

Distribution of *Typhula* spp. and *Typhula ishkariensis* Varieties in Wisconsin, Utah, Michigan, and Minnesota

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ABSTRACT

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Snow molds are psychrophilic fungi that grow under snow cover by taking advantage of carbohydrate-depleted, dormant plants. *Typhula* snow molds caused by *Typhula incarnata*, *T. phacorrhiza*, and *T. ishkariensis* are the most important winter diseases of perennial grasses and winter cereals in the United States. Colonized turfgrass samples with sclerotia were collected from 135 golf courses in Wisconsin, Utah, Michigan, and Minnesota in spring 2001 and 2002. Species and varieties from a total of 2,864 samples were identified using *Typhula* spp.-specific polymerase

chain reaction markers. All three species were found throughout the states sampled, except *T. phacorrhiza*, which was not found in Minnesota. *T. incarnata* was distributed in areas of shorter snow cover duration and higher mean temperature than *T. ishkariensis*. Canonical correspondence analysis indicated that snow cover days and mean temperature were significantly correlated with frequency of *Typhula* spp. and *T. ishkariensis* varieties infecting turfgrasses on golf courses in Wisconsin, and that *T. incarnata*, *T. phacorrhiza*, and *T. ishkariensis* were ecologically distinct based on the 2001 data. However, because these two variables accounted for a relatively small proportion of total variation, other environmental variables also may be important in characterizing the distribution of these pathogens and require further study.

Typhula snow molds are the most important winter diseases of perennial grasses and winter cereals in the cool climates of the northern hemisphere (2,4,27,28,34). *Typhula* fungi are Basidiomycetes in which three species, *Typhula incarnata* Lasch ex. Fr., *T. ishkariensis* Imai, and *T. phacorrhiza* Fries, have been problematic worldwide, including within the United States (30,34). *Typhula* snow molds caused by *T. incarnata* and *T. ishkariensis* are also known as gray snow mold, speckled snow mold, and *Typhula* blight (10).

These fungi have the ability to attack plants at low temperatures under persistent snow cover. During winter dormancy, the carbohydrate reserves of the plants are depleted, and the plant becomes less resistant to disease. Weakened plant defenses allow these psychrophilic organisms to take advantage of a host while escaping competition and antagonism from other microorganisms (3,9,14,23). In intensively managed turfgrasses, particularly creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.), symptoms and signs of the disease appear in spring after snow melt as straw-colored circular patches of either dead tissue embedded with sclerotia in the leaves, crowns, or roots of the plant, or sclerotia loosely detached, suspended in mycelium between leaves (32,34).

In most golf courses in the United States, control of these diseases generally relies on fungicide applications. Fungicides are applied one or two times prior to permanent snowfall to protect turfgrass from infection and colonization by *Typhula* spp. throughout long winters. However, due to the difficulty of fungicide reapplication in winter caused by snow cover, the fungicide

application rate is much higher for snow mold treatments compared with rates used to control summer turfgrass diseases. The most common method of chemical control of snow mold disease is a combination of fungicides with different active ingredients. The combination of active ingredients is necessary due to the diversity of organisms causing snow mold disease (34). Snow mold outbreaks incited by *T. ishkariensis* are considered harder to control than those caused by *T. incarnata* (36,37). This may be explained by the fact that *T. ishkariensis* is found primarily in regions with longer snow cover duration, where plant carbohydrate reserves are more depleted, which increases host susceptibility (6). Furthermore, some fungicides may control only a specific species or variety of *Typhula* (14,34). Therefore, information on ecological aspects, including distribution of each species and variety in relation to environmental factors, is needed for effective control and management of *Typhula* snow molds.

Historically, identification of *Typhula* spp. and varieties has relied upon morphological characteristics, such as sclerotial size, color, shape and rind pattern, and size and color of sporocarp, as well as mating compatibility studies. However, the identification of *Typhula* spp. and, especially, *T. ishkariensis* varieties using morphological characters and mating compatibility alone has proven to be confusing and complex (10,22). The difficulty involved in distinguishing different morphotypes and biologically distinct strains has resulted in taxonomic confusion among researchers (3,13,18). Taxonomically synonymous subspecies of *T. ishkariensis* have been classified into several different categories, progressing from varieties to biotypes (17) to groups (20) and, most recently, to biological species (15).

This taxonomic confusion is amplified by adaptations of *T. ishkariensis* varieties to different habitats and environments. Matsumoto and Tajimi (19) found that *T. ishkariensis* populations in uncultivated lands were more diverse than in areas

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planted with perennial grasses. Weather conditions also are likely to affect the distribution of *Typhula* spp. (5). Matsumoto et al. (17) described that, in Japan, different *T. ishikariensis* biotypes were geographically distinct according to amount of snow cover and temperature fluctuations. Similarly, Millett (21) indicated that correlation to temperature and snow cover is found in development and distribution of the causative agents of *Typhula* snow molds in Wisconsin.

So far, most studies have focused on *Typhula* spp. infecting winter cereal crops and not on those infecting intensively managed turfgrasses. Understanding the impact of these conditions could prove to be a key for controlling *Typhula* spp. on turf, because previous studies indicate that the distribution of *Typhula* spp. and varieties varies according to regions with different habitats (14,19) and substrates (31).

Therefore, in this study, *Typhula* spp. and *T. ishikariensis* varieties in samples collected from 135 golf courses in Wisconsin, Utah, Michigan, and Minnesota were identified using species- and variety-specific polymerase chain reaction (PCR) markers (11). Using specific molecular markers will alleviate identification problems that have occurred in the past when only morphological and mating studies methods were available (8,29). We have identified where each *Typhula* sp. and variety occurs frequently within our study area, and have correlated various abiotic (climatic conditions) and biotic (species competition and interaction) factors to the distribution data. With a better understanding how *Typhula* spp. and varieties are influenced by their environment, more effective strategies of snow mold control can be developed and implemented. The wide variation among habitats in Wisconsin, Minnesota, Michigan, and Utah offers an ideal opportunity to evaluate the distribution of *Typhula* spp. This information then

can lead to improved cultural manipulation and judicious fungicide selection for more effective snow mold control strategies on turfgrass.

MATERIALS AND METHODS

Collection of *Typhula sclerotia*. In Wisconsin, 100 public golf courses, which may not be able to afford snow mold fungicide applications on fairways, were sampled. Courses were chosen randomly by consideration of United States Department of Agriculture plant hardiness zones and estimated annual snow cover days (National Operational Hydrologic Remote Sensing Center, 1996 and 1997). In addition, 35 golf courses (6 in Utah, 15 in Michigan, and 14 in Minnesota) were selected based on the likelihood of snow mold damage as assessed by local pathologists (Fig. 1). We collected data on two environmental factors that have been suggested as major factors in snow mold disease development (17). Mean ambient air temperature from 1 October through 1 May in 2000–01 and 2001–02 was obtained from each of 93 weather stations located throughout Wisconsin (Midwestern Regional Climate Center; 2001 and 2002). The mean temperature at each weather station was matched to the nearest golf course. The total cumulative number of days with snow cover (SCD) for each site, without considering frequency and duration of snow melts, were estimated based on daily satellite snow cover images for each winter (24,25). The SCD data from 100 locations were categorized into 20-day periods ranging from 71 to 90 days to 151 to 170 days.

Courses were visited in the spring of 2001 or 2002 to collect turfgrass tissues that were colonized with mycelia and sclerotia. In Wisconsin, all 100 courses were visited for the samples during

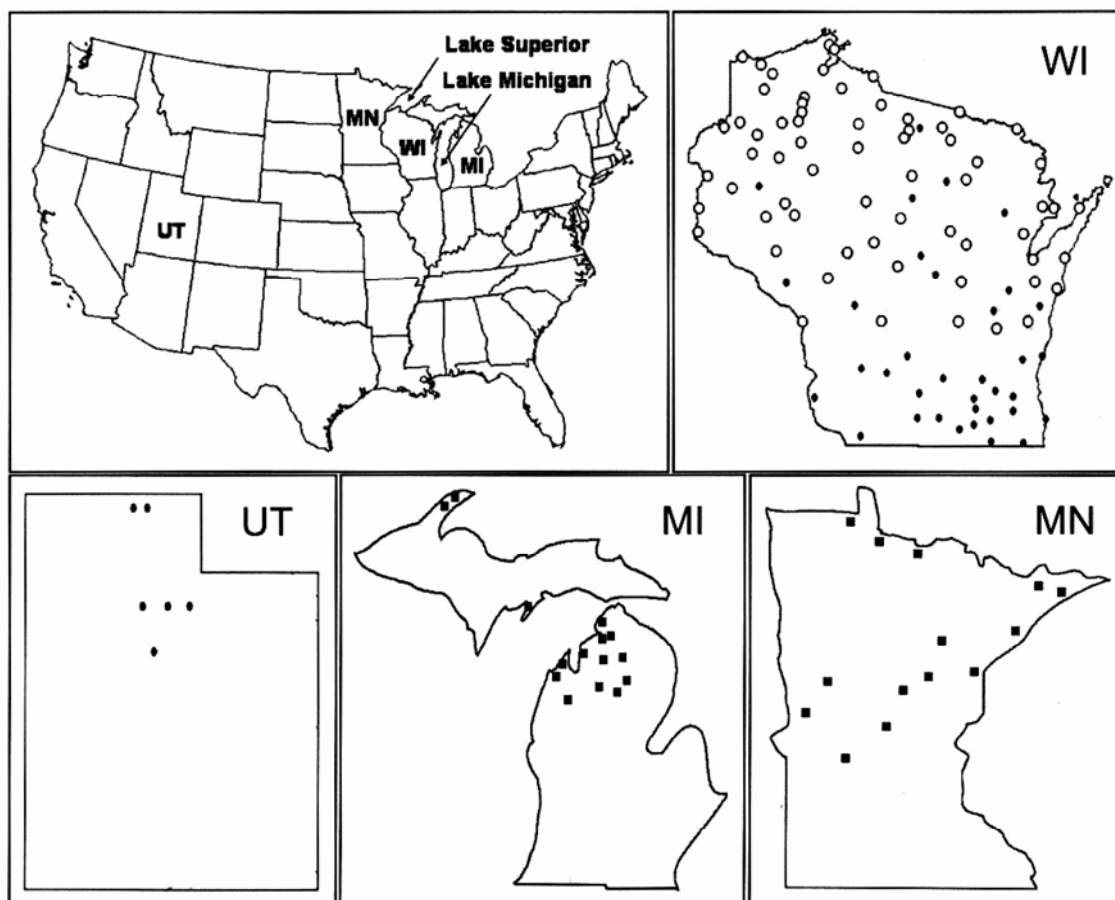


Fig. 1. Sampling sites of Wisconsin (WI), Utah (UT), Michigan (MI), and Minnesota (MN) in 2001 and 2002. The symbols on the map of each state represent sampling sites, golf courses. Sites sampled in 2001 = ●, 2002 = ■, and both 2001 and 2002 = ◐.

spring 2001. Due to a lack of snow in spring 2002, not all courses showed disease symptoms. Therefore, sampling was limited to golf courses where there was sufficient evidence of snow mold development (Fig. 1). This sampling strategy was devised through phone interviews with golf course superintendents and personal visits to assess snow mold damage. All courses in the northern region of the state were sampled in 2002 (Fig. 1). We randomly chose 10 golf courses located in the southern regions of the state and asked superintendents to assess snow mold damage. If damage was reported, we included those courses in our sampling strategy. If no disease was reported, we visited the site to confirm the lack of snow mold development before excluding it from the study. Based on this information, in 2002, 79 courses initially were selected and visited. Of these, 65 courses had snow mold damage and subsequently were sampled. Golf courses in Utah were sampled in spring 2001 only, and those in Michigan and Minnesota were sampled during spring 2002.

At each golf course, four fairways were selected randomly for sampling. Samples of 10 to 15 infected grass leaves with mycelia and sclerotia in each fairway were taken from five sites located equidistantly along a transect from green to tee. In 2002, the same fairways were sampled in Wisconsin as in 2001. Samples were stored in a small coin envelope and classified by golf course, fairway, and sample number within each fairway. The geographical coordinates of each sampling site were recorded using a Garmin Int. eTrex geographical positioning system (GPS) unit (Olathe, KS). If no symptoms were present on the fairway turf, areas of the rough directly adjacent to the fairway sampling location were assessed for symptoms. If no symptoms were found in the rough, no sample was taken from that site.

Culturing of *Typhula* isolates and mycelial DNA extraction. Sclerotia were surfaced sterilized in 10% bleach for 20 s, then rinsed three times in sterile water. Five sclerotia per sample were plated on 1.5% water agar (WA; Difco Laboratories, Detroit) medium and incubated at 10°C. Mycelial growth from a single sclerotium was transferred from WA to potato dextrose agar (PDA; Difco Laboratories, Detroit) medium and incubated at 10°C. Mycelial growth was transferred from PDA to 6 ml of potato dextrose broth (PDB; Difco) in 15-ml tubes and moderately shaken at 10°C until the volume of mycelial growth reached between 0.5 and 1 ml.

Mycelial samples were spun at 1,300 relative centrifugal force (rcf) for 5 min. The supernatant was removed, and the mycelium was resuspended in 500 µl of 0.1× Tris + EDTA (TE). Samples were shaken briefly, then spun at 1,300 rcf for 5 min. The mycelium was transferred to a 2-ml screw-cap Eppendorf tube and frozen at -80°C for at least 30 min. Samples were lyophilized overnight and then ground in 600 µl of Dellaporta extraction buffer (7) with a ceramic bead, using a FastPrep FP120 machine (BIO 101 Inc., Carlsbad, CA). Then, 70 µl of 20% sodium dodecyl sulfate was added to each sample, and the samples were incubated in a 65°C water bath for at least 1 h. Samples were centrifuged at 11,800 rcf for 10 min and the supernatant was transferred to a clean 1.5-ml microcentrifuge tube. Debris was precipitated with 400 µl of 5 M potassium acetate for 20 min at room temperature. Samples then were centrifuged at 11,800 rcf for 10 min and supernatant was transferred to a clean 1.5-ml microcentrifuge tube. DNA was precipitated with 100% isopropanol for 30 min. To retrieve DNA pellets, the samples were centrifuged at 13,800 rcf for 10 min and the supernatant was discarded. The DNA was washed with 70% ethanol and the DNA pellets were collected through centrifugation. The DNA was resuspended in 50 µl of 0.1× TE. Finally, the DNA was quantified using a DNA Fluorometer Model TKO-100 (Hoefer Scientific Instruments, San Francisco) and then diluted to 4 ng/µl in 10 µM Tartrazine + 0.1× TE for PCR reactions.

Single sclerotium DNA extraction. For samples that failed to germinate in pure culture, DNA was extracted from a single field-

collected sclerotium following the methods of Ragatz et al. (26). A single sclerotium was ground in a 1.5 ml tube in 30 µl of TPS buffer (100 mM Tris-Cl + 1 M KCl + 100 mM EDTA) using a disposable Kontes pestle and incubated at 95°C for 10 min. Then, 30 µl of chloroform/octanol (24:1 in volume) was added to the supernatant and samples were centrifuged at 2,040 rcf for 5 min. The supernatant was transferred to a new 1.5-ml tube, filled with EtOH and 3 M NaOAc (10:1 in volume) and incubated at room temperature for 20 min for DNA precipitation. The samples were centrifuged at 8,200 rcf for 3 min and supernatant was discarded. The DNA pellet was washed in 70% EtOH and air dried. Then, 10 µl of 0.1× TE was added to the DNA pellet and incubated at 37°C for 20 min for use in PCR.

Sample identification using PCR-based markers. Species-specific PCR markers designed to identify *T. incarnata*, *T. phacorrhiza*, *T. ishihariensis*, *T. ishihariensis* var. *ishihariensis*, and *T. ishihariensis* var. *canadensis* were used to determine *Typhula* spp. and varieties using sample DNA (11). In all, 100 *T. ishihariensis*, 48 *T. incarnata*, and 4 *T. phacorrhiza* isolates collected from golf courses throughout Wisconsin (21), and 9 reference isolates of *T. ishihariensis* (5 from the collection of D. Smith and 4 from the American Type Culture Collection), were used to design the primers. Sequences of the primer pairs are as follows: *T. incarnata*, forward (F): 5'-CCCCACCACCACACTCTATACTGTCC-3' and reverse (R): 5'-TCCGTAGGTGAACCTGCGG-3'; *T. ishihariensis*, F: 5'-TCCGTAGGTGAACCTGCGG-3' and R: 5'-GCATTTAAAGAGGAGTCGATCTCG-3'; *T. ishihariensis* var. *canadensis*, F: 5'-AGGGCCGTCTACCGTTCTAC-3' and R: 5'-AGGGCCGTCTGTGGGACCTTG-3'; *T. ishihariensis* var. *ishihariensis*, F: 5'-AGGGCCGTCTGAGGCTCTCC-3' and R: 5'-AGGGCCGTCTTTATAACGGT-3'; and *T. phacorrhiza*, F: 5'-TGCGGCTGAGTGGAGTTTC-3' and R: 5'-TGCGGCTGAGGCGAAGATGC-3'. Primers identifying *T. ishihariensis* var. *ishihariensis*, *T. ishihariensis* var. *canadensis*, and *T. phacorrhiza* are sequenced characterized amplified region (SCAR) primers converted from random amplified polymorphic DNA (RAPD) bands unique to each *Typhula* sp. or variety following the procedure outlined by Scheef et al. (29). Primers identifying *T. incarnata* and *T. ishihariensis* were designed from the internal transcribed spacer region (ITS) sequence of the nuclear ribosomal DNA (nrDNA) (11,21). Primers were grouped for two multiplex PCR reactions by combining the primers for *T. ishihariensis*, *T. ishihariensis* var. *ishihariensis*, and *T. ishihariensis* var. *canadensis* into one group and the primers for *T. incarnata* and *T. phacorrhiza* into another group. The PCR analyses were performed in 10-µl volumes in 96-well plates in a MJ PTC-100 (MJ Research, Watertown, MA) with thermal cycling conditions of 91°C for denaturation, 65°C for annealing, and 72°C for elongation. Forty cycles were performed with the first cycle timing of 60 s for denaturation, 15 s for annealing, and 70 s for elongation. Subsequent cycles were the same, with the exception of a 15-s denaturation period. PCR products were visualized in 1.5% agarose gels stained in distilled water containing ethidium bromide at 0.5 µg/ml for 10 to 20 min. Single bright bands for each *Typhula* sp. and variety were visually scored.

Statistical analysis. The number of each species or variety in each golf course or SCD zone according to the presence of the species- and variety-specific PCR bands was counted. The observed number was compared using χ^2 tests at the significance level. The χ^2 tests were not performed on a sample size of less than 10 due to lack of statistical power (SAS Institute, Cary, NC).

Correlation of species distribution with environmental factors. Canonical correspondence analysis (CCA) was used to examine the relationship between *Typhula* spp. or varieties and environmental factors in Wisconsin (35). The species matrix consisted of the absolute frequency of each species and variety present at each of the 100 sites in 2001 and 65 sites in 2002, and the environmental matrix was composed of the two environmental

variables at each site (SCD and mean temperature in degrees). The statistical significance of eigenvalues and species–environment correlations within each axis were tested with a Monte Carlo simulation of 1,000 runs. The resulting *P* value was computed as the proportion of randomized runs with an eigenvalue greater than or equal to the observed. CCA was performed using the PC-ORD software (version 4.30; MJM Software Design Gleneden Beach, OR).

RESULTS

Environmental conditions. In Wisconsin, total SCD ranged from 80 to 170 days in 2001 and from 40 to 150 days in 2002 (Table 1). In all, 72 golf courses (72%) had more than 130 SCD in 2001 compared with 9 courses (7%) in 2002. In addition, 2002 was an unusually warm winter, with frequent snow melts over most of the state.

Estimated mean temperatures for 2001 and 2002 were calculated by averaging daily air temperature for each month from 1 October through 1 May during each winter in Wisconsin. Mean temperature ranged from –4.4 to 1.8°C (mean –1.6°C) during 2001 and from –2.3 to 2.9°C (mean 0°C) during 2002.

Typhula spp. composition. Of the 2,019 samples collected in 2001 from 100 Wisconsin golf courses, 947 (46.9%) were cultured successfully for the production of mycelia and sclerotia. These samples were used for DNA extraction using the modified Dellaporta method. When sclerotial samples did not germinate in culture, single-sclerotium DNA extraction was performed. Of all 2,019 samples from 2001, 1,835 (90.9%) were processed successfully by one of the two DNA extraction techniques and could be identified as *Typhula* spp. through species-specific PCR primers (Table 2). Of these 1,835 samples, 51.9, 45.4, and 2.7% were identified as *T. incarnata*, *T. ishikariensis*, and *T. phacorrhiza*, respectively, which deviated significantly from a 1:1:1 ratio

(*P* < 0.001). Within *T. ishikariensis*, 43.3% of 834 were identified as var. *ishikariensis*, 38.7% as var. *canadensis*, and 18.0% as var. *idahoensis*, which deviated significantly from a 1:1:1 ratio (*P* < 0.001). However, excluding var. *idahoensis* resulted in a frequency distribution of 43.3 and 38.7% for var. *ishikariensis* and *canadensis*, respectively, and this distribution did not deviate significantly from a 1:1 ratio.

In total, 184 samples could not be identified using the PCR primers. Of these, 32.1% were attempted using mycelium-extracted DNA, and the rest used DNA processed from field-collected single sclerotia.

Of the 680 samples collected from 65 Wisconsin golf courses in 2002, 37.5% were cultured successfully for DNA extraction. DNA was extracted from 46.2% of the samples using the previously described single-sclerotium method; therefore, 83.7% of the total samples were able to be identified as *Typhula* spp. through species-specific PCR primers (Table 2). The observed frequencies of the three *Typhula* spp., 37.6, 0.3, and 59.9% for *T. incarnata*, *T. phacorrhiza*, and *T. ishikariensis*, respectively, deviated significantly from a 1:1:1 ratio (*P* < 0.01). The occurrence of three *T. ishikariensis* varieties, 37.8, 51.3, and 10.9% for var. *ishikariensis*, *canadensis*, and *idahoensis*, respectively, also deviated significantly from a 1:1:1 ratio (*P* < 0.01), as did the frequencies of var. *ishikariensis* and *canadensis* (*P* < 0.01). In all, 111 samples could not be identified using the PCR primers. Of these, 19 were from a total of 255 DNA samples from PDB-cultured mycelium. The rest of the unidentified samples were from DNA samples processed from field-collected sclerotia.

In 2002, the ratio of *T. incarnata* and *T. ishikariensis* significantly deviated from the expected ratio (1:1) (*P* < 0.01), but not in 2001. *T. incarnata* was higher in frequency than *T. ishikariensis* in 2001 but lower in 2002 (Table 2).

Of the 495 samples collected from 35 golf courses in Utah, Michigan, and Minnesota, 77.4% were cultured successfully for

TABLE 1. Frequency of occurrence of *Typhula* spp. and *Typhula ishikariensis* varieties according to estimated snow cover days (SCD) in golf courses (GCs) of Wisconsin in 2001 and 2002^a

No. of SCD	No. of GCs	<i>Typhula</i> spp.		<i>T. ishikariensis</i> varieties			
		No. tested	TIN:TISH	No. tested	TISI:TCAN:TIDA	No. tested	TISI:TCAN
Year 2001							
71–90	5	94	84:6***	6	2:2:2	4	2:2
91–110	13	179	151:12***	12	6:0:6**	6	6:0
111–130	10	187	154:31***	31	15:14:2**	29	15:14 ^{NS}
131–150	31	525	196:217 ^{NS}	217	86:81:50**	167	86:81 ^{NS}
151–170	41	850	267:568**	568	252:226:90***	478	252:226 ^{NS}
Year 2002							
71–90	19	97	49:45 ^{NS}	45	22:19:4**	41	22:19 ^{NS}
91–110	16	165	38:123***	123	57:59:7***	116	57:59 ^{NS}
111–130	19	180	77:100 ^{NS}	100	27:57:16***	84	27:57**
131–150	9	127	50:73*	73	23:40:10***	63	23:40*

^a TIN: *T. incarnata*, TISH: *T. ishikariensis*, TISI: *T. ishikariensis* var. *ishikariensis*, TCAN: *T. ishikariensis* var. *canadensis*, and TIDA: *T. ishikariensis* var. *idahoensis*. NS = nonsignificant and *, **, and *** significant at *P* < 0.05, 0.01, and 0.001, respectively, based on χ^2 of either 1:1 or 1:1:1 expected ratio.

TABLE 2. Frequency of occurrence of *Typhula* spp. and *Typhula ishikariensis* varieties sampled from golf courses (GCs) in Wisconsin (WI, 2001 and 2002), Utah (UT, 2001), Michigan (MI, 2002), and Minnesota (MN, 2002)^a

State, year	No. of GCs	<i>Typhula</i> spp.			<i>T. ishikariensis</i> varieties			
		No. tested	TIN:TPA:TISH	TIN:TISH	No. tested	TISI:TCAN:TIDA	No. tested	TISI:TCAN
WI								
2001	100	1,835	952:49:834***	952:834**	834	361:323:150***	684	361:323 ^{NS}
2002	65	569	214:14:341**	214:341***	341	129:175:37***	304	129:175**
UT, 2001	6	50	15:4:31**	15:220***	31	29:2:0***	31	29:2***
MI, 2002	15	266	59:3:204**	59:204***	204	136:54:14***	190	136:54***
MN, 2002	14	144	25:0:119**	25:119***	119	47:57:15***	104	47:57 ^{NS}

^a TIN: *T. incarnata*, TPA: *T. phacorrhiza*, TISH: *T. ishikariensis*, TISI: *T. ishikariensis* var. *ishikariensis*, TCAN: *T. ishikariensis* var. *canadensis*, and TIDA: *T. ishikariensis* var. *idahoensis*. NS = nonsignificant and *, **, and *** = significant at *P* < 0.05, 0.01, and 0.001, respectively, based on χ^2 of either 1:1 or 1:1:1 expected ratio.

DNA extraction. For the 22.6% of the samples that failed to grow in culture, DNA was re-extracted from a single field-collected sclerotium. Of the 495 samples processed, 92.9% were identified successfully as *Typhula* spp. through species-specific PCR primers (Table 2). Of the 236 *Typhula* DNA samples in Utah, Michigan, and Minnesota, 15, 59, and 25, respectively, were identified as *T. incarnata*; 4, 3, and 0 as *T. phacorrhiza*; and 31, 204, and 119 as *T. ishkariensis*. Within *T. ishkariensis*, 29, 136, and 47 were identified as var. *ishkariensis* in Utah, Michigan, and Minnesota, respectively; 2, 54, and 57 as var. *canadensis*; and 0, 14, and 15 as var. *idahoensis*. In all three states, the distribution of the three *Typhula* spp. deviated significantly from an expected 1:1:1 ratio.

The frequency of *T. incarnata* was significantly lower than of *T. ishkariensis* in all states except in Wisconsin in 2001 only. In Utah and Michigan, frequency of var. *ishkariensis* was significantly higher than the other varieties. However, frequency of var. *ishkariensis* and *canadensis* did not differ significantly in Minnesota or in Wisconsin in 2001. In all, 35 samples in Minnesota, Michigan, and Utah were not able to be identified using the PCR primers.

Distribution of *Typhula* spp. and *T. ishkariensis* varieties. Based on estimated SCD during both the Wisconsin 2001 and 2002 winters, *T. incarnata* was distributed largely in areas with less SCD across the collection sites, whereas *T. ishkariensis* occurred more frequently in areas of longer SCD (Table 1). *T. phacorrhiza* samples were not included in the χ^2 test analysis due to low frequency in all states (Table 1).

In all four SCD zones in Wisconsin during 2001, the frequencies of *T. incarnata* and *T. ishkariensis* were significantly different from each other (Table 1). The sole exception to this was where the total SCD numbered between 131 and 150, in which case the ratio of *T. incarnata* to *T. ishkariensis* approached 1:1.

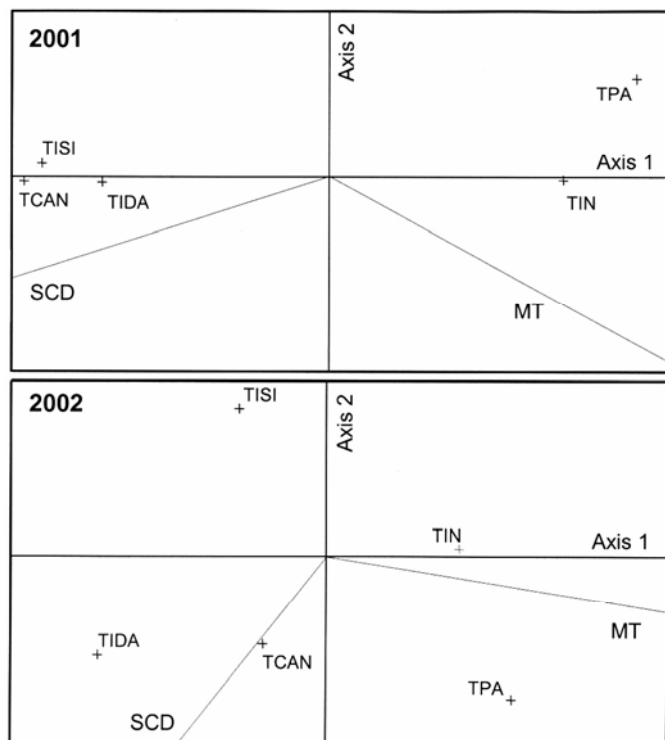


Fig. 2. Biplot from canonical correspondence analysis showing relationship between *Typhula* spp. and *Typhula ishkariensis* varieties and environmental factors (SCD = snow cover days and MT = mean temperature) along axis 1 and axis 2 in 2001 and 2002 in Wisconsin. Solid lines represent variable vectors. TISI = *T. ishkariensis* var. *ishkariensis*, TCAN = *T. ishkariensis* var. *canadensis*, TIDA = *T. ishkariensis* var. *idahoensis*, TIN = *T. incarnata*, and TPA = *T. phacorrhiza*.

Observed frequencies of the two species in zones of 91 to 110 and 131 to 150 SCD during 2002 in Wisconsin were 38:123 and 50:73, which deviated significantly from a 1:1 ratio. In SCD zones 71 to 90 and 111 to 130, however, the frequency of the two species did not statistically differ from each other.

Observed frequencies of the three *T. ishkariensis* varieties in all SCD zones, except that of 71 to 90 SCD in Wisconsin in 2001, significantly deviated from a 1:1:1 ratio in both years. The frequency of *T. ishkariensis* var. *ishkariensis* and *canadensis* did not deviate from a 1:1 ratio in all zones in both years, except two zones (111 to 130 and 131 to 150) in 2002. Two zones, 71 to 90 and 91 to 110, in 2001 were not included in the statistical analysis due to low frequency of *T. ishkariensis* var. *ishkariensis* and *canadensis*.

Relationships between environmental factors and *Typhula* spp. and *T. ishkariensis* varieties.

Canonical correspondence analysis identified two axes which showed relationships between environmental variables and *Typhula* spp. using 2001 and 2002 Wisconsin data (Fig. 2). In 2001, axis 1 was highly correlated with two environmental factors, SCD and mean temperature, and axis 2 was moderately correlated with both variables (Table 3). In 2002, axis 1 was correlated with both SCD and mean temperature; however, axis 2 was highly correlated to SCD only (Table 3). The eigenvalues of each axis were low, explaining only 20.0 and 2.1% of the variation for axes 1 and 2, respectively, in 2001, while explaining 0.1 and 1.3% of the variation in 2002. Eigenvalues represent the variance in the community matrix that is attributed to a particular axis. For the 2001 data, a Monte-Carlo randomization test of the eigenvalues showed that 0.1% of the 1,000 runs were greater than the observed values for axis 1 and, for axis 2, 57.7% of 1,000 runs were greater than the observed values. In 2002, the test showed that 76.3 and 10.6% of the 1,000 runs were greater than the observed values for axis 1 and 2, respectively (Table 4). Two vectors were constructed within the biplot-one corresponding to SCD and one to mean temperature (Fig. 2). The direction of the vectors from the plot origin indicates a positive, higher value for each environmental variable, with negative (lower) values represented in the direction opposite the drawn vector.

In 2001, all *T. ishkariensis* varieties were grouped together and separated from *T. incarnata* and *T. phacorrhiza* along axis 1, which showed high correlation with SCD and mean temperature (Fig. 2, 2001). *T. incarnata* and *T. phacorrhiza* were separated

TABLE 3. Intraspecific correlations between environmental factors, snow cover days (SCD), and mean temperature (MT) of Wisconsin in 2001 and 2002 and axes of canonical correspondence analysis

Variable	2001		2002	
	Axis 1	Axis 2	Axis 1	Axis 2
SCD	-0.953	-0.302	-0.626	-0.780
MT	0.878	-0.478	0.987	-0.159

TABLE 4. Eigenvalues and species-environment correlations (Corr.) from matrix used in canonical correspondence analysis

Year, axis	Real data	Randomized data Monte Carlo test
	Eigenvalue (Corr.)	P^a
2001		
1	0.200 (0.753)	0.001 (0.001)
2	0.001 (0.097)	0.763 (0.597)
2002		
1	0.021 (0.241)	0.577 (0.820)
2	0.013 (0.237)	0.106 (0.162)

^a P = proportion of randomized runs with eigenvalue greater than or equal to the observed eigenvalue; that is, $P = 1 + \text{number of permutations} \geq \text{observed} / (1 + \text{number of permutations})$. Monte Carlo test based on 999 runs.

along axis 2, which was moderately correlated to SCD and mean temperature, but not along axis 1. *T. incarnata* and *T. phacorrhiza* were more separated along the SCD vector than the mean temperature vector. Because three *T. ishikariensis* varieties were tightly clustered in 2001 (Fig. 2, 2001), further canonical correspondence analysis using the three varieties only was performed and resulted in weak relationships between two environmental variables and the variety data (*data not shown*). In 2002, the three varieties of *T. ishikariensis* were separated from *T. incarnata* and *T. phacorrhiza* along axis 1, which again was correlated to both SCD and mean temperature. The grouping of the three *T. ishikariensis* varieties in 2002 differed from 2001. *T. ishikariensis* var. *ishikariensis* was separated from *T. ishikariensis* var. *canadensis* and *idahoensis* along axis 2, which was correlated to SCD (Fig. 2, 2002). *T. incarnata* and *T. phacorrhiza* were separated based on SCD, given their respective positions along the SCD vector and along axis 2 (Fig. 2). Winter conditions were less harsh, with higher mean temperature and less SCD, across Wisconsin in 2002 than in 2001.

DISCUSSION

This is the first report of the frequency of *Typhula* spp. on golf courses in Wisconsin, Utah, Michigan, and Minnesota and the first correlation of *Typhula* distributions with environmental factors in Wisconsin. All three *Typhula* spp. were found across all states, except Minnesota, where *T. phacorrhiza* was not reported (Table 2). *T. incarnata* and *T. ishikariensis* occurred frequently in all states, whereas *T. phacorrhiza* was rare or absent. All three varieties of *T. ishikariensis* were found in all states except Utah, where var. *idahoensis* was not reported. The absence of species and varieties reported from Minnesota and Utah may be due to sampling error or the effect of environmental conditions during the sample years.

In 2001, *T. incarnata* was the most frequent species identified, and was found relatively evenly across Wisconsin (99 golf courses), with broad distribution across SCD zones, compared with *T. ishikariensis* (76 golf courses) (Table 1). These findings correspond to a study by Masumoto et al. (16), which stated that the wide geographic range of *T. incarnata* in Japan is ascribed to its ecological versatility. Furthermore, as SCD increased the relative frequency of *T. incarnata* decreased, whereas the frequency of *T. ishikariensis* increased significantly, suggesting that the habitats of the two species were different (Table 1; Fig. 2). Smith (33) similarly noted that *T. incarnata* could adapt to less favorable environments for snow mold development, such as a shallower snow cover and shorter snow cover days, compared with *T. ishikariensis*. Although *T. incarnata* is considered less virulent than *T. ishikariensis*, the ecological advantage to *T. incarnata* may be a higher saprotrophic ability that allows the fungus to occupy a broader ecological niche (14).

The frequency of *T. incarnata* in Wisconsin in 2002 was significantly lower than *T. ishikariensis* (Tables 1 and 2), indicating the evidence of the major effect of environment on *Typhula* spp. occurrence overall. The golf courses sampled in 2002 are located in northern regions of the state, which have served as ideal habitats for *T. ishikariensis* for a long time; therefore, the higher relative frequency of *T. ishikariensis* in 2002 might be due to its greater inoculum potential. A combination of environmental factors other than those studied here also may play a role in the higher frequency in 2002. In Utah, Minnesota, and Michigan, the lower frequency of *T. incarnata* seems to be related to the more compatible habitat for *T. ishikariensis*, due to heavy snowfall caused by high altitude or lake effect (Utah), lake effect (Michigan), and northern climate (Minnesota) (24,25). CCA analysis of the 2001 Wisconsin data shows that *T. incarnata* was separated in the opposite direction of *T. ishikariensis* varieties along vectors representing SCD and mean temperature. *T. incarnata* was situ-

ated away from the SCD vector and along the positive mean temperature vector, whereas the *T. ishikariensis* varieties were positively positioned along the SCD vector but away from the mean temperature vector, meaning that *T. incarnata* favored a habitat less affected by snow and cold temperatures. The exception to this occurs in 2002, where *T. ishikariensis* var. *ishikariensis* is positioned away from the SCD vector, suggesting that SCD were not a significant factor in distribution of this variety during the unusual 2002 winter (Fig. 2; Table 1).

T. phacorrhiza showed the lowest frequency of occurrence in all states, and was not found at all in Minnesota. This species has been shown to have low saprotrophic and pathogenic ability on winter grasses compared with both *T. incarnata* and *T. ishikariensis* (21,31,38). In a recent study, Millett (21) reported that *T. phacorrhiza* was found only in northern and central Wisconsin. However, in our study with a larger sampling scheme, *T. phacorrhiza* occurred relatively evenly throughout Wisconsin in both years. A similar result has been noted by Remsburg (27) that *T. phacorrhiza* was widely distributed in temperate climates due to its saprotrophic ability on leaf debris of grasses and deciduous plants. The reported distribution of *T. phacorrhiza* throughout temperate winter climates agrees with the CCA analysis, where the fungus is found in areas of less SCD and higher mean temperature. This niche may reflect the saprotrophic activity of *T. phacorrhiza*.

T. ishikariensis has been reported from northern Japan, northern Scandinavia, South Korea, and northern North America, including Canada and the northern United States (12,34). As previously reported by Millett (21), *T. ishikariensis* was distributed across all four states included in this study and occurred more frequently in regions with longer SCD and lower mean temperature (Table 1; Fig. 2). Our result was similar to the report of Årsvoll (1) that *T. ishikariensis* was common in areas with more than 100 SCD. Interestingly, however, in our study, the fungus also was found in areas with less than 100 SCD. In particular, the frequency of *T. ishikariensis* was similar to that of *T. incarnata* in areas with 71 to 90 SCD in 2002 (Table 1). It is possible that weather conditions other than SCD, mean temperature, and host species also play important roles in the survival and occurrence of *T. ishikariensis*. Based on these results, we must conclude that *T. ishikariensis* can be a potential problematic pathogen in warmer areas with less SCD, given the proper habitat. More research must be performed to identify which, if any, of these other factors are key to *T. ishikariensis* occurrence during warmer winters.

All three varieties were found in closely related ecological habitats in 2001 (Fig. 2). This may be the result of harsh environmental conditions which resulted in an ecological separation of *T. ishikariensis* varieties from *T. incarnata* and *T. phacorrhiza*, but no separation within the species.

T. ishikariensis var. *idahoensis* showed the lowest frequency in all states compared with the other two varieties. The low frequency of this variety could be explained by its unstable genetic background causing a reduction of the proliferation ability (18), or their specific habitat could be different than var. *canadensis* as well as var. *ishikariensis*. In Wisconsin during 2002, *T. ishikariensis* var. *ishikariensis* occurred less frequently than var. *canadensis*. The distribution of *T. ishikariensis* var. *idahoensis* among habitats based on SCD and mean temperature was more similar to that of var. *canadensis* than that of var. *ishikariensis* (Fig. 2, 2002). In 2001, there was much less separation of the varieties based on environmental factors than in 2002. Interestingly, this result suggests that the distribution of *T. ishikariensis* varieties can be affected significantly by harsh environmental conditions. These trends also were present in the data from the other states. In areas with more SCD but higher temperatures, such as Utah and Michigan, *T. ishikariensis* var. *ishikariensis* was found more often. In colder habitats with less SCD,

such as Minnesota, *T. ishkariensis* var. *ishkariensis* and *canadensis* were found with equal frequency throughout the entire collection area.

In conclusion, *T. incarnata*, *T. phacorrhiza*, and *T. ishkariensis* occupy unique ecological niches. *T. incarnata* and *T. ishkariensis* were the most common species. *T. incarnata* favored warmer areas, but showed strong ecological adaptability, whereas *T. ishkariensis* varieties were more frequently distributed in colder regions with longer snow cover. Based on CCA, SCD and mean temperature were highly correlated with the frequency of *Typhula* species and *T. ishkariensis* varieties in Wisconsin in 2001 (Fig. 2). The analysis showed that *T. incarnata*, *T. phacorrhiza*, and *T. ishkariensis* were ecologically distinct based on the 2001 data, but that the three *T. ishkariensis* varieties were not adapted to different environments, especially given the 2002 data. Although SCD was the best variable based on this study, the fact that it did not explain a majority of the variation suggests more precise measurements (depth, longest duration, and frequency of snow melts) of snow cover and soil temperature may give more detail in future studies.

Future research should focus on investigating potential competition among *Typhula* spp. and varieties. Additionally, this study did not focus on the effect of turfgrass species composition on frequency of *Typhula* spp. and *T. ishkariensis* varieties, despite reports on differences in *Typhula* susceptibility among turfgrass species or cultivars within species (4,38). Virulence screening of *Typhula* spp. on turfgrass species and the reproductive and pathogenic evaluation of monokaryon and dikaryon as survival strategies can provide more insights into competition among the potential pathogens and the effect of inoculum potential of each, as well as help determine strategies of disease management of *Typhula* spp. More information on the genetic variation within *T. ishkariensis* at the subspecies and population levels using mating and molecular techniques will further our understanding of the dynamics of these fungi in the field.

Another area needing further study is the robustness of current *Typhula* spp. identification methods. Future efforts will investigate samples left unidentified using current species- and variety-specific PCR markers. Several samples had *Typhula*-like structure, but were not identified as *Typhula* spp. by molecular methods or microscopic evaluation. In fact, some of them were identified as *T. phacorrhiza* based on sequences of ITS region despite morphological characters, such as sclerotial color and shape, that did not seem to support their species identity (data not shown). It is necessary to determine whether samples that were not identified as *Typhula* spp. represent other fungi that could be associated with the disease or whether the total DNA quantity was insufficient. Identification of these turf pathogens is important for understanding other psychrophilic fungi that potentially could interact with *Typhula* spp., and for designing effective management strategies.

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