Fertile sporophore production of *Typhula phacorrhiza* in the field is related to temperatures near freezing

Y. Yang, F. Chen, and T. Hsiang

Abstract: Two field tests and one lab test were conducted to examine the environmental factors affecting sporophore production in *Typhula phacorrhiza* and to compare these results with those documented for *T. ishikariensis* and *T. incarnata.* In the 2001 lab test where lighting, soil moisture, and soil–sand media were tested in 50 mL screw-cap tubes incubated at 4 °C, the limiting factor for *Typhula* sporophore production was found to be moisture. In the fall 2001 field test, 100 sclerotia of six isolates from three *Typhula* spp. were placed into pots filled with a sand and soil mixture. The pots were monitored weekly, and maximum sporophore production for all six isolates and for watered and unwatered pots was observed at 11 weeks, which was soon after mean daily temperatures fell below 0 °C. In the second field test in fall 2003, five isolates of the three species were tested with similar procedures, but peak sporophore production was observed after 6 weeks, and again only after mean daily temperatures fell below 0 °C. In the field, sporophore production of *T. phacorrhiza* seems to require the same environmental cues as those of *T. ishikariensis* or *T. incarnata*, namely high moisture and temperatures near freezing.

Key words: snow mold, fruiting, basidiocarp, cold.

Résumé : Deux tests sur le terrain et un test en laboratoire ont été réalisés afin d'examiner les facteurs environnementaux qui affectent la production de sporophores chez *Typhula phacorrhiza* et comparer ces résultats avec les résultats documentés chez *T. ishikariensis* et *T. incarnata*. En 2001, où l'effet de l'éclairage, de l'humidité du sol et d'un mélange terre–sable a été testé en laboratoire dans des tubes vissés de 50 mL incubés à 4 °C, il s'est avéré que le facteur limitant la production de sporophore de *Typhula* était l'humidité. Lors des études sur le terrain menées à l'automne 2001, 100 sclérotes de six isolats de trois espèces de *Typhula* ont été placés dans des pots remplis d'un mélange terre–sable. Ces pots ont été examinés hebdomadairement et la production maximale de sporophores pour les six isolats cultivés dans des pots arrosés et non arrosés a été obtenue après 11 semaines, soit peu après la période où les températures moyennes durant le jour descendent sous 0 °C. Lors d'un deuxième test sur le terrain, à l'automne 2003, cinq isolats de trois espèces ont été testés selon le même protocole; cependant la production maximale de sporophore est survenue après 6 semaines, encore après la chute des températures moyennes durant le jour sous 0 °C. Sur le terrain, la production de sporophores de *T. phacorrhiza* semble requérir les mêmes signaux environnementaux que *T. ishikariensis* ou *T. incarnata*, soit une humidité élevée et des températures avoisinant le point de congélation.

Mots clés : anthracnoses, fructification, basidiocarpe, froid.

[Traduit par la Rédaction]

Introduction

The basidiomycetous genus *Typhula* contains over 60 species (Remsberg 1940), but there are two species of major economic importance, *T. ishikariensis* Imai and *T. incarnata* Lasch ex. Fries (Bruehl and Cunfer 1971; Detiffe et al. 1981), which cause diseases of grasses and cereals. A third, *T. phacorrhiza* Fries, has been implicated as a biological control agent for the first two (Hsiang et al. 1999). Variants of *T. ishikariensis* include var. *ishikariensis*, var. *canadensis* (also known as *T. canadensis*), and var. *idahoensis* (also known as *T. idahoensis* Remsberg). *Typhula ishikariensis* is probably a complex of biological species (Matsumoto 1997; Hsiang et al. 1999), but these variants did not show genetic differences at a species level using various molecular markers (Hsiang and Wu 2000).

Sexual spore-producing structures of *Typhula* species are called clavula because of the club-shaped head on a narrow cylindrical stipe. These sporophores arise from sclerotia and mature to produce basidiospores at the fertile head (Remsberg 1940; Matsumoto and Tajimi 1985). For *T. ishikariensis*, there is usually just one sporophore per sclerotium (Hsiang et al. 1999), while there may be multiple sporophores per sclerotium for other species (Remsberg 1940; Koske 1975; Berthier 1976). Although sclerotia of *Typhula* spp. can be found in abundance in nature, the sporophores are seldom observed (Remsberg 1940). Among *Typhula* spp., sporophores

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Table 1. *Typhula* species and strains used in this study.

Isolate	Species	2001 field test	2001 lab test	2003 field test
M07×M24	*T. phacorrhiza*	—	—	Yes
M44×M53	*T. ishikariensis*	—	—	Yes
M78×M87	*T. incarnata*	—	—	Yes
M78×M99	*T. incarnata*	—	—	Yes
M90×M99	*T. incarnata*	—	—	Yes
TN20	*T. incarnata*	Yes	Yes	—
TN45	*T. incarnata*	Yes	Yes	—
TP94613	*T. phacorrhiza*	Yes	Yes	—
TP94671	*T. phacorrhiza*	Yes	Yes	—
TS94072	*T. ishikariensis*	Yes	Yes	—
TS95216	*T. ishikariensis*	Yes	Yes	—

Note: Except for isolate TS94072, which originated from Washington State, all other isolates came from southern Ontario.

of *T. incarnata* are most frequently observed perhaps because of their conspicuous delicate pink appearance up to 2 cm long and their association with economically important hosts, such as turfgrasses (Hsiang et al. 1999).

Sclerotia of *Typhula* spp. can germinate carpogenically to produce sporophores or myceliogenically to produce hyphae (Coley-Smith and Cooke 1971). In years that are dry and warm prior to snowfall, sclerotia of *T. ishikariensis* and *T. incarnata* are thought to germinate under snowcover to produce infective mycelia (Jackson and Fenstermacher 1969; Vargas 1994). Conditions that favor the germination of sclerotia of *T. incarnata* include cool, wet conditions in the fall (Remsberg 1940; Jackson and Fenstermacher 1969), with sporophore production favored by short wavelength light (Remsberg 1940). Sporophores of *T. incarnata* can persist for several weeks and can even resume spore production after a dry period (Jackson and Fenstermacher 1969). Sporophores of *T. incarnata* have been observed from September to December in northern temperate regions. Sporophores of *Typhula* spp. are usually found during rainy cold weather in the fall or, for some species such as *T. gyrans*, in early spring (MacDonald 1934; Remsberg 1940). Sporophores of *T. phacorrhiza* have been observed in the fall in nature (Remsberg 1940).

Remsberg (1940) reviewed the early literature on *Typhula* spp. and concluded that the optimum conditions for sporophore production are cold weather and abundant moisture. Cunfer and Bruehl (1973) investigated the environmental conditions that affect the formation of sporophores of *T. idahoensis* and found that sporophores could form rapidly when sclerotia were placed outdoors in October, if the weather remained cool and moist, with sporulation reaching a peak just prior to the start of winter snowcover. Koske (1975) found that sclerotia of *T. erythropus* Fr. could achieve over 95% carpogenic germination at temperatures of 4 or 10 °C. Kawakami et al. (2004) demonstrated that low temperatures (10 °C day and 5 °C night) and high humidity promoted stipe elongation of *T. ishikariensis*, while light exposure and moderate day length (8 h of light per day) promoted formation of fertile heads under laboratory conditions. Remsberg (1940) found that short wavelength light from 2700 to 3250 Å (1 Å = 0.1 nm) would stimulate sporophore production of several *Typhula* spp. The light intensity required for sporophore development of *T. idahoensis* is very low, since straw-covered sclerotia placed in the field could still produce sporophores (Cunfer and Bruehl 1973). Light is required to initiate and allow maturation of sporophores in other basidiomycetes (Ballou and Holton 1985; Ellis et al. 1999). However, darkness is also necessary for normal development of sporophores in *Coprinus cinereus* (Schaeff.) Gray (Ballou and Holton 1985). Aeration may also affect sclerotial germination. Sanogo and Pennypacker (1997) found that sclerotia of *Colletotrichum coccodes* (Wallr.) Hughes in Petri dishes sealed with Parafilm germinated primarily to produce mycelium, whereas in unsealed Petri dishes, sclerotial germination was strictly carpogenic. Sporophore development in basidiomycetes is often induced after drastic changes in the environment (Kues and Liu 2000).

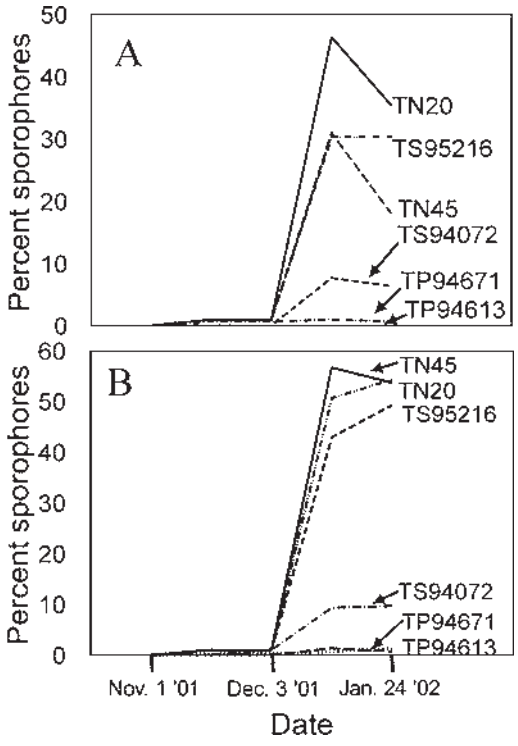
In culture, sclerotia of *Typhula* species are easily produced, but fertile sporophores are seldom found (Remsberg 1940; Berthier 1976). Kawakami et al. (2004) were able to induce fertile sporophore production in culture, and found that the use of low temperatures (5–10 °C), lower light intensity (45 μE m^{-2} s^{-1}), and an unsterilized soil promoted the formation of fertile sporophores and allowed for basidiospore production in less than 5 weeks. Cunfer and Bruehl (1973) examined the production of basidiospores of *T. idahoensis* under field conditions and found that a minimum of 35 days was required for sclerotia to produce mature sporophores under favorable conditions, which they described as wet and cool. The objective of the present study was to examine the environmental factors affecting sporophore production in *T. phacorrhiza* and compare them to those documented for *T. ishikariensis* and *T. incarnata.*

Materials and methods

Fungal isolates

The fungal isolates used in this study are listed in Table 1. Stock cultures were maintained for short periods at 4 °C on potato dextrose agar (PDA) slants (Difco Laboratories, Detroit, Michigan) or for long term at –22 °C on mixed grains (an equal mixture of wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and corn (*Zea mays* L.)). To produce sclerotia, the isolates were cultured at 10 °C for 8–10 weeks, and the sclerotia were picked off each plate and stored at 4 °C until needed for the experi-

Fig. 1. Production of sporophores of three species of *Typhula* (TN = *T. incarnata*, TP = *T. phacorrhiza*, TS = *T. ishikariensis*) under watered (A) or unwatered (B) conditions placed outdoors in fall 2001. One hundred sclerotia were placed in each pot with three replicate pots per isolate.



ments. Harvested sclerotia were stored less than 1 week before use.

Sand–soil preparation

A golf course top-dressing sand (Hutchison sand mix, pH 8.8) was combined with a Fox Sandy Loam soil obtained from the University of Guelph Arboretum (Guelph, Ontario). The soil had been passed through a 3 mm mesh to remove large particles. For the field experiments, the sand and soil were mixed 1:1 (*v*/*v*) and placed into pots (10 cm diameter) filled to a depth of 4 cm. The distance between the soil surface and the top of the pot was 5 cm. For laboratory experiments, 15 mL of the sand–soil mixture or sand alone was placed into 50 mL polypropylene centrifuge tubes with a screw-cap lid (Fisherbrand 06-443-20, Fisher Scientific, Nepean, Ontario). Water was added to tubes containing sand to achieve gravimetric soil moisture contents of 2% or 20%. Soil moisture content was determined by taking a sample of the sand or the soil–sand mixture with the water added and then air-drying it at 80 °C for 24 h, and calculating soil moisture as (wet weight – dry weight)/wet weight.

Effect of environmental conditions on sporophore production

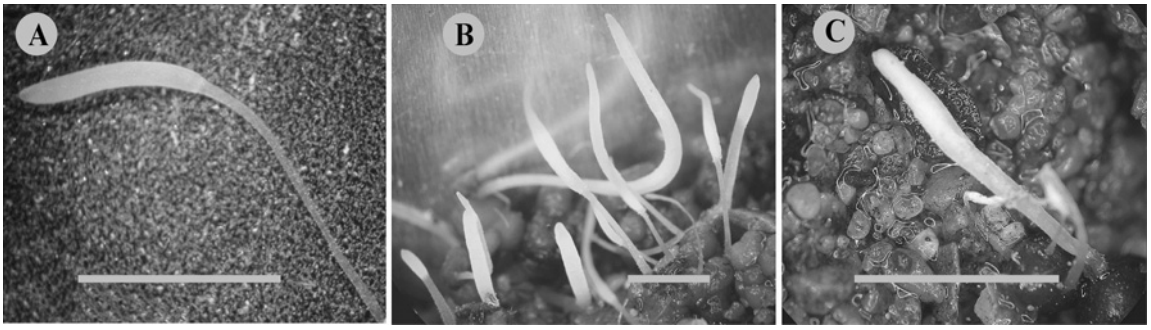
To examine the effect of moisture on the sporophore formation in the field, 100 sclerotia from 8-week-old cultures were seeded onto the surface of each pot, described above. Two layers of cheesecloth were placed over the top and sides of each pot to allow rainfall and filtered light to pass through and also to wick water up from the trays to maintain a moist environment. The pots were placed outdoors on 18 October 2001. One set of pots was placed into plastic trays and watered periodically to ensure the soil and cheesecloth remained moist. The other set of pots did not receive additional water. Sporophore development was observed weekly for 16 weeks, and the number of sclerotia with sporophores was recorded. Only sporophores with unwithered full heads were counted. Sporophores were sampled periodically to check for basidiospore production. Six isolates were tested here, two of each species, with three replicate pots per isolate (Table 1, 2001 field experiment).

A lab experiment (Table 1, 2001 lab experiment) was set up in a 4 °C walk-in incubator to examine the effects of light, moisture, air exchange, soil sterilization, and soil content on sporophore production. Five treatments were replicated under dark and light conditions: (1) sand–soil mix; (2) sterilized sand–soil mix, autoclaved twice with a 24 h interval; (3) sand–soil mix but with the caps tightly screwed on and wrapped with Parafilm to prevent air exchange (all other tubes were loosely capped); (4) air-dried sand with 0.5 mL of autoclaved water; and (5) air-dried sand with 7.0 mL of autoclaved water. Each 50 mL tube was filled with 15 mL of the appropriate sand and (or) soil mix, the mix leveled while the tubes were in an upright position, and sclerotia were placed on the sand and (or) soil surface. For *T. incarnata* and *T. ishikariensis*, 50 sclerotia were placed in each tube, while only 10 sclerotia per tube were used for *T. phacorrhiza* because of their larger size. The tubes were kept upright in styrofoam containers, and six replicates were made for each isolate by treatment combination. Half of the tubes were placed under a fluorescent light (50 μE m^{-2} s^{-1}, 10 h of light, 14 h of dark); the other half were wrapped with aluminum foil for dark conditions. The tubes were checked weekly for 16 weeks, and the number of sporophores per tube was recorded. Only sporophores with unwithered full heads were counted. Sporophores were sampled periodically for basidiospore production.

Effect of sclerotial pretreatment on sporophore production

The effects of pre-treatment on sclerotial germination in the field were investigated starting in early October 2003 for five isolates of the three *Typhula* species (Table 1, 2003 field experiment). The first two treatments involved sclerotia from 8-week-old PDA cultures grown at 10 °C. One hundred of these sclerotia were placed into each pot (8 cm diameter) filled with sand–soil mix as described above. Half of the pots were incubated at 22 °C for 2 weeks without watering, and the other half at –22 °C for 2 weeks. These pots were then moved outdoors. A third treatment used freshly harvested sclerotia that had been grown at 10 °C for 10 weeks on PDA. These were placed into plots with the same sand–soil mix described above, and placed outdoors. All treatments were replicated four times, and the pots were placed in trays and exposed to outdoor environmental conditions with temperatures ranging from 10 to 15 °C during the day and 0 to 7 °C at night. They were watered periodically to ensure that the soil remained moist. The pots were evaluated weekly for 6 weeks, and the number of sclerotia bearing sporophores from the 100 sclerotia seeded per pot was

Fig. 2. Sporophores of (A) *Typhula incarnata*, (B) *T. phacorrhiza*, and (C) *T. ishikariensis*. Scale bar at bottom of each picture represents 5 mm.



recorded. Only sporophores with unwithered full heads were counted. Sporophores were sampled periodically for basidiospore production.

Data analyses

Data were subjected to analysis of variance using SAS® PROC GLM (SAS Institute Inc., Cary, North Carolina). When treatment effects were significant in the analysis of variance ($p \leq 0.05$), means were separated by the test of least significant difference (LSD, $p = 0.05$).

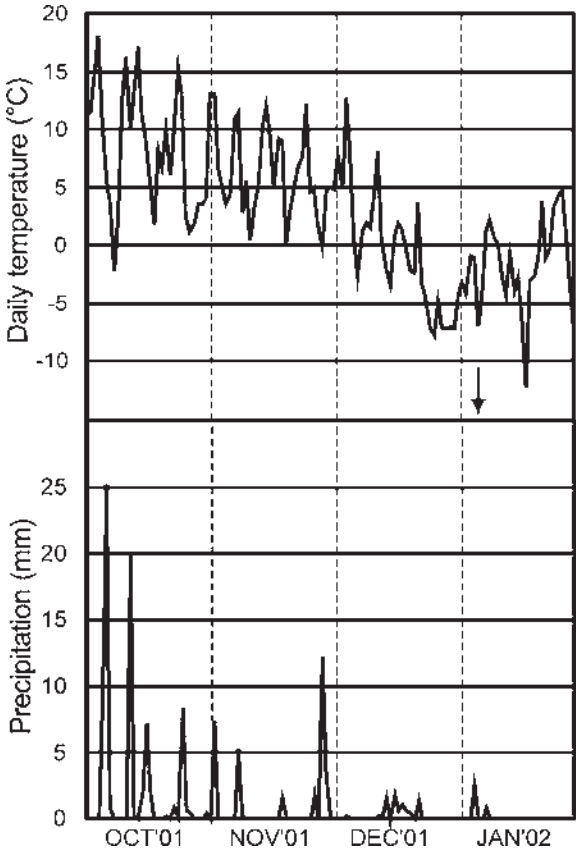
Results and discussion

Sporophore production in the field

The six isolates showed different levels of sporophore production in both the watered and unwatered outdoor tests in 2001 (Fig. 1). In general, sclerotia of *T. incarnata* produced the most sporophores and *T. phacorrhiza*, the least. Examples of *Typhula* sporophores are presented in Fig. 2. The environmental conditions that triggered sporophore production in the field seemed to be similar for all three *Typhula* species, since peak production of sporophores was seen on 3 January 2002 for all three species, although sporadic sporophore production was first seen in late November. Sporophore counts decreased after this peak, probably because some sporophores had already completed their sporulation and shrivelled up. The peak production of sporophores was seen within 2 weeks of mean daily temperatures falling below 0 °C (Fig. 3). Some snowfall had occurred in December (<5 cm), but it melted by early January, with no long term snowcover until mid-January (Cook and Hsiang 2003).

Cunfer and Bruehl (1973) found that regardless of when sclerotia of *T. idahoensis* were first placed outdoors (starting at 10-day intervals from early September), the peak production occurred in mid-November in eastern Washington State. Bruehl and Cunfer (1975) recorded that sporophores of *T. idahoensis*, *T. ishikariensis*, and *T. incarnata* were produced from 10 to 15 of November in 1969 to 1971, even though the sclerotia were placed outdoors in late summer. According to the Web site with historical weather data of eastern Washington State (http://www.wunderground.com/US/WA/Pullman.html), mid-November is when the mean temperatures in autumn first fall below 0 °C in that area. These data from eastern Washington State also support a connection between temperatures falling below 0 °C and the production of sporophores in the field.

Fig. 3. Environmental conditions at Guelph, Ontario, from October 2001 through January 2002, showing average daily temperatures and precipitation. The downward arrow indicates the time when peak sporophore production was observed.



Sporophore production in culture

In screw-cap tubes incubated at 4 °C in a walk-in growth chamber, fertile sporophores were also produced (Table 2). Tubes kept under a fluorescent light led to significantly fewer sporophores than those kept in the dark (Table 2). Koske (1975) found that darkness inhibited hymenial development in *T. erythropus*. Kawakami et al. (2004) found that darkness inhibited fertile head development in *T. ishikariensis*, while

Table 2. Average sporophore production of six *Typhula* isolates on a soil–sand mixture.

Treatment	Content	Autoclaved	Moisture content (%)*	% Sporophores per tube†	
				Light	Dark
1	Soil & sand	No	8.5	5.1b	14.1a
2	Soil & sand	Yes	7.0	2.1bc	12.8a
3	Soil & sand	No	8.5	10.8a	12.8a
4	Sand	No	2.0 (plus 0.5 mL H_2O)	0.1c	5.8b
5	Sand	No	20.0 (plus 7.0 mL H_2O)	12.4a	17.6a

Note: Isolates were incubated at 4 °C for 11 weeks in 50 mL screw-cap tubes containing 15 mL of a soil–sand mixture.
*The gravimetric soil moisture content was measured at the start of the experiment, and no additional water was provided during the course of the experiment. Except for Treatment 3 where the tubes were tightly capped and sealed with Parafilm, the tubes of the other treatments were loosely capped.
†These means are the average of six replicate tubes for six isolates from three *Typhula* species, and means within a column followed by the same letter are not significantly different at $p = 0.05$. The light was produced by a fluorescent light and the tubes were exposed to a 50 μE m^{-2} s^{-1} light intensity. The tubes were wrapped with aluminum foil for the dark treatment.

Table 3. Sporophore production of six *Typhula* isolates incubated in the dark.

Treatment	% Sporophores per tube					
	TN20	TN45	TP94613	TP94671	TS94072	TS95216
1	27.3a	32.7a	3.3a	6.7a	2.0a	12.7b
2	27.3a	37.3a	0	0	0	12.0bc
3	28.0a	32.0a	0	10.0a	2.0a	4.7bc
4	12.0a	19.3a	0	0	0	3.3c
5	29.3a	25.3a	0	6.7a	9.3b	34.7a

Note: Isolates were incubated at 4 °C in 50 mL tubes for 11 weeks. The descriptions of each treatment are listed in Table 2. Each mean is the average of six replicates, and means within a column followed by the same letter are not significantly different at $p = 0.05$.

Table 4. Effect of sclerotial pretreatment on sporophore production of five *Typhula* isolates.

Sclerotial treatment	% Sporophores per pot				
	M07×M24 *T. phacorrhiza*	M44×M53 *T. ishikariensis*	M78×M87 *T. incarnata*	M90×M99 *T. incarnata*	M78×M99 *T. incarnata*
Pre-incubated at 22 °C for 2 weeks	5.8a	30.8a	41.7a	27.5a	32.5a
Fresh sclerotia grown at 10 °C for 10 weeks	7.5a	40.0a	43.0a	43.3a	42.5a
Pre-incubated at –22 °C for 2 weeks	2.5a	26.7a	28.3a	26.7a	28.3a

Note: The *Typhula* isolates were seeded with 100 sclerotia in 10 cm pots and were placed outdoors on 4 October 2003 and assessed on 13 November 2003. Each mean is based on four replicate pots, and means within a column followed by the same letter are not significantly different at $p = 0.05$.

lower light levels (46 μE m^{-2} s^{-1}) promoted fertile head development more than higher light levels (137 μE m^{-2} s^{-1}). Cunfer and Bruehl (1973) found that the light intensity required for sporophore development is very low. Perhaps the weekly exposure to diffuse light when the aluminum-wrapped tubes were being checked for sporophore formation was sufficient to allow development of sporophores in our test, whereas Kawakami et al. (2004) found that sporophores did not mature in darkness.

For tubes that were kept in the dark (Table 3), the two isolates of *T. incarnata* seemed to be unaffected by the range of moisture conditions and soil content in this test, with all conditions allowing for successful production of sporophores. For the two isolates of *T. phacorrhiza* kept in the dark, sporophore production was inhibited in this test, with several treatments showing no sporophore production at all. Perhaps *T. phacorrhiza* is more sensitive to light effects than the other species. For the two isolates of *T. ishikariensis* kept in the dark, moisture content seemed to be the major limiting factor, with Treatment 5, containing the highest moisture level at the start of the test (20%), showing significantly higher levels of sporophore production than the other treatments that ranged from 2.0% moisture (significantly lowest sporophore production for *T. ishikariensis* isolate TS95216) to 8.5% moisture.

Sclerotial pretreatment

Before being placed outside in pots, sclerotia of five *Typhula* isolates (Table 1, 2003 field experiment) were pretreated at 22 °C for 2 weeks, –22 °C for 2 weeks, or freshly picked from PDA plates used to produce sclerotia. There were no significant differences in sporophore production among any of these pretreatments (Table 4), with average percent sporophore production of 5.3%, 32.5%, and 34.9% for the isolates of *T. phacorrhiza*, *T. ishikariensis*, and *T. incarnata*, respectively. Sporophores for all three species showed peak

production after 6 weeks of incubation outdoors (13 November 2003), but the difference between this test and the 2001 field experiment where sporophore production required 11 weeks (18 October 2001 – 3 January 2002) was that the onset of winter was sooner in 2003 than in 2001. In both field experiments, sporophore peak production occurred soon after average daily temperatures fell below 0 °C (Figs. 3 and 4).

Basidiospore production

From every experiment, a subsample of sporophores was examined for the presence of basidiospores. All mature sporophores were found to bear basidiospores. Also stipes from germinating sclerotia were often observed in culture on PDA plates or on mixed-grain media stored at 4 °C in dark incubators, but none of these were found to be fertile. In stock cultures of isolates TP94671, TS9216, TN45, and TN20 that were incubated at 10 °C for up to 16 weeks, stipe production averaged 27.0%, 68.8%, 72.5%, and 67.5%, respectively, out of hundreds of sclerotia, but none of the ones sampled were found to bear basidiospores. Remsberg (1940) noted that in many *Typhula* spp., atypical sporophores may be formed in culture from sclerotia, stromatic crusts, or even mycelial mats, but these usually remain sterile.

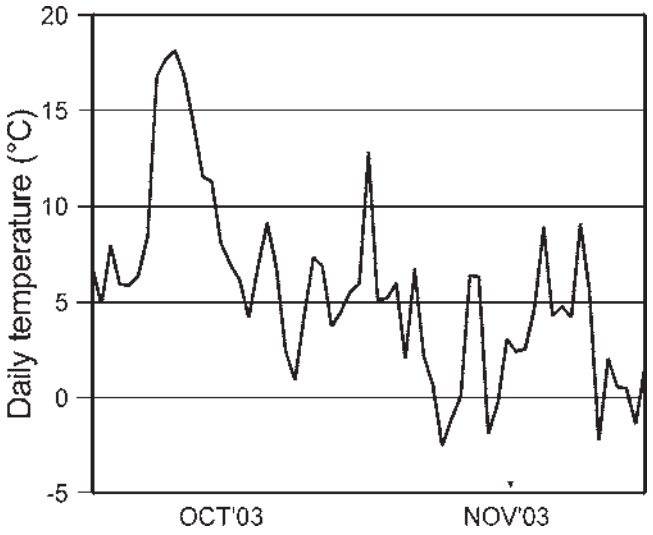
Moisture and temperature effects

The results from the pot tests in the field and the tube tests in the laboratory indicate that sporophores of *Typhula* species can be formed under a variety of conditions in culture, with moisture playing a key role in the extent of sporophore formation. However, in our tests, a much higher level of sporophore production was found with sclerotia incubated outdoors than in the lab. Kawakami et al. (2004) were able to induce over 80% sporophore formation of *T. ishikariensis* within 4 weeks in Petri plates incubated at 5–10 °C. None of our isolates under any of the test conditions achieved this level of sporophore formation, with less than 40% in laboratory tests and less than 60% in field tests for our *Typhula* isolates.

One of the limiting factors on sporophore production in our lab tests was moisture level. The soil moisture levels in our laboratory studies more closely approach the moisture levels found in field soils where sclerotia of *T. incarnata* and *T. ishikariensis* are often found. For example, turfgrass putting greens are normally composed of 80% sand and 20% peat by weight, and the moisture levels found in these soils in autumn is usually less than 20%, and field capacity for such soils is less than 30% (Carey 2004). In previous laboratory studies (Cunfer and Bruehl 1973; Kawakami et al. 2004), the sclerotia were placed in Petri plates where relative humidity may have approached 100%, as evidenced by the production of hyphae from infertile sporophores. We did not observe hyphal strands produced from sporophores in our laboratory tube experiment.

A strong relationship was found between the onset of sporophore production in the field and air temperatures falling to 0 °C, both from data presented in this study and from a re-analysis of data from other studies (Cunfer and Bruehl 1973; Bruehl and Cunfer 1975). Snow mold fungi, such as many of the *Typhula* spp., are adapted for growth under snowcover, and they are poor competitors against other fungi, except when temperatures are near freezing (Hsiang et al. 1999; Snider et al. 2000). Perhaps one of the triggers for carpogenic germination in the field is when temperatures fall to 0 °C. As air temperatures approach 0 °C, the chances of snowfall increase. snowcover would limit the dispersal of propagules, so an evolutionary strategy for psychrophilic organisms would be to disperse their propagules right before snowfall, when cold temperatures also limit competition.

Fig. 4. Maximum and minimum daily temperatures at Guelph, Ontario, from October 2003 through November 2003. The downward arrow indicates the time when peak sporophore production was observed.



Although sclerotia of *T. ishikariensis*, *T. incarnata*, and *T. phacorrhiza* are abundant in their respective habitats after snowmelt, particularly in years with several months of snowcover, the sclerotia are not observed to germinate at that time. Koske (1975) found that sclerotia of *T. erythropus* need to be turgid, with an exposure to 15–20 °C for 10–14 days before they can germinate. Perhaps this heat requirement for carpogenic germination is also found with other *Typhula* species in the field, although it was not necessary under lab conditions for the three *Typhula* spp. examined here.

Special conditions are needed for the production of sporophores of *Typhula* species, just as they are for other fungi (Kues and Liu 2000). In the field, sporophore production in *T. phacorrhiza* seems to involve the same environmental cues as those of *T. ishikariensis* or *T. incarnata*, namely abundant moisture and temperatures falling below freezing. Future research on sclerotial germination of *Typhula* species should address the issue of heat requirements for carpogenic germination as well as examining the physiological changes in *Typhula* sclerotia as temperatures fall below freezing.

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References

Ballou, L.R., and Holton, R.W. 1985. Synchronous initiation and sporulation of fruitbodies by *Coprinus cinereus* on a defined medium. Mycologia, **77**: 103–108.

Berthier, J. 1976. Monographie des *Typhula* Fr., *Pistillaria* Fr. et genres voisins. Bulletin de la Société Linn. Lyon. No. 45.

Bruehl, G.W., and Cunfer, B.M. 1971. Physiologic and environmental factors that affect the severity of snow mold of wheat. Phytopathology, **61**: 792–79.

Bruehl, G.W., and Cunfer, B.M. 1975. *Typhula* species pathogenic to wheat in the Pacific Northwest. Phytopathology, **65**: 755–760.

Carey, K. 2004. (University of Guelph.) Personal Communication.

Coley-Smith, J.R., and Cooke, R.C. 1971. Survival and germination of fungal sclerotia. Ann. Rev. Phytopath. **9**: 65–92.

Cook, S., and Hsiang, T. 2003. Snow mold disease control on creeping bentgrass at putting green height, November 2001 – April 2002. Guelph Turfgrass Institute Ann. Res. Rep. **17**: 65–66.

Cunfer, B.M., and Bruehl, G.W. 1973. Role of basidiospores as propagules and observations on sporophores of *Typhula idahoensis*. Phytopathology, **63**: 115–120.

Detiffe, H., Maraite, H., and Meyer, J.A. 1981. Evolution de l'infection de *Typhula incarnata* Lasch ex. Fries sur escourgeon en Belgique. Meded. Fac. Landbouwwet, Rijksunir. Gent. **46**: 841–849.

Ellis, R.J., Bragdon, G.A., and Schlosser, B.J. 1999. Properties of the blue light requirements for primordia initiation and basidiocarp maturation in *Coprinus stercorarius*. Mycol. Res. **103**: 779–784.

Hsiang, T., and Wu, C. 2000. Genetic relationships of pathogenic *Typhula* species assessed by RAPD, ITS-RFLP and ITS sequencing. Mycol. Res. **104**: 16–22.

Hsiang, T., Matsumoto, N., and Millett, S.M. 1999. Biology and management of *Typhula* snow molds of turfgrass. Plant Dis. **83**: 788–798.

Jackson, N., and Fenstermacher, J.M. 1969. *Typhula* blight: its cause, epidemiology and control. J. Sports Turf Res. Inst. **45**: 67–73.

Kawakami, A., Matsumoto, N., and Naito, S. 2004. Environmental factors influencing sporocarp formation in *Typhula ishikariensis*. J. Gen. Plant Pathol. **70**: 1–6.

Koske, R.E. 1975. *Typhula erythropus*: II Sclerotial germination and basidiocarp production. Mycologia, **67**: 128–146.

Kues, U., and Liu, Y. 2000. Fruiting body production in basidiomycetes. Appl. Microbiol. Biotech. **54**: 141–152.

MacDonald, J.A. 1934. The life history and cultural characteristics of *Typhula gyrans* (Batsch) Fries. Ann. Appl. Biol. **21**: 590–613.

Matsumoto, N. 1997. Evolution and adaptation in snow mold fungi. Soil Microorganisms, **50**: 13–19 (in Japanese).

Matsumoto, N., and Tajimi, A. 1985. Field survival of sclerotia of *Typhula incarnata* and *T. ishikariensis* biotype A. Can. J. Bot. **63**: 1126–1128.

Remsberg, R.E. 1940. Studies in the genus *Typhula*. Mycologia, **32**: 52–96.

Sanogo, S., and Pennypacker, S.P. 1997. Weather variables associated with infection of tomato fruit by *Colletotrichum coccodes*. Plant Dis. **81**: 753–756.

Snider, C.S., Hsiang, T., Zhao, G., and Griffith, M. 2000. Role of ice nucleation and antifreeze activities in pathogenesis and growth of snow molds. Phytopathology, **90**: 354–361.

Vargas, J.M. 1994. Management of turfgrass diseases. Lewis Publishers, Ann Arbor, Michigan.