積雪条件下におけるイネ科牧草雪腐病菌の行動と発生推移

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Field Observation of Snow Mold Pathogens of Grasses under Snow Cover in Sapporo

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Summary

The behavior of snow mold pathogens of grasses under snow cover was observed for three winters in the fields of Sapporo, Hokkaido. *Sclerotinia borealis* prevailed on upper leaves, while *Typhula incarnata* thrived from lower leaves to upper leaves. *S. borealis* infected just after lasting snow cover had begun and culminated in the first half of winter. *T. incarnata*, on the contrary, prevailed in the latter half of winter although it occasionally infected even before snow cover. *Fusarium nivale* appeared just before thawing. *T. incarnata* monokaryons were occasionally isolated from infected leaves. *T. ishikariensis* was never isolated although its sclerotia were found in the fields.

Introduction

Although many studies have been made on snow mold pathogens of winter cereals[1,10] and forage crops[5,7,9], precise observation in relation to their behavior under snow cover appears not too much. Only a few findings on a certain phase of the life history are available. OZAKI[7] revealed that ascospores of *Scleotinia borealis* Bubák & Vleugel infected immediately after host plants had been covered with snow, but no observation was made as to how the pathogen prevailed and ceased to develop under snow cover. No such observation on *Typhula* spp., another major snow mold pathogen, has been made either. Only the significance of basidiospores[3] and that of sclerotia[4] in the epidemiology of *T. idahoensis* Remsberg have been clarified by experiments. We observed the behavior of snow mold pathogens under snow cover for three successive winters. During the periods, climatic conditions were recorded.

We thank T. SAKURADANI for providing us with the meteorology data of the field. Acknowledgement is due to the Second Forage Crop Laboratory for offering one of the fields.

Materials and methods

Observation was made during the winter months of 1975 - 1978. Two fields of Hokkaido National Agricultural Expriment Station in Hitsujigaoka, Sapporo, where *Typhula* spp. are dominant as snow mold pathogen[10], were selected as sampling sites. One was an experimental field No.23 (referred to as Field A) with meadow fescue (abbr. MF, *Festuca pratensis* L.) sown in 1974, and the other was No.9 (Field B) sown with perennial ryegrass (PR, *Lolium perenne* L.) in 1975 and with MF in 1976.

In the first winter, leaves of MF in Field A were sampled for isolation of the pathogens. The following winter, MF in Field A and PR in Field B were sampled, and sampling of MF in Field B was

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added in the third winter. Ten plants were collected from each field each time and rinsed in water. One upper leaf and one lower leaf were detached from each plant. Five leaf segments about 1 cm long were obtained at ranfom from each leaf. In the first winter, 10 segments were obtained from two green leaves of one plant.

Leaf segments were longitudinally cut into two, and one was sterilized in 70 % ethanol for five sec and in sodium hypochlorite solution (0.125 % active chlorine) for five min, and the other remained unsterilized. Sample segments were rinsed three times in autoclaved water, transferred to potato-dextrose agar, and incubated at 10℃ for 12 - 13 days. Mycelia arising from the segments were compared with reference cultures of the pathogens. For *Typhula* isolates, di-mon mating experiments[2] were conducted as an aid for identification of species.

Records of air temperature and snow depth during the three winters were obtained from the monthly weather report of our experiment station. In the second winter, temperatures of PR stem 2.5 cm above the ground, ground surface, and soil 10 cm deep were recorded in Field B.

Results

Changes in the isolation frequencies of *S. borealis* and *T. incarnata* Lasch ex Fr. are shown in Figs. 1 - 6. The *Typhula* isolates were all proved to be *T. incarnata*. *S. borealis* prevailed in January and February, while *T. incarnata* thrived thereafter until thawing (Figs. 1 - 3). However, these phenomena were indistinct in the third winter (Figs. 4 - 6). Although not shown in the Figs., *Fusarium nivale* (Fr.) Cesati was frequently isolated just after *T. incarnata* had culminated. The microflora on the plants under snow cover excluding the pathogens consisted of *F. roseum* Lk. emnd. Snyd. & Hans., *Cladosporium* sp., *Epicoccum purpurascens* Ehrenb. ex Schlecht, *Mucor* sp., and unidentified fungi. Their isolation frequency tended to diminish while *S. borealis* and *T. incarnata* were prevalent.

S. borealis infected the host plants after lasting snow cover had occurred. The first winter observation indicated that the pathogen infected in Dec. between 15 and 24 (Fig. 1), while snow cover started on Dec. 13. The following winter, the fungus was first isolated from sterilized samples on Dec. 27 in Field A (Fig. 2) and on Dec. 21 in Field B (Fig. 3), and snow cover began on Dec. 10. In the third

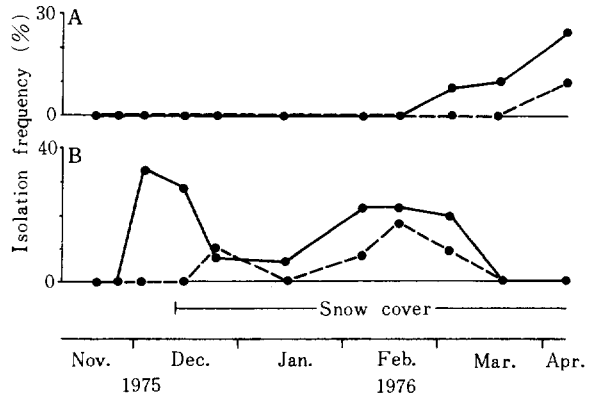

Fig. 1. Change in isolation frequency of *Typhula incarnata* (A) and *Sclerotinia borealis* (B) from green leaves of meadow fescue in Field A. ●——●, unsterilized; ●·····●, sterilized.

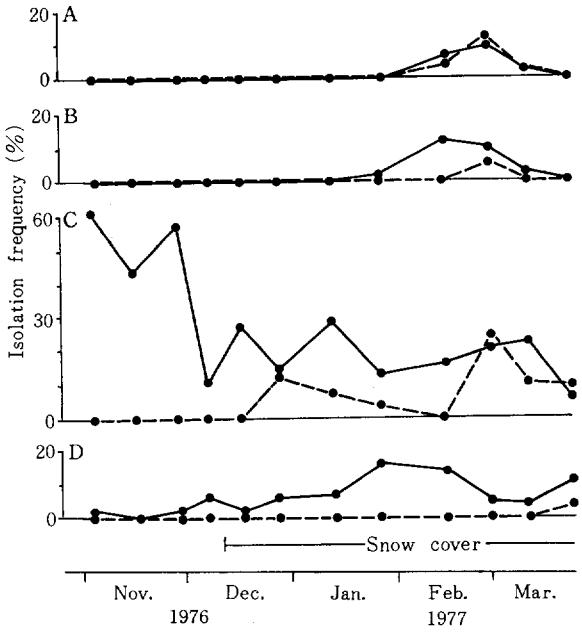

Fig. 2. Change in isolation frequency of *Typhula incarnata* (A, B) and *Sclerotinia borealis* (C, D) from upper leaves (A, C) or lower leaves (B, D) of meadow fescue in Field A. ●——●, unsterilized; ●·····●, sterilized.

winter, *S. borealis* took longer time to infect. Although persistent snow cover began on Dec. 20, 1977, the pathogen was first isolated from sterilized samples on Jan. 11, 1978 in Field A (Fig. 4) and on Jan. 17 in Field B (Fig. 5). The isolation frequency of the pathogen from unsterilized samples was very low during the first half of the third winter. In every case *S. borealis* was isolated more frequently from upper leaves than from lower ones. *T. incarnata* had already infected the lower leaves of PR before lasting snow cover began in the second winter (Fig. 3). The pathogen developed from lower to upper leaves, and this was most evident on PR (Fig. 5). Monokaryons of *T. incarnata* were often isolated from sterilized samples as well as unsterilized ones.

S. borealis and *T. incarnata* on PR exhibited the following transition in the second winter; persistent snow cover started on Dec. 10, and PR leaves which had so far been frozen thawed the following day. The upper leaves were partially water-soaked, and the tips of them were dead. The lower leaves were similar but somewhat yellowish, and *T. incarnata* mycelia were visible on some of them. On Dec. 21, the lower leaves further yellowed. The ground had been frozen until Jan. 11 but was found thawed two weeks later. The lower leaves were mostly dead, and the upper leaves deteriorated with water-soaked portions extended. On Feb. 9, the plants were badly rotten, and the snow layer covering them were stained brownish with plant exudate. Sclerotia of *S. borealis* and *T. incarnata* were found for the first time this day. White immature sclerotia of *S. borealis* surrounded by the mycelium were formed on the base of the leaf blades of the upper leaves, while *T. incarnata* produced sclerotia on the tips of the lower leaves. *S. borealis* sclerotia were darkened on Feb. 21. In March, air temperature rose in the daytime, and the leaves were too badly decomposed to use for isolation.

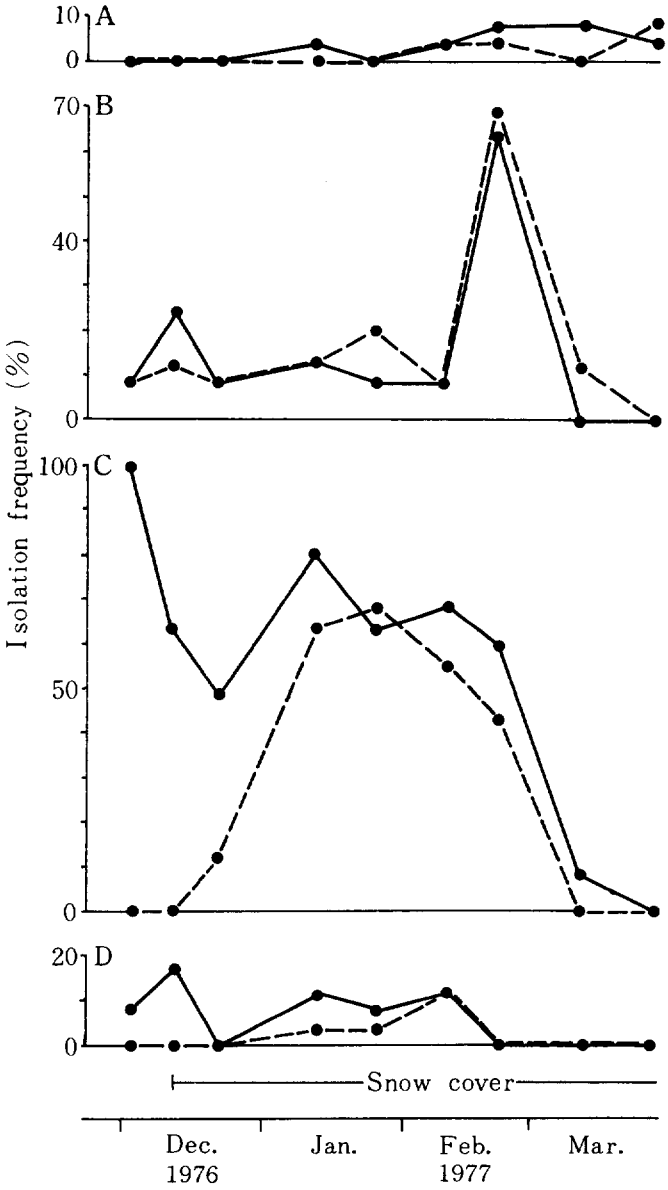

Fig. 3. Change in isolation frequency of *Typhula incarnata* (A, B) and *Sclerotinia borealis* (C,D) from upper leaves (A, C) or lower leaves (B, D) of perennial ryegrass in Field B. ●——●, unsterilized; ●·····●, sterilized.

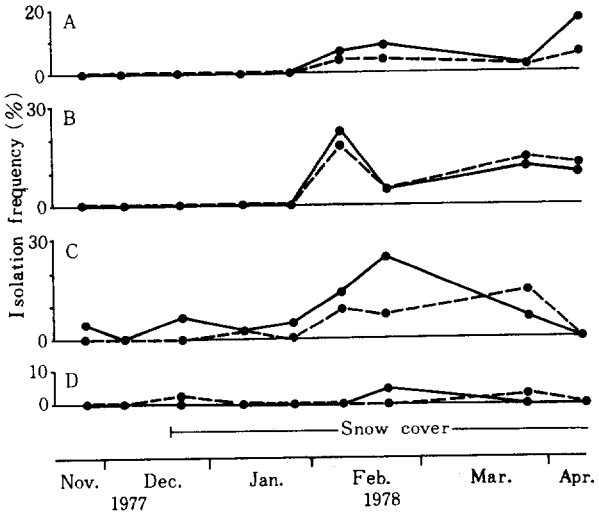

Fig. 4. Change in isolation frenquency of *Typhula incarnata* (A, B) and *Sclerotinia borealis* (C, D) from upper leaves (A, C) or lower leaves (B, D) of meadow fescue in Field A. ● —— ●, unsterilized ; ● ····· ●, sterilized.

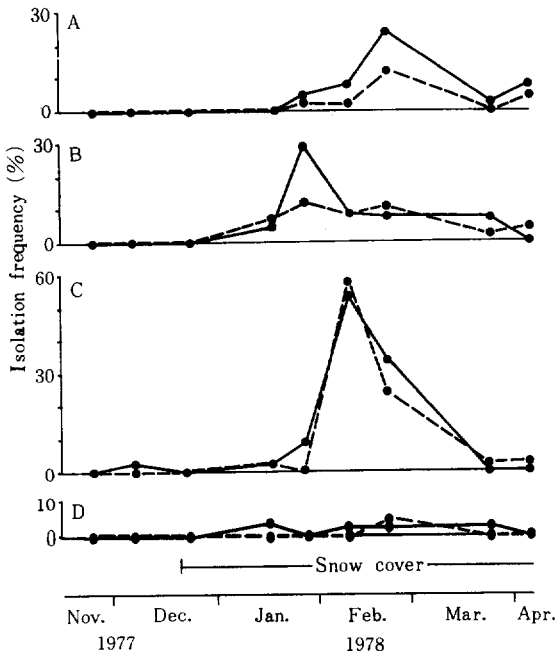

Fig. 5. Change in isolation frequency of *Typhula incarnata* (A, B) and *Sclerotinia borealis* (C, D) from upper leaves (A, C) or lower leaves (B, D) of perennial ryegrass in Field B. ● —— ●, unsterilized ; ● ····· ●, sterilized.

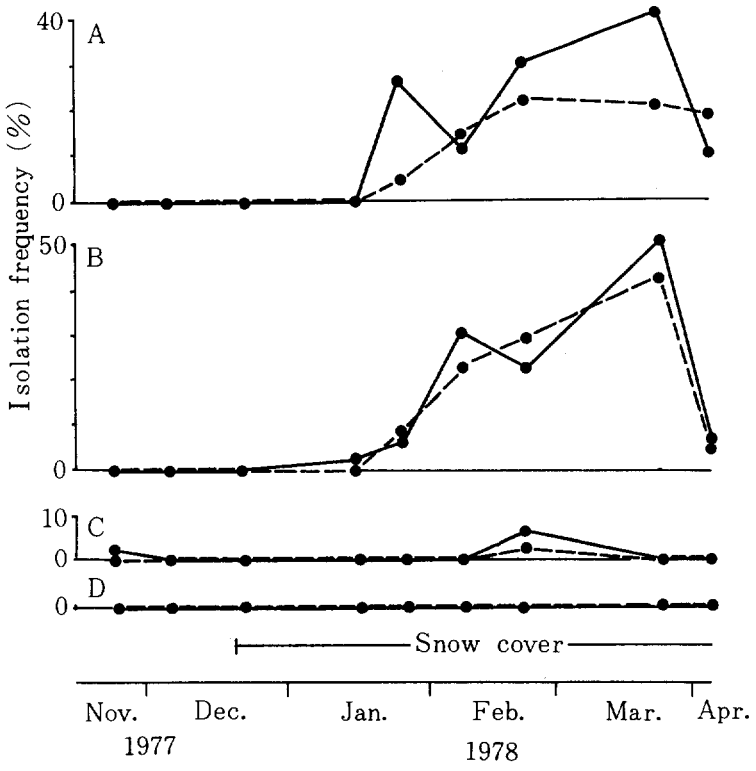

Fig. 6. Change in isolation frequency of *Typhula incarnata* (A, B) and *Sclerotinia borealis* (C, D) from upper leaves (A, C) or lower leaves (B, D) of meadow fescue in Field B. ●——●, unsterilized; ●·····●, sterilized.

Average air temperature was low in Jan. and Feb., and the second winter was coldest (Fig. 7). Changes in snow depth in the first and second winters were similar (Fig. 7), and snow cover was deeper from Jan. to mid-Mar. In the third winter, however, snow cover was not deep until late Jan. and thereafter became deep to last until Apr. (Fig. 7). The stem temperature of PR was sub-zero until mid-Jan. (Fig. 8 A). The ground - surface temperature fluctuated with the change in weather and decreased gradually until the ground was covered with snow(Fig.8 B). The temperature under snow cover was almost 0℃, and then started to rise gradually in mid-Jan., and decreased again to 0℃ about a month before thawing. The soil temperature was always plus: snow cover kept the temperature at 1℃ until it decreased to about 0℃ just before thawing (Fig.8 C).

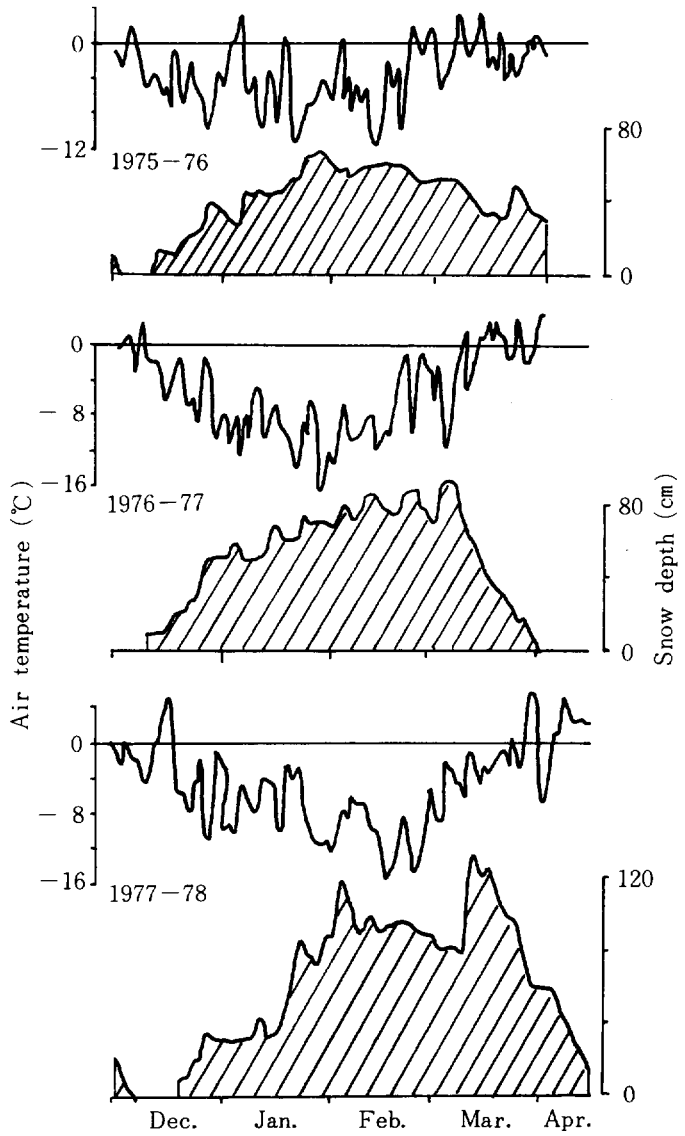

Fig. 7. Changes in air temperature and snow depth during the observation periods.

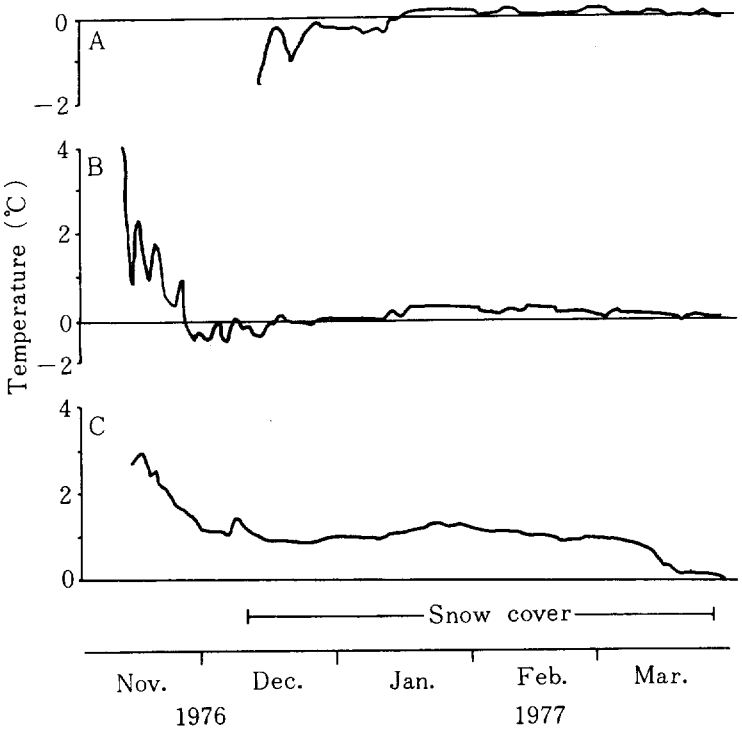

Fig. 8. Changes in temperatures of perennial ryegrass stem 2.5 cm above the ground (A), ground surface (B), and soil 10 cm deep (C) in Field B during the winter months of 1976 - 77.

Discussion

The use of PR enabled us to observe the snow mold pathogens: PR was severely damaged under the milder winter conditions of Sapporo so that *S. borealis* could occur. The results revealed that there were chronological differences in the prevalence of the pathogens as well as geographical differences in distribution shown by TOMIYAMA[10] and LEBEAU & LOGSDON[5]. *S. borealis* was the first snow mold pathogen to prevail, followed by *T. incarnata*, and *F. nivale* appeared just before thawing. *F. nivale* spread too rapidly on the isolation medium used to determine isolation frequency after 12 - 13 days' incubation. The delay of *S. borealis* in reaching maximum prevalence in the third winter brought about vague results. This delay would be attributed to two reasons; 1) *S. borealis* could hardly be detected even on the plant surface during the first half of the winter. Consequently, the scarse exsistance of the pathogen on the host plant might have caused to delay in spreading over the whole plant. 2) Snow cover was about 30 cm deep in Jan., causing the delay of infection. Snow temperature 30 - 40 cm deep is affected by air temperature in Hokkaido[8]. OZAKI[7] suggested that snow cover more than 40 cm deep was essential for the pathogen to prevail.

S. borealis prevailed in the first half of winter, and *T. incarnata* in the latter half, despite that there were no remarkable changes in the temperature of the plant or ground surface, which was approximately 0℃ throughout the season. Such temperature conditions fluctuating within 0.5℃ would not affect the incidence of the pathogens greatly. *S. borealis* infects the host plant which has sustained cold injury and, subsequently, has been covered with snow[7]. On the contrary, *T. incarnata* begins to attack the host plant when it deteriorates[10]. This difference in the pathogenesis between the two pathogens is considered one of the reasons for the difference in the time of culmination in relation to the physiological changes of the host under snow cover. The second-winter observation of PR indicates that lower leaves can be regarded as already-deteriorated before lasting snow cover, while upper leaves may gradually deteriorate due to inhibition of photosynthesis under snow cover. *T. incarnata* may follow the process of host deterioration, showing the translocation from lower to upper leaves.

The fact that *T. incarnata* monokaryons, which are considered derived from basidiospores, were obtained from infected plant may imply the potential of basidiospores as inoculum.

T. ishikariensis S. Imai was never isolated throughout the periods, although its sclerotia were occasionally found in the fields after thawing. This can not be a mere chance but is hard to explain. The niche of *T. ishikariensis* could be considered to lie between *S. borealis* and *T. incarnata*. *S. borealis*, which does not occur so commonly in Sapporo, might have replaced the habitat of *T. ishikariensis* when such a less winter hardy host like PR was involved in snow mold. *T. incarnata*, on the other hand, has established its own niche with a high competitive saprophytic ability[6], and no other fungi might compete for the habitat. This is, we believe, one of the reasons for the ubiquity of *T. incarnata*.

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積雪条件下におけるイネ科牧草雪腐病菌の行動と発生推移

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摘　要

寒冷積雪地帯のイネ科牧草の重要病害である雪腐病を起こす雪腐大粒菌核病菌（*Sclerotinia borealis* Bubák & Vleugel）と雪腐褐色小粒菌核病菌（*Typhula incarnata* Lasch ex Fr.）の積雪下における行動と発生推移をメドウフェスクとペレニアルライグラスを用い，1975年から1978年にわたり札幌の圃場条件下で観察した．*S. borealis* は根雪直後に宿主植物の上位葉から感染し，真冬に至って盛んに蔓延した．本菌が下位葉から分離されることはまれであった．これに対して *T. incarnata* は下位葉から感染してその後上位葉へと移行し，真冬以後に蔓延した．先端が地面に接触している下位葉では根雪前に本菌が既に感染している例も観察された．*T. incarnata* では一核体の菌株もしばしば検出された．この観察期間を通じて雪腐黒色小粒菌核病菌（*T. ishikariensis* S. Imai）は検出されなかったが，融雪後には菌核がわずかに散見された．融雪直前になって紅色雪腐病菌（*Fusarium nivale* (Fr.) Cesati）が蔓延した．