

**Effect of temperature on the production of sclerotia  
by the psychrotrophic fungus *Typhula incarnata* in Poland**

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Isolates of the snow mold fungus *Typhula incarnata* from Radzików, Blonie, near Warsaw in Poland formed many small-sized sclerotia (< 1 mm) at 0 °C. This phenomenon was not observed in other isolates from different locations that have regions with annual snow cover. The formation of small-sized sclerotia by Polish isolates decreased with rising temperature, but the formation of large-sized sclerotia increased.

**Key words:** sclerotium size, snow mold fungi, *Typhula incarnata*

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Kmeny druhu *Typhula incarnata* pocházející z Radzikówa u Blonia poblíž Varšavy v Polsku tvořily při teplotě 0 °C mnoho malých sklerocií (< 1 mm). Tento jev nebyl pozorován u kmenů pocházejících z oblastí, kde sněhová pokrývka vytrvává po delší období. Tvorba malých sklerocií u polských kmenů se snižovala s rostoucí teplotou, zatímco tvorba velkých sklerocií se zvyšovala.

#### INTRODUCTION

Snow mold fungi are psychrophilic or psychrotrophic fungal pathogens of perennial grasses and winter cereals in the Northern Hemisphere (Hsiang et al. 1999, Smith 1986). The genus *Typhula* (Basidiomycota) includes five species of snow mold fungi, *T. incarnata* Lasch ex Fr., *T. ishikariensis* S. Imai, *T. phacorrhiza* (Rich.: Fr.) Fr., *T. trifolii* Rostr. and *T. variabilis* Riess. The first two species have serious economic impact, and *T. incarnata* requires less than three months of snow cover to cause a serious injury to fodder grasses and winter cereals. Injuries caused

by this fungus have been observed even without snow cover (Årsvoll 1973, Bruehl and Cunfer 1971, Detiffe et al. 1981). *T. ishkariensis* is a psychrophilic fungus and is found mainly in areas with more than 5 months of snow cover (Årsvoll 1973, Bruehl and Cunfer 1971).

It has been reported that *T. incarnata* is a versatile pathogen with a different ecological behaviour in different environments (Matsumoto et al. 1995). In contrast, *T. ishkariensis* has evolved several infraspecific taxa adapted to different winter climate (Matsumoto 1992, 1994). Size variation of sclerotia in *T. ishkariensis* is as great as that in *T. incarnata*, but this is due to strain variability and the character is stable within the strain (Matsumoto et al. 2001). Matsumoto and Tajimi (1990) reported that there is a correlation between winter climate and sclerotium size in *T. ishkariensis*. Isolates from snowy regions had large sclerotia, while those from regions with less snow had small sclerotia. This has not been found in *T. incarnata*.

Dynowska (1983, 1984, 1992) reported *T. incarnata*, *T. ishkariensis*, *T. phacorrhiza*, *T. sclerotoides* (Pers.) Fr., *T. subvariabilis* Berthier and *T. variabilis* from Olsztyn in northern Poland. In April 2000, we found *T. incarnata* in Radzików, Blonie, *T. phacorrhiza* and *T. variabilis* in Bartązek, Bartąg in Poland. The winter climate in Poland is variable, and consequently the periods of snow cover are variable (Dynowska 1983, Prończuk and Zagdańska 1993). Polish isolates of *T. incarnata* seem to have adapted to shorter periods of snow cover compared with strains from other regions. In this study, we tried to elucidate the variation in morphological characteristics of *T. incarnata* from Poland, which are considered adaptations to shorter periods of snow cover.

#### MATERIALS AND METHODS

##### Isolation of *Typhula incarnata* from overwintering grass leaves in Poland

Fungal sclerotia were collected from decayed leaves or stems of perennial ryegrass (*Lolium perenne* L.) from the Plant Breeding and Acclimatization Institute in Radzików, Blonie, 30 km east of Warsaw, Poland on May 19-21, 2000. The fungal sclerotia were placed in paper envelopes and dried at room temperature during transportation. In the laboratory (AIST, Japan), the fungal sclerotia were surface-sterilized in 70 % (v/v) ethanol and 0.5 % (as active chlorine) sodium hypochlorite solution and thoroughly washed with sterilized distilled water. They were then cut with sterilized razor blades, placed on potato dextrose agar (PDA, Difco, Becton Dickinson Microbiology Systems, MD, USA) so that cut surfaces were in contact with the agar, and incubated at 5 °C. Mycelia from growing margins of the colonies were transferred to new PDA plates (each 9 cm in diameter).

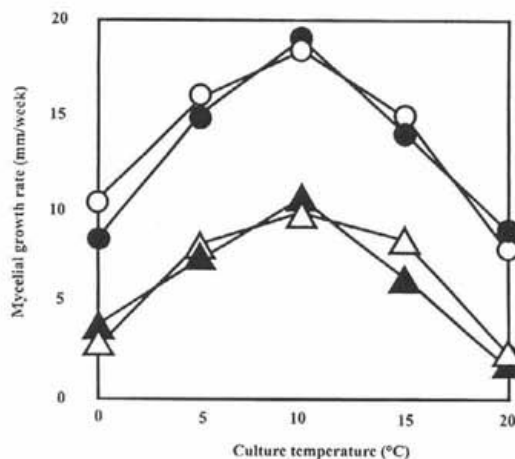


Fig. 1. Thermal dependence of mycelial growth rate of *Typhula incarnata* from Poland and Russia. Open circles: strain R-1, closed circles: strain R-2 from Poland (Radzików, Blonie), open triangles: strain SPB-1, closed triangles: strain SPB-2 from Russia (St. Petersburg).

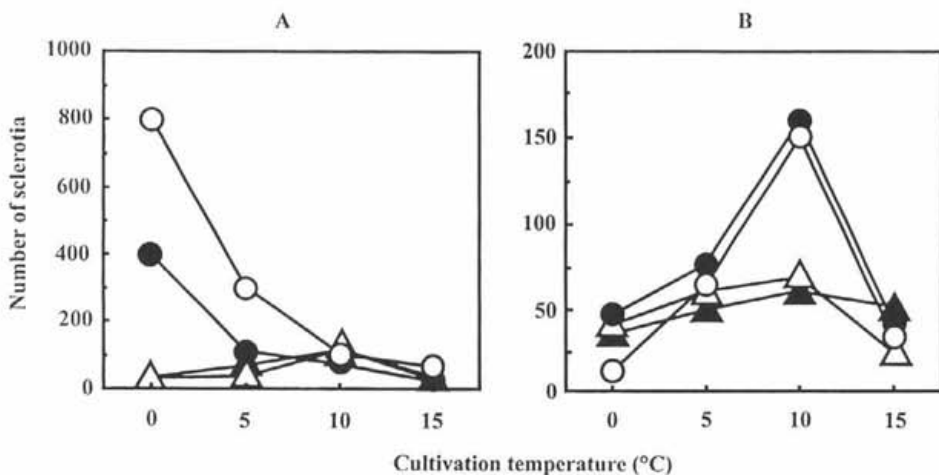


Fig. 2. Effects of temperature on sclerotium formation of cultures of *Typhula incarnata* in Poland and Russia. A. Number of small-sized sclerotia (< 1 mm). B. Number of normal-sized sclerotia (> 1 mm). Open and closed circles: isolates from Poland (Radzików, Blonie), open and closed triangles: isolates from Russia (St. Petersburg).

## Fungal strains in this research

Two strains of *T. incarnata* from different sclerotia collected in Radzików, Poland were used in our study. Another two strains of *T. incarnata* from St. Petersburg in Russia were used for comparison in our research. Isolates from St. Petersburg were prepared from fungal sclerotia in winter wheat from the experimental field of N. I. Vavilov Research Institute of Plant Industry. We obtained these fungal sclerotia on May 2000. All isolates were maintained on PDA slant cultures at 0 °C.

## Growth temperature of mycelia and sclerotium formation

Mycelial discs of 5 mm diameter were cut from the margin of an actively growing colony, transferred to the centre of PDA plates (I. D., 9 cm), and inoculated in duplicate at five different temperatures from 0 to 20 °C. During mycelial growth, the colony diameter was observed daily for up to 21 days after inoculation. The linear mycelial growth rate per week was calculated after the initial log period. After 2 months of incubation at 0, 5, 10 and 15 °C, the number and diameters of sclerotia on each plate were measured, in duplicate.

## RESULTS AND DISCUSSION

Four pathogenic *Typhula* species, *T. incarnata*, *T. ishikariensis*, *T. phacorrhiza* and *T. variabilis*, have been found in Olsztyn in northern Poland (Dynowska 1983, 1984). We found a few infections of *T. incarnata* in overwintering leaves of perennial ryegrass (*Lolium perenne* L.) in Radzików, Błonie, in the central part of Poland. We also collected sclerotia of *T. phacorrhiza* and *T. variabilis* from perennial ryegrass in Bartązek, Bartąg, northern Poland. However, *T. ishikariensis* was not found in those two areas, probably because it needs a longer period (more than 5 months) of snow cover (Årsvoll 1973, Bruehl and Cunfer 1971). Therefore, *T. ishikariensis* might not have adapted to the current warm climate in Poland (Prończuk and Zagdańska 1993).

The sclerotia of *T. incarnata* collected from Radzików, Błonie were red to dark brown in colour and globose to oval in shape and were formed on the surface and inside leaves. The average size of 100 sclerotia was 0.51–0.79 (av. 0.62) × 0.39–0.47 (av. 0.44) mm. The morphology of sclerotia was almost the same as that described by Dynowska (1983, 0.45–3.5 × 0.5–2 mm), but the average sclerotium size was smaller than those in other localities (3–4 mm: Berthier 1976 from unknown localities, 0.5–4.5 × 0.5–2 mm: Ito 1955 from Japan, 0.5–2 × 1.5–4 mm: Remsberg

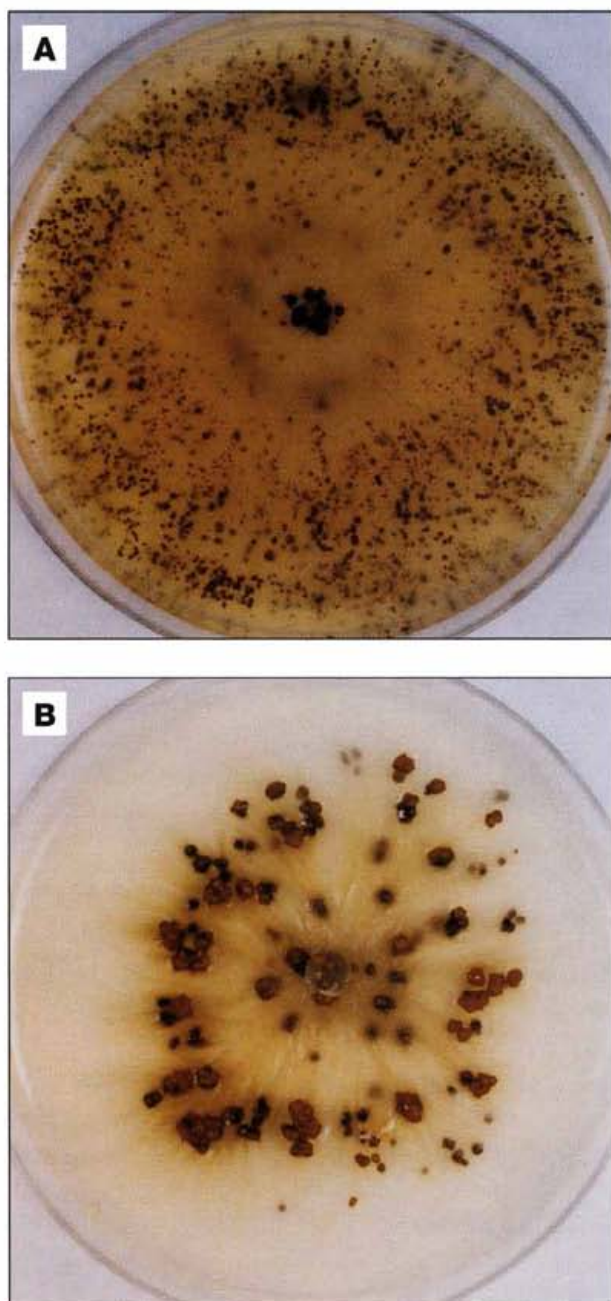


Fig. 3. Morphology of colonies of *Typhula incarnata* on PDA, grown at 0 °C over a period of 1 month. A. Strain R-1 from Poland (Radzików, Blonie). B. Strain SPB-1 from St. Petersburg, Russia.

1940 from Finland). Host plants of this fungus had been only slightly damaged by fungal infections, suggesting that snow mold diseases caused by *T. incarnata* do not have a great impact on overwintering grasses in Radzików, Błonie.

Figure 1 shows the thermal dependence of mycelial growth rates of several isolates collected in Poland and one other locality (St. Petersburg, Russia). Isolates from Poland and Russia showed the same optimum growth temperature, 10 °C. The same results were obtained from isolates from other countries (e.g. Smith 1986). However, mycelial growth rates of Polish isolates were faster than those of Russian isolates. In addition, Polish isolates formed many small-sized sclerotia (< 1 mm) on PDA at 0 °C (Fig. 2A and Fig. 3A). The same results were obtained by using other cultural media such as corn meal agar, lima bean agar, malt extract agar, oat meal agar and tomato juice agar (data not shown). We did not observe a high production of small-sized sclerotia from other isolates of *T. incarnata* in our cultural collection from various localities such as Japan, Russia and Nordic countries (Faroe Islands, Greenland, Iceland and Norway). Wu and Hsiang (1999) and Kim et al. (1992) reported that *T. incarnata* produced abundant sclerotia at temperatures ranging from 0 to 15 °C. A similar pattern was observed in the formation of normal-sized sclerotia (> 1 mm) of Polish isolates (Fig. 2B). However, the formation of small-sized sclerotia decreased with increasing cultivation temperature.

*T. ishikariensis* biotype C, which is highly adapted to the condition of short snow cover in Japan, is also known to produce small-sized sclerotia (Honkura et al. 1986). However, the sclerotium size of *T. ishikariensis* is not dependent on environmental conditions including culture temperature. On the other hand, sclerotium production of Polish isolates of *T. incarnata* was greatly changed by low temperatures. Small and large sclerotia of *T. incarnata* from Poland did not show any differences in morphological characteristics (rind pattern and basidiocarp formation, data not shown). Therefore, small-sized sclerotia of *T. incarnata* probably had the same ecological role as that of large-sized sclerotia.

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