Mycoscience 40: 373–375, 1999

Short Communication

Change in isolation frequency of *Typhula ishikariensis* from turfgrass under snow cover on golf courses

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Accepted for publication 1 June, 1999

The change in isolation frequency of *Typhula ishikariensis* from bentgrass leaves under snow cover on golf courses near Sapporo, Hokkaido was investigated over three consecutive winters. *Agrostis palustris* Huds. cv. Penncross growing at these sites was severely infested with *T. ishikariensis* biotype B. Isolation tests showed that *T. ishikariensis* biotype B was first isolated 9 to 34 d after snow cover in December-January, and isolation frequency peaked in February-March. The fungus was never isolated from leaves after snowmelt. The behavior of *T. ishikariensis* on bentgrass was discussed in terms of winter hardiness of plants and its epidemiology.

Key Words——*Agrostis palustris*; bentgrass; epidemiology; snow mold.

Snow mold caused by *Typhula ishikariensis* S. Imai is one of the most serious diseases of turfgrasses, forage crops, and winter cereals in northern Japan. However, the behavior of the pathogen on turfgrass has remained unknown because of the difficulty in collecting it from plants under snow. Only a little information on this matter is available: Matsumoto and Araki (1982) repeated isolations for three years to find that *Sclerotinia borealis* Bubáck & Vleugel prevailed on upper leaves in the former half of winter and that *Typhula incarnata* Lasch ex Fr. on lower leaves in the latter half from snow-covered ryegrass and meadow fescue in Hokkaido. They were unable to detect *T. ishikariensis*.

In this study, I examined the change in isolation frequency of *T. ishikariensis* under snow cover on golf courses near Sapporo, Hokkaido over three consecutive winters to obtain fundamental information on the epidemiology of the pathogen under snow.

Three test sites, Field A (1991–92), Field B (1992–93), and Field C (1993–94), were chosen. These sites were planted with *Agrostis palustris* Huds. cv. Penncross and severely infested with *T. ishikariensis* biotype B. A sod (ca. 50×50 cm^2) was removed every other week from Field A and every month from Fields B and C. Snow was first removed from the sod. Fifty bentgrass leaves were randomly chosen from each sod. They were cut into pieces of ca. 5 mm in length, sterilized in 70% ethanol for 10 seconds, rinsed in sterile water three times, transferred to 1.5% water agar, and incubated at 0°C for 30 days. Samples from Fields A and C and some from Field B were not sterilized later, since preliminary tests revealed that isolation frequencies from unsterilized and sterilized leaves were almost the same.

Each mycelium developed from leaves was observed under light microscopy (Nikon OPTIPHOTO-2). Hyphae considered to be *Typhula* with clamp connections were transferred to MYPG agar medium (0.3% yeast extract, 0.3% malt extract, 0.5% polypeptone, 1.0% glucose, 2.0% agar, pH 7.0; Yamazato et al., 1986) to produce sclerotia. All putative *Typhula* hyphae were identified as *T. ishikariensis* biotype B by its characteristic black sclerotia. No other snow mold fungi were isolated, and other fungi, for example *Mucor* sp., were rarely isolated. Isolation frequency was determined as follows:

Isolation frequency (%) = 100 × (Number of leaf fragments from which *T. ishikariensis* was isolated)/50

Temperature was recorded on the ground surface and in the air at noon in Fields A and B by an automated measurement machine, and snow depths were daily recorded in the three fields, as these parameters were considered to relate deeply with changes in isolation frequency.

Changes in isolation frequency of *T. ishikariensis* biotype B from bentgrass leaves of Fields A (1991–92), B (1992–93), and C (1993–94) are shown in Figs. 1, 2, and 3, respectively. Snow depth in the fields is also shown in the figures. The isolation frequency of *T. ishikariensis* from sterilized bentgrass leaves under snow cover was the same as that from unsterilized leaves in Field B (Fig. 2). Therefore, the results in Fields A and C are considered to reflect the exact population change of *T. ishikariensis*.

Typhula ishikariensis was never isolated before snow cover in any of the fields. It was first isolated 9 to 34 d after snow cover in December–January, with frequencies ranging 20–53% from unsterilized leaves

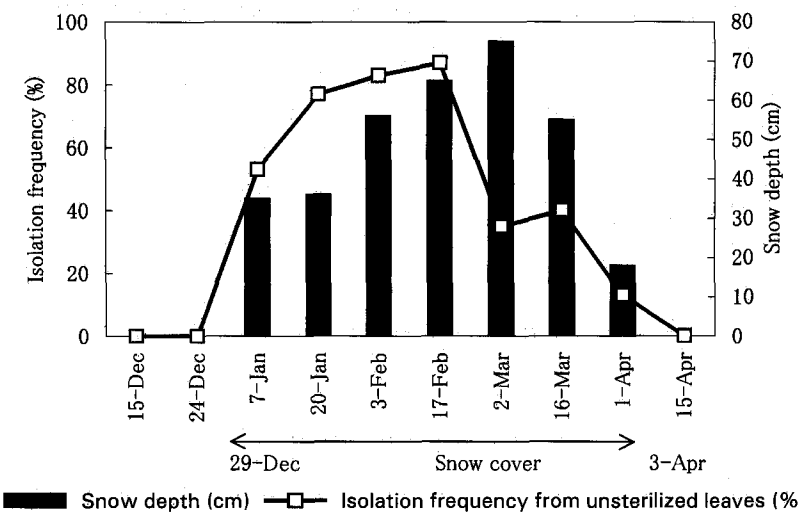

Fig. 1. Change in isolation frequency of *Typhula ishikariensis* biotype B from bentgrass leaves and snow depth in Field A (1991–92).

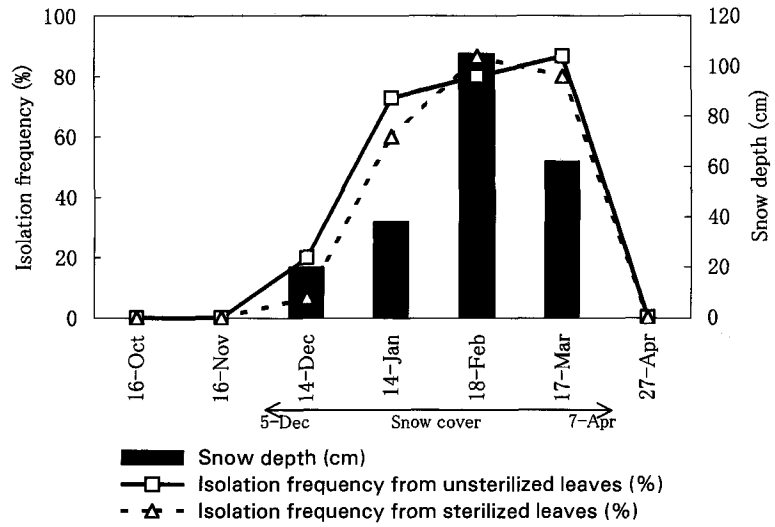

Fig. 2. Change in isolation frequency of *Typhula ishikariensis* biotype B from bentgrass leaves and snow depth in Field B (1992–93).

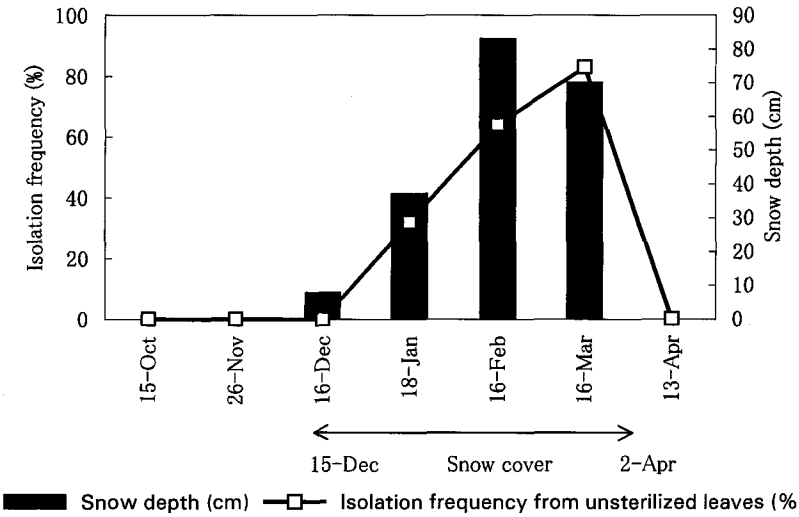

Fig. 3. Change in isolation frequency of *Typhula ishikariensis* biotype B from bentgrass leaves and snow depth in Field C (1993–94).

(Figs. 1–3). These results suggested that the fungal inoculum was present close to the plants, since the mycelial growth rate of *T. ishikariensis* biotype B was estimated to be only 0.80 mm/d on potato dextrose agar at 0°C (Matsumoto, 1989). Matsumoto and Araki (1982) first isolated *T. incarnata* 1 mo or more after snow cover from meadow fescue and perennial ryegrass, much later than in the case *T. ishikariensis*.

Three reasons are considered to account for the earlier isolation of *T. ishikariensis*. 1) *T. ishikariensis* is more virulent than *T. incarnata* and capable of attack before host resistance deteriorates. Nakajima and Abe (1994) found that continuous snow cover gradually reduced the resistance of wheat to pink snow mold over 120 d. Pathogenesis of snow mold depends on the practical absence of antagonists and the gradual decline in host resistance under snow (Matsumoto, 1994). 2) The number of sclerotia surviving in the field and consequent inoculum potential are higher in *T. ishikariensis* than *T. incarnata* (Matsumoto and Tajimi, 1985). 3) It is possible that used meadow fescue and perennial ryegrass were more resistant than bentgrass to snow mold, although their susceptibilities differ largely between cultivars (Hsiang et al., 1999).

Isolation frequency rose in the latter half of snow cover and peaked in February or March. Isolation frequency declined in March in Field A (Fig. 1) presumably due to fluctuation of incubation temperature. During the snow cover period, air temperatures ranged from −6.7°C to 13.9°C in Field A, and from −6.7°C to 11.1°C in Field B. However, ground surface temperature fluctuated only between −0.8°C and 0.7°C in both fields A and B. *T. ishikariensis* was never isolated from leaves in any field 11–20 d after snowmelt.

No information is available on the germination or production of sclerotia under snow cover. Hyphae of *T. ishikariensis* were consistently isolated from bentgrass leaves under snow cover (Figs. 1–3), but sclerotia were never observed from sod leaf samples under snow cover throughout the experiments (data not shown). However, sclerotia attached to the leaves were observed infrequently in all fields after snowmelt. There are two possible explanations of these observations: 1) Sclerotia are produced on or under the ground surface and liable to be overlooked; 2) The fungus produces hyphae rather than sclerotia under snow cover and starts to produce sclerotia just before the snow thaws. The production of sclerotia represents one of the least studied areas in the pathogenic species of *Typhula* (Matsumoto and Tajimi, 1988). The sclerotium is the sole propagule to pass dormancy, and further investigations on the ecology and physiology of sclerotium production should provide us with critical clues to the control of *T. ishikariensis*.

Acknowledgements——I greatly thank Dr. N. Matsumoto, National Institute of Agro-Environmental Sciences, for his critical reading of the manuscript. I am indebted to Dr. H. Kunoh, Mie University, for his cooperation in conducting this study. Thanks are due to Mr. T. Ohta, Mr. M. Takahashi, Hokkaido Green Agricultural Materials Co., Ltd., Mr. T. Egawa, Snow Brand Seed Co., Ltd., and greenkeepers of golf courses for their cooperation in the field experiments. Invaluable support from Dr. H. Shigemitsu and Mr. H. Kobori, Sumitomo Forestry Co., Ltd., is appreciated.

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