in mote detail.

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