Chapter 2

Distribution of Typhula Snow Molds in Wisconsin

ABSTRACT

A systematic random sampling technique was used to estimate the distribution of Typhula snow molds that caused Typhula blights in Wisconsin golf courses during the 1996-1997 winter season. The sampling frame divided the state into three climate zones based on annual snowfall, USDA plant hardiness zones, estimated annual snow cover days and frost depth zones. Within these three zones, seven golf courses within a 70 kilometer radius of Madison (43° 04', southern zone), Stevens Point (44° 31', central zone) and Woodruff (45° 54', northern zone), were randomly selected to survey. Four fairways were sampled for a total of 20 samples per golf course. Turfgrass samples were air dried, crushed and sieved to collect sclerotia. The sclerotia were identified as either *Typhula incarnata*, *T. ishikariensis* complex or *T. phacorrhiza*. In general, the Typhula blight pressure was mild to moderate in the southern zone and moderate to severe in the central and northern zones. *T. incarnata* was the most frequently collected *Typhula* species in the southern zone, although *T. ishikariensis* was evident in small numbers. *Typhula ishikariensis* was the dominant species in the central and northern zones. *Typhula phacorrhiza* Reichard ex. Fr. was also found associated with distinctive patches in the central and northern zones. Complexes were common in the central and northern zones, but not in southern zone. The understanding of the distribution patterns of the *Typhula* species will aid other researchers in developing management practices for a particular area of the State.

INTRODUCTION

This investigation aimed to determine which *Typhula* species are present in Wisconsin golf courses and where they are located. Based on a biogeographical analysis of *Sclerotinia sclerotiorum*, Reichert (1958) suggested that fungal plant pathogens are geographically limited. Reichert considered this mycogeographical approach useful in describing the ecology of the pathogen and believed this information to be useful in disease management. The distribution of fungi is largely habitat controlled (Cooke, 1979). The Typhula snow molds have a world-wide distribution pattern that ranges from approximately 35° to 65° north latitude, (Fig. 2.1). The fungus *Typhula incarnata* Lasch ex Fr. is believed to be more common in southern Wisconsin (Worf, 1988), while *Typhula ishikariensis* Imai is believed to be more common in the northern regions of the state. Matsumoto et al. (1982) found that in Japan the distribution of *T. ishikariensis* and its biotypes are strongly restricted by the duration of snow cover.

Typhula blights have been reported in North America, Europe and Asia. In North America, Typhula blights have been reported in Washington, Idaho, Montana, Wyoming, Utah (Bruehl and Machtmes, 1980), Pennsylvania (Dejardin and Ward, 1970), Wisconsin (Dahl, 1933; Worf, 1988), Michigan (Beard, 1966), Minnesota (Stienstra, 1980), Illinois (Shurtleff et al., 1964), Ohio (Muse, 1970), Indiana (Patterson et al., 1990), and Massachusetts (Torello, 1987), Ontario and British Columbia, Canada (Cormak and LeBeau, 1959), Ottawa (Dejardin and Ward, 1970), Connecticut, New York, and Pennsylvania (Wernham and Chelton, 1943), Edmonton, Alberta, Canada (Lebeau and Dickson, 1955), and Whitehorse, Yukon Territory, Canada (Lebeau and Logsdon, 1958). In Europe, Typhula blights have been reported from Northeastern Bavaria (Andres et al., 1987), Norway (Årsvoll, 1973), 1964), Czechoslovakia, Moravia, Slovakia (Benada, 1976), Alnarp, Sweeden (Bengtsson, 1989), Switzerland (Cavelier and Auquier, 1980; Smith et al., 1989), Scotland (Gray , 1964), Poland (Furgal et al., 1974), Belgium (Mariate et al., 1981), Germany (Metzler, 1987), Russia (Tkachenko et al., 1997), United Kingdom (Woodbridge and Coley-Smith, 1991), Finland (Ylimäki, 1962),

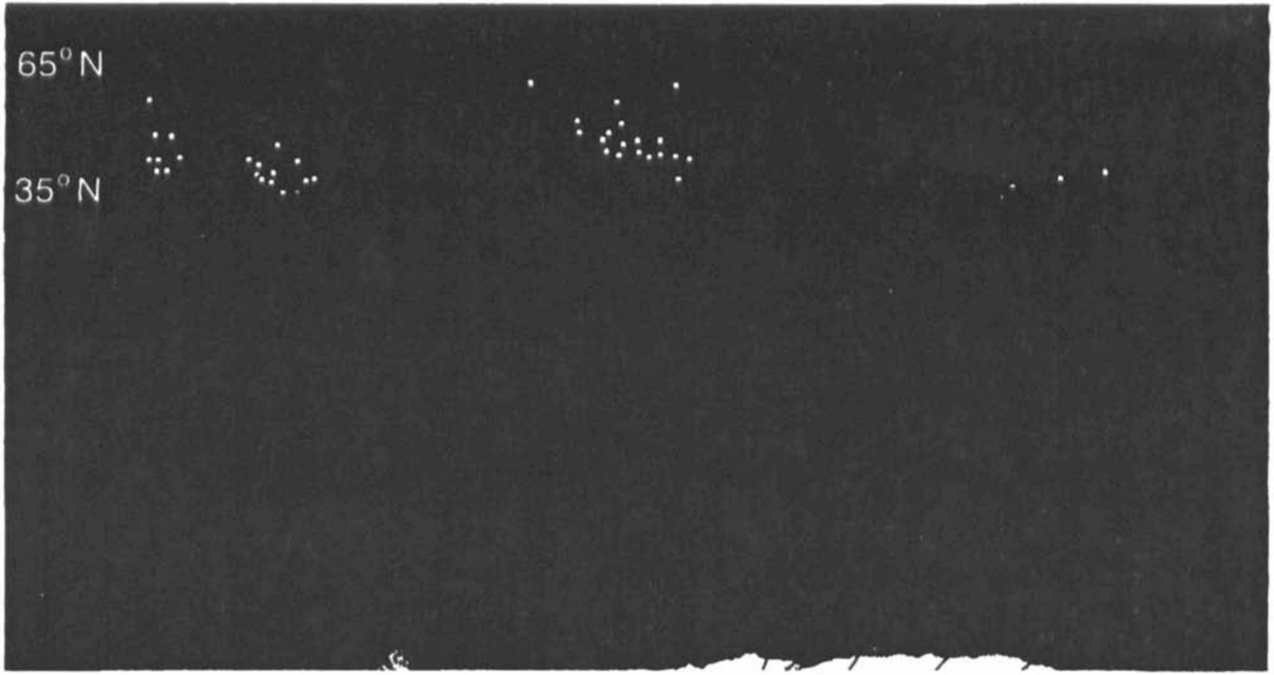


Figure 2.1 World-wide distribution of Typhula snow molds. Location of documented reports of Typhula snow molds indicated (o). References cited in text.

Denmark (Jensen, 1974), Netherlands (De Leeuw and Vos, 1970), Austria (Köck, 1976), Iceland (Kristinsson and Gudleifsson, 1975), Bulgaria (Dimov and Vasilev, 1983), France (Glemas et al., 1977), Romania (Ilias and Diaconu, 1988) and Slavonia (Yugoslavia) (Zugec et al., 1986). In Asia, Typhula blights have been reported in Hokkaido, Japan (Abumiya and Akai, 1964), China (Ye et al., 1987) and Kunpo, Korea (Kim et al., 1992).

Dependable control of Typhula blights are often difficult to achieve because several different fungal pathogens can act both alone or in a complex. The fungi *T. incarnata* and the *T. ishikariensis* complex respond differently to the environment and to the chemicals used to control them (Matsumoto, 1992; Tani and Beard, 1997). Furthermore, field observations of fungicide research plots indicate that Typhula blights caused by *T. ishikariensis* are more difficult to control than blights caused by *T. incarnata* (Stienstra, 1980; Worf, 1988; Tani and Beard, 1997). However, *T. ishikariensis* is found primarily in regions where, due to longer periods of snow cover, plant energy reserves are depleted, increasing the plant's susceptibility to Typhula blights (Couch, 1995).

Advancements in management can be made by describing the distribution patterns of the *Typhula* species. In Wisconsin, it is imperative to the successful management of Typhula blights that we know which pathogens are present throughout the state. Typhula blight fungicide trials conducted by personnel from the University of Wisconsin, Department of Plant Pathology, between 1982 and 1993 were located at various golf courses throughout the state (Table 2.1). This study will also help locate the most appropriate sites for Typhula blight fungicide trials.

The objective of this experiment was to estimate the distribution of *T. incarnata* and the *T. ishikariensis* complex in Wisconsin golf courses. The hypothesis being tested was that *T. incarnata* is found throughout Wisconsin while the *T. ishikariensis* complex is found in the northern two-thirds of the state.

A stratified systematic random sampling technique was used to estimate the distribution

Table 2.1. Location of Typhula snow mold fungicide trials conducted by personnel from the University of Wisconsin, Department of Plant Pathology from 1982 to 1993[a].

EXPERIMENT			PATHOGEN
Year	**Location**	**Latitude**	**Genus species [b]**
1982	Appelton	44° 16'	no disease
	Madison	43° 04'	no disease
1983	Wausau	44° 57'	unknown *Typhula* spp.
	Waukesha	43° 01'	*M. nivale* only
1984	Wausau	44° 57'	*M. nivale* only
	Waukesha	43° 01'	*M. nivale* only
1985	Stevens Point	44° 31'	Unknown *Typhula* spp.
1986	Stevens Point	44° 31'	*T. ishikariensis* var. *canadensis*
1987	Stevens Point	44° 31'	*T. ishikariensis* var. *canadensis*
	Waukesha	43° 01'	no disease
1988	Stevens Point	44° 31'	*T. ishikariensis* var. *canadensis*
1989	Walworth	42° 32'	*M. nivale* only
1990	Langlade	45° 15'	*T. canadensis*
	Eagle River	45° 55'	*T. canadensis*
	Madison	43° 04'	no disease
1991	Langlade	45° 15'	*T. ishikariensis*
	Eagle River	45° 55'	*T. ishikariensis*
	Madison	43° 04'	no disease
1992	Rhinelander	45° 39'	Unknown *Typhula* spp.
	Plum Lake	46° 03'	Unknown *Typhula* spp.
1993	Sarona	45° 43'	Unknown *Typhula* spp.
	Madison	43° 04'	Unknown *Typhula* spp.

[a] Worf et al., 1983; Worf et al., 1984; Worf et al., 1985; Worf et al., 1986; Worf et al., 1987; Worf et al., 1988; Worf et al., 1989; Worf et al., 1990; Worf et al., 1991; Worf et al., 1992; Meyer et al., 1993; Meyer et al., 1994. There were no published fungicide trials 1994 to 1996.
[b] Genus-species as stated by the authors.

of *Typhula* species that are pathogenic to turfgrasses in Wisconsin. The sampling frame divided the state into three climate zones based on annual snowfall, USDA plant hardiness zones, estimated annual snow cover days and frost depth zones. Within these three zones, seven golf courses within a 70 kilometer radius of Madison (43° 04', southern zone), Stevens Point (44° 31', central zone) and Woodruff (45° 54', northern zone), were randomly selected to survey. Four fairways were sampled for a total of 20 samples per golf course. In general, the Typhula blight pressure was mild to moderate in the southern zone and moderate to severe in the central and northern zones. *T. incarnata* was the most frequently collected *Typhula* species in the southern zone, although *T. ishikariensis* was evident in small numbers. *Typhula ishikariensis* was the dominant species in the central and northern zones. *Typhula phacorrhiza* Reichard ex. Fr. was also found in the central and northern zones. Complexes were common in the central and northern zones, but not in southern zone. The *Typhula* spp. isolates collected in this survey were used in the dikaryon-monokaryon mating experiments (Chapter 3), the molecular characterization experiments (Chapter 4) and in the aggressiveness assay (Chapter 5).

MATERIALS AND METHODS

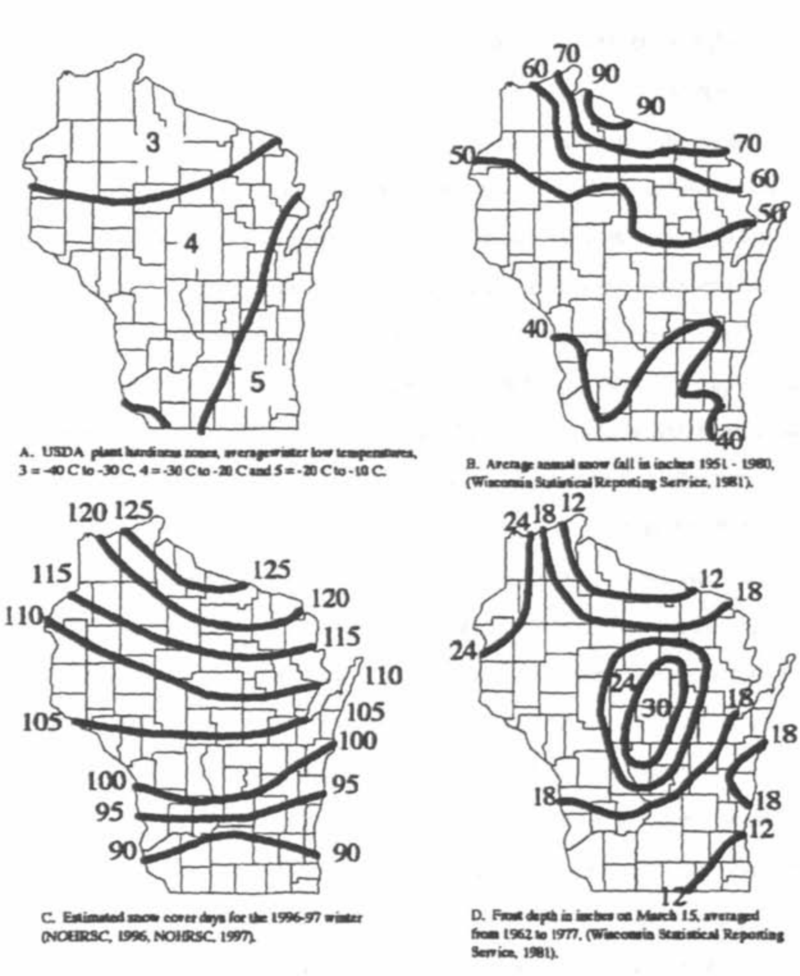
Sampling Frame

A stratified systematic random sampling technique (Cochran, 1977; Foreman, 1991; Chaudhuri and Stenger, 1992) was used to estimate the distribution of *Typhula* species that are pathogenic to turfgrasses in Wisconsin. The sampling frame divided the state into three climate zones based on annual snowfall, USDA plant hardiness zones, estimated annual snow cover days and frost depth zones, (Fig. 2.2). Within the three zones, seven golf courses within a 70 kilometer radius of Madison (southern zone), Stevens Point (central zone) and Woodruff (northern zone) were randomly selected to survey, (Fig. 2.3). The surveyed golf courses were at least two years old. The survey was conducted in the early spring of 1997.

Sampling Procedures

Four fairways were sampled on each of the seven golf courses within each zone. and within each golf course, four fairways were sampled. From each fairway, four Typhula blight patch-samples (symptomatic) and one asymptomatic sample were taken. Two 10 x 10 meter areas were selected for sampling within the fairways, one taken from a higher elevation than the other. The symptomless sample was taken from outside the lower and higher elevation areas. The percentage of the area of each sampled fairway that was damaged by Typhula blight was estimated to the nearest 1% by dividing the sampled fairway into four quadrants and visually grouping the patches into one area. An average estimated Typhula blight damaged area percentage was then calculated for each golf course and zone.

From the snow mold patches, 5 cm by 5 cm samples with approximately 0.5 cm of top soil were cut from the fairway and placed in a labeled paper bag. The samples were later air dried and stored at 4° C until ready for processing. The field samples were crushed and sieved through a 10 mesh (2 mm), 18 mesh (1 mm), 50 mesh (300 micron) and a 100 mesh (150 micron) brass/steel sieves. The 50 and 100 mesh sieved debris was collected, placed in vials and stored at -20° C. Twenty-five sclerotia were taken from each sample and placed in a



A. USDA plant hardiness zones, average winter low temperatures, 3 = -40 C to -30 C, 4 = -30 C to -20 C and 5 = -20 C to -10 C.

B. Average annual snow fall in inches 1951 - 1980, (Wisconsin Statistical Reporting Service, 1981).

C. Estimated snow cover days for the 1996-97 winter (NOHRSC, 1996, NOHRSC, 1997).

D. Frost depth in inches on March 15, averaged from 1962 to 1977. (Wisconsin Statistical Reporting Service, 1981).

Figure 2.2. Climatological maps used to devise the three climate zones of Wisconsin to be surveyed. A) USDA plant hardiness zones, B) average annual snowfall in inches, C) estimated snow cover days, D) frost depth in inches on December 15th.

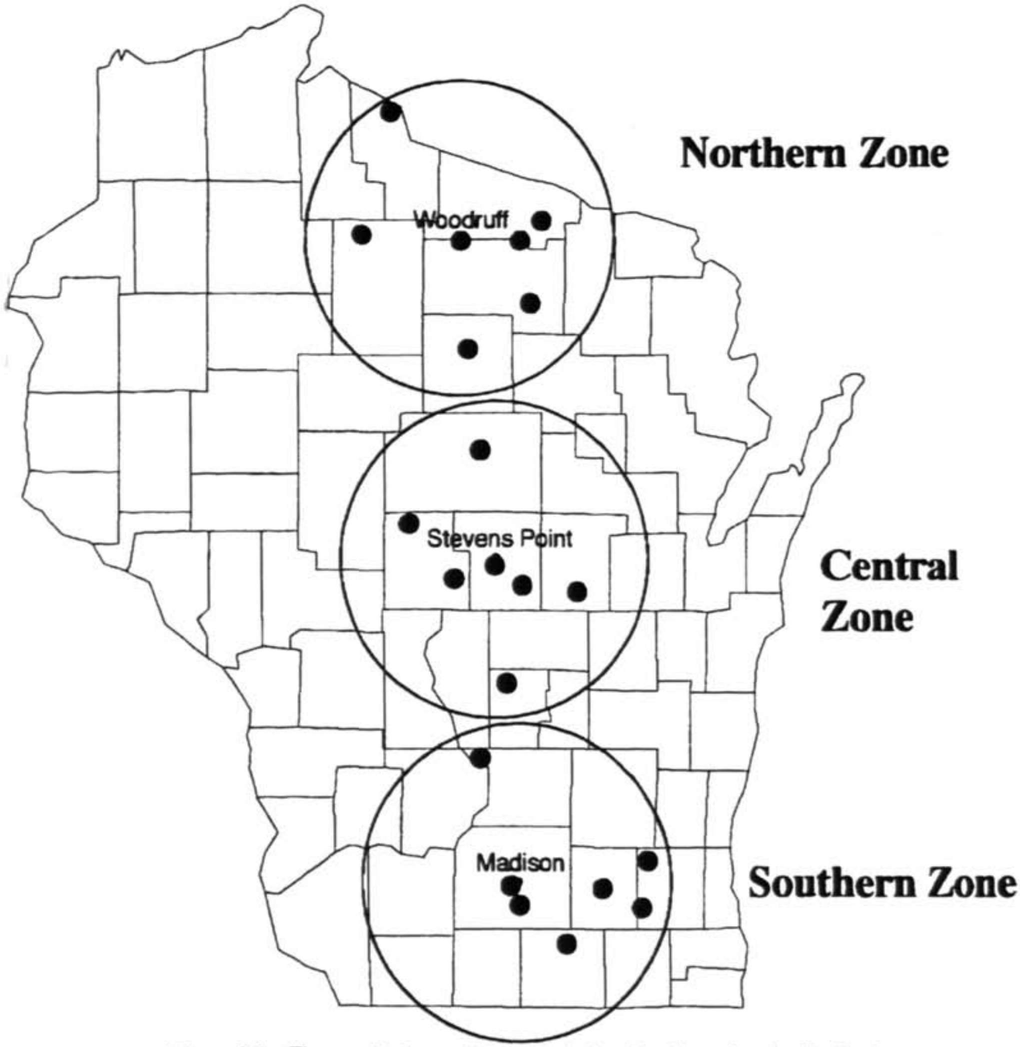


Figure 2.3 . The stratified sampling frame designed to determine the distribution of *Typhula* species associated with Typhula blights of Wisconsin golf courses. Apporximate locations of the golf courses surveyed are indicated (●).

microfuge tube, labeled and stored at -10° C.

The approximate size, shape, numbers, attachment and color of the sclerotia were used for identification (Hsiang et al., 1999). From the dried and crushed 5 cm by 5 cm samples, the numbers of sclerotia were estimated from the 50, 100 and 18 mesh sieves. The attachment of the sclerotia was recorded as being embedded, easily detached, loose or attached by a stipe. The location of the sclerotia on the plant parts was recorded as being attached to either the leaves, stems, stolons or roots.

Sclerotia were identified as either *T. incarnata*, the *T. ishikariensis* complex or *T. phacorrhiza*, based on color, size, debris attachment, rind cell patterns and relative abundance. Corner (1950) stated that, "from the practical point of view one must add that sclerotia are often found rather than fruit-bodies, particularly in diseases caused by species of *Typhula*, so that pathologists require a sclerotium-genus and a means of identifying sclerotia as Remsburg has developed."

Gray snow mold is caused by *T. incarnata*. Sclerotia of *T. incarnata* are initially light colored (pink, yellow or brown) but darken with age, drying to a reddish-brown. They are spherical, subglobose or irregularly shaped, and up to 5 mm in diameter but usually less then 3 mm. Sclerotia often remain attached to plant tissues. The rind cells are golden to reddish-brown and moderately interlocked. "The fungus grows in culture at 0° to 18° C with an optimum temperature of 9° to 12° C. Mycelial growth is abundant, white, webby, radiating, concentrically banded, and fan-shaped. Sclerotia, which appear in 5 to 10 days, are pinkish orange when young and tawny to hazel brown when mature, single or coalesced with a tendency to develop in concentric rings. Sterile white sporophores frequently develop in culture from sclerotia (Remsburg, 1940)."

Speckled snow mold is caused by the *T. ishikariensis* complex. In field conditions, sclerotia of *T. ishikariensis* are small (0.5 to 2.0 mm across), chestnut-brown to bone-brown to black when dry, generally spherical, and loosely attached to leaves or embedded in tissues.

On turf that is cut at a putting green height (< 7.5 mm), masses of sclerotia may be visible on the patch surface. On longer cut turf, the sclerotia often drop off from leaf blades and may not be obvious even right after snow melt. Sclerotia are usually observed in the field as small black spots on plant tissues, but when soaked with water, they become light to dark brown. The surface rind cells of sclerotia show variations in pattern from rounded to lobate, but these patterns cannot be used as the sole basis of differentiating subgroups within the *T. ishikariensis* complex (Matsumoto et al., 1996). *In vitro*, "the organism grows in culture over a range of 0° to 18° C, with an optimum temperature of 9° to 12° C. Sclerotia, which appear in 5 to 10 days, are clustered or in concentric rings, always single, never coalesced into masses, light-amber when young, chestnut-brown when mature. Sterile brown sporophores develop from sclerotia abundantly in culture (Remsburg, 1940)."

A biological control agent that has demonstrated efficacy against Typhula blights (Burpee et al., 1987; Wu and Hsiang, 1998), *Typhula phacorrhiza*, has large, pyriform to irregularly shaped, pedicellate sclerotia, often up to 7 mm in diameter and firmly attached to debris. The rind of *T. phacorrhiza* sclerotia are yellow to reddish-brown with heavily digitate and conspicuous rind cell patterns. *In vitro*, "the fungus grows from 0° to 21° C, with an optimum temperature of 12° to 15° C. Mycelial growth is appressed, granular, often concentrically banded and fan-shaped, forming a rough cartilaginous mat over the surface of the agar. Sclerotia, which appear in 7 to 12 days, are very adherent to the surface of the agar, clustered or in concentric rings, typically pyriform and flattened laterally, white at first, later russet to cinnamon-brown. Long sterile, yellowish-brown sporophores develop abundantly in culture from either sclerotia or the mycelium (Remsburg, 1940)."

Fungal isolates

Dikaryotic isolates were obtained from sclerotia produced on the turfgrass samples. Ten sclerotia were randomly selected from the sieved debris of the survey samples, imbibed in sterile distilled water for 2 min., rinsed in sterile distilled water for 5 to 10 sec., surface disinfested with 70% ethanol for 5 to 10 sec. and then in a 10% commercial bleach solution for 5 to 10 min., and rinsed twice in sterile distilled water. The sclerotia were then lightly pinched with sharp forceps, the excess water blotted off by placing them on autoclaved filter paper. The sclerotia were then evenly placed in one 100-mm x 15-mm petri dish containing one-half strength potato dextrose agar (1/2 PDA) amended with gentamicin sulfate (50 parts per million) and incubated at 10° C for one to two weeks. A single colony was selected from each plate and subcultured on full strength potato dextrose agar (PDA) and incubated at 10° C without light.

RESULTS

The areas sampled had three different types of patches (Fig. 2.4). The gray snow mold patches, (Fig. 2.4.A), were circular, bleached and matted often had wefts of mycelia on the outer margins (see Chapter 1, pp. 3-4). Speckled snow mold patches (Fig. 2.4.B) had a similar circular, bleached and matted appearance but were often covered with a crust of mycelia containing abundant small black sclerotia (see Chapter 1, pp. 3-4). A third patch type was the associated snow mold (Fig. 2.4.C). This associated snow mold was usually found as a patch on top of other patches, although the associated snow mold patches were sometimes found alone. The associated snow mold patches had a thicker crust of mycelia and plant debris, appearing whiter than the surrounding patches, which were either gray, or speckled or a mixture of the two snow molds. Several cracks in the mycelial mat were visible, resembling a dry, cracked and thick algal crust. A few large and pedicellate sclerotia were found on the upper surface of the crust.

Three different sclerotia types were collected from the air dried samples (Fig. 2.5.A). All isolates plated onto potato dextrose agar and some of the fresh samples were checked for presence of clamp connections before drying (Fig. 2.5.B). Sclerotia of *T. incarnata* were chestnut to dark-brown, globular to flattened, embedded in leaves and leaf sheaths (Figs. 2.6.A, B, C and D). Rind cell patterns were lobate and interlocking (Fig. 2.6E). Sclerotia of *T. ishikariensis* were small (0.3 to 2 mm), abundant and dark brown to almost black. They were attached loosely, or sometimes embedded in stolons (Figs. 2.7.B, C and D). The rind cell patterns of *T. ishikariensis* were fairly regular in outline, moderately lobate and sometimes digitate (Fig. 2.7.E). Sclerotia of *T. phacorrhiza* were dark, reddish-brown, large (1 to 3 mm x 2 to 7 mm), pyriform (pear-shaped) and firmly attached to the plant debris by a well developed stalk or stipe (Fig. 2.8A). Few sclerotia of *T. phacorrhiza* were usually found on the surface of the atypical patches (Fig. 2.8.B). The rind cell patterns of *T. phacorrhiza* were conspicuously ornate and deeply lobate to digitate (Fig. 2.8.C). From the northern zone,

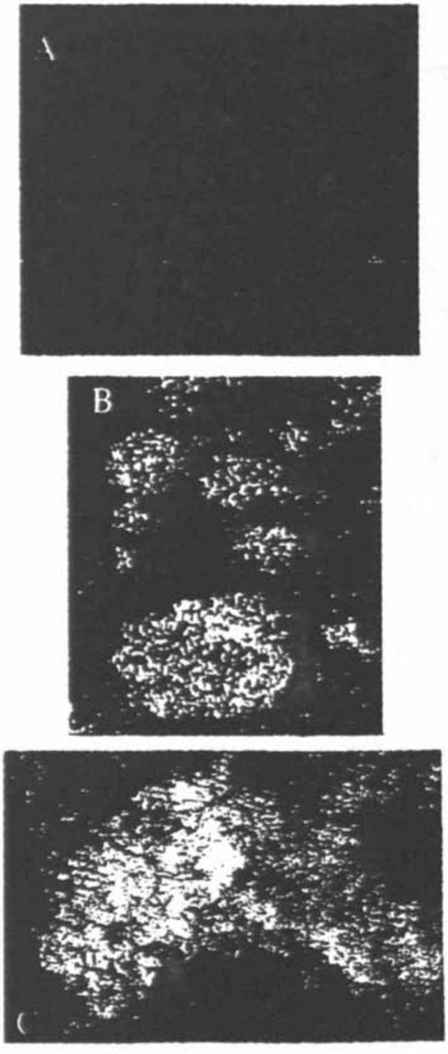


Figure 2.4. Patches of A) gray snow mold caused by *Typhula incarnata*. B) speckled snow mold caused by *T. ishikariensis* and C) an associated snow mold caused by *T. phacorrhiza*.

Figure 2.5. A) Sclerotia of *Typhula* species associated with Typhula blights of turfgrasses. 1. *T. incarnata*, 2. *T. ishikariensis* and 3. *T. phacorrhiza*. B) and C) Clamp connections of *Typhula* spp.

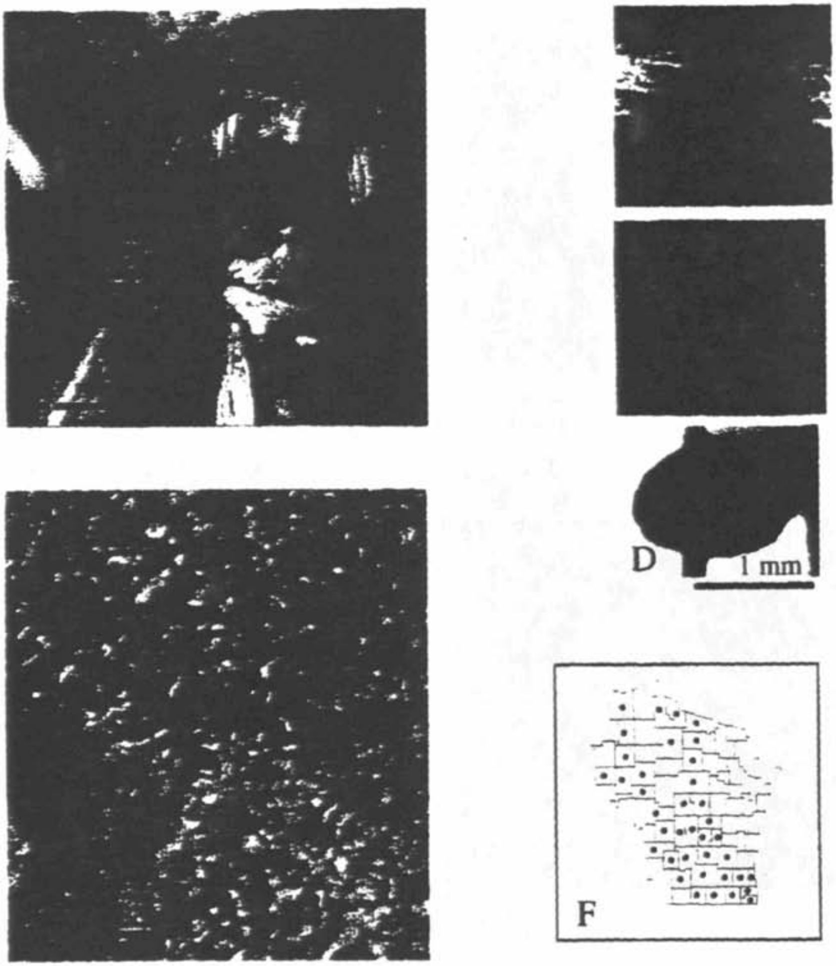


Figure 2.6 A) and B) Sclerotia of *Typhula incarnata*, causal agent of gray snow mold, are chesnut to dark-brown, globular to flattened, embedded in leaves and leaf sheaths, C) smooth and gelatinous when fresh, D) wrinkled when dry (0.5 to 5 mm). E) Sclerotia rind cell patterns are lobate and interlocking. F) Distribution pattern of *T. incarnata* based on field observations from 1994 to 1999.

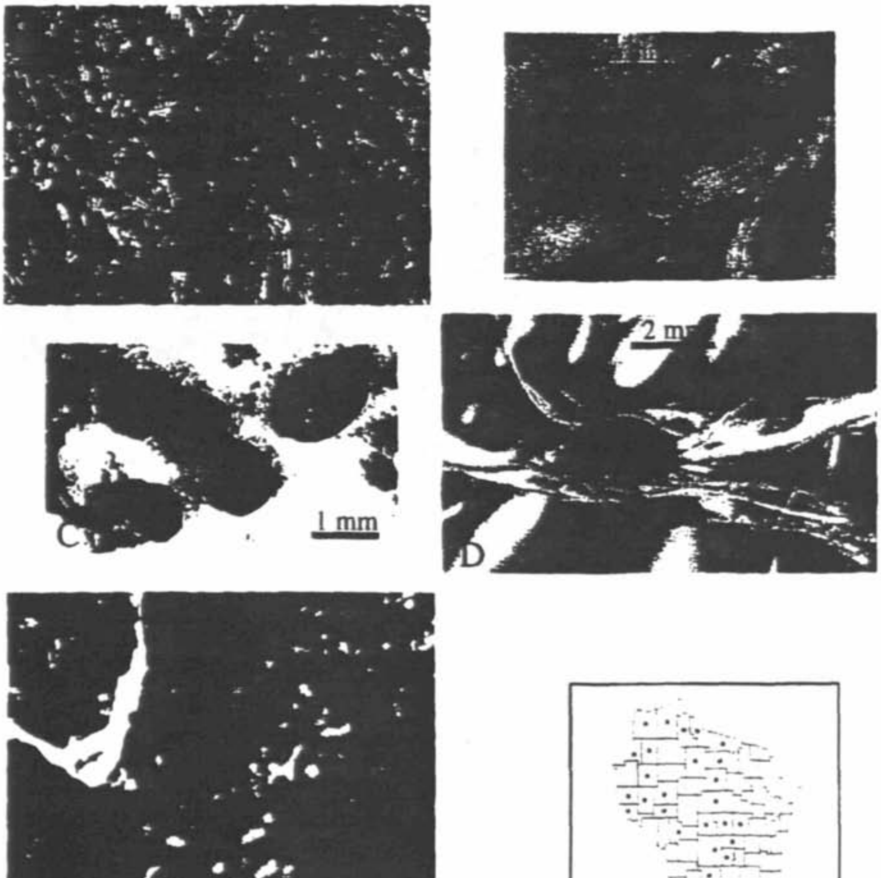


Figure 2.7 A) Numerous small (0.3 to 2 mm), dark brown to almost black sclerotia of *Typhula ishikariensis* , causal agent of speckled snow mold, which are B) loosely attached to leaves, C) loose and D) sometimes embedded in stolons. E) Sclerotial rind cell patterns are fairly regular in outline, moderately lobate and sometimes digitate. F) Distribution pattern of *T. ishikariensis* based on field observations from 1994 to 1999.

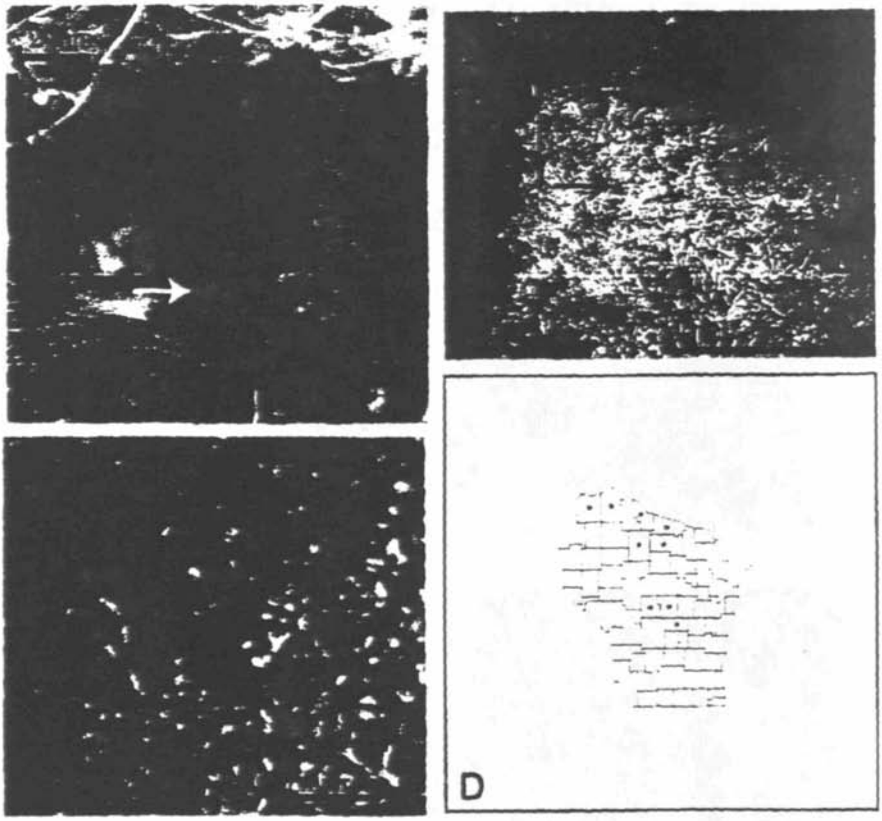


Figure 2.8. A) Sclerotia of *Typhula phacorrhiza*, an associated snow mold, are dark, reddish-brown, large (1 to 3 mm x 2 to 7 mm), pear-shaped and firmly attached to plant debris by a well-developed stalk (arrow). B) Few sclerotia found associated with bleached patches. C) Rind cells are deeply lobate to digitiate. D) Distribution of *T. phacorrhiza* based on field observations from 1994 to 1999.

there were three unidentified *Typhula*-like isolates, (Timber Ridge Golf Course, fairways 12 and 18; and Trout Lake Golf and Country Club, fairway 1) and one *Sclerotinia borealis* isolate (Timber Ridge Golf Course, fairway 4).

The *in vitro* characteristics of the *Typhula* spp. isolates collected are presented in Fig. 2.9. The *T. incarnata* and the *T. phacorrhiza* isolates were uniform in their cultural characteristics. However, there were two distinct types of *T. ishikariensis* isolates. These two different types will be discussed later in Chapter 3. Also, one isolate of *Sclerotinia borealis* (3.256) was found with *T. ishikariensis* in the northern zone and an image of the isolate *in vitro* is presented in Fig. 2.10.

The fungal pathogens identified from the 21 golf courses are presented in Tables A. 2 to A.22, and the percentage of snow mold fungi collected from each zone is presented in Fig. 2.11. The estimated percentage of diseased area for the southern zone was 2% (with a range of 1 to 4%), for the central zone it was 11% (with a range of 2 to 30%) and for the northern zone, 18% (with a range of 4 to 45%).

Typhula incarnata was the most frequently collected fungus in the northern zone (80%) and *T. ishikariensis* was found as far south as Jefferson, WI (Meadow Springs). *Typhula ishikariensis* was found alone (3%) and in combination with *T. incarnata* (2%). Some golf courses had no snow mold damage (7%). Also, pink snow mold, caused by *Microdochium nivale*, was collected from a few golf courses in the southern zone (8%).

The central zone had more damage (11%) than the southern zone (2%). *Typhula ishikariensis* was the dominant fungus collected (63%), while *T. incarnata* was the second most frequently collected fungus (19%). Complexes were common (16%) and *T. phacorrhiza* was collected in 4% of the samples.

The northern zone had a higher estimated diseased area (18%) than the central zone (11%). Again, *T. ishikariensis* was the most frequently collected fungus (52%) while *T. incarnata* was the second most frequent (18%). Complexes were more common (28%) than in

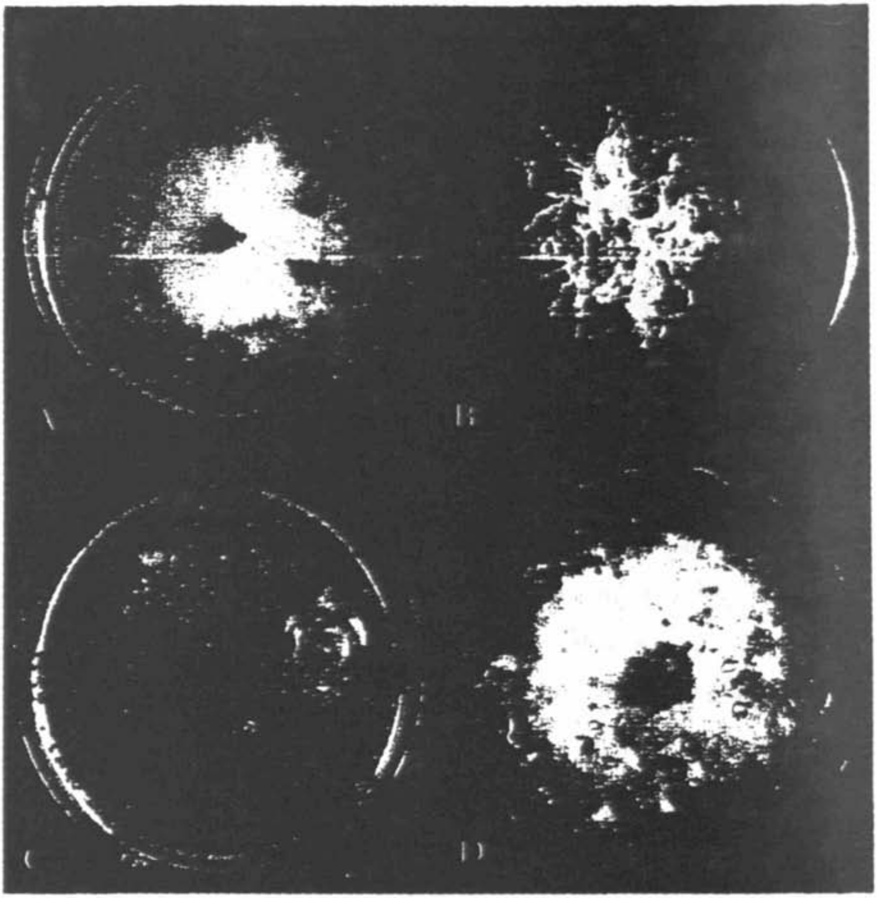


Figure 2.9. *In vitro* characteristics of A) *Typhula incarnata* (1.35.MS) B) *T. phacorrhiza* (3.120B.TL), C) *T. ishikariensis* Wisconsin group 1 (3.120A.TL) and D) *T. ishikariensis* Wisconsin group 2 (2.104.SW) growing on potato dextrose agar at 10°C for 60 days.

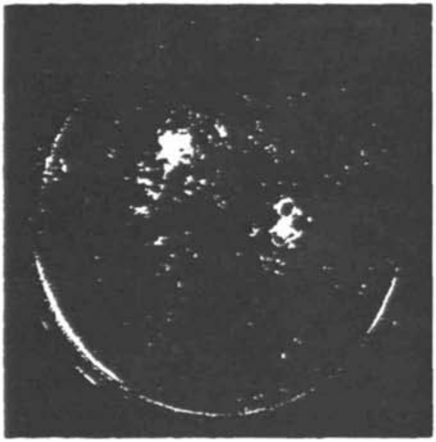


Figure 2.10. *Sclerotinia borealis* isolate culture 3.256.TR growing in PDA for 60 days at 5° C.

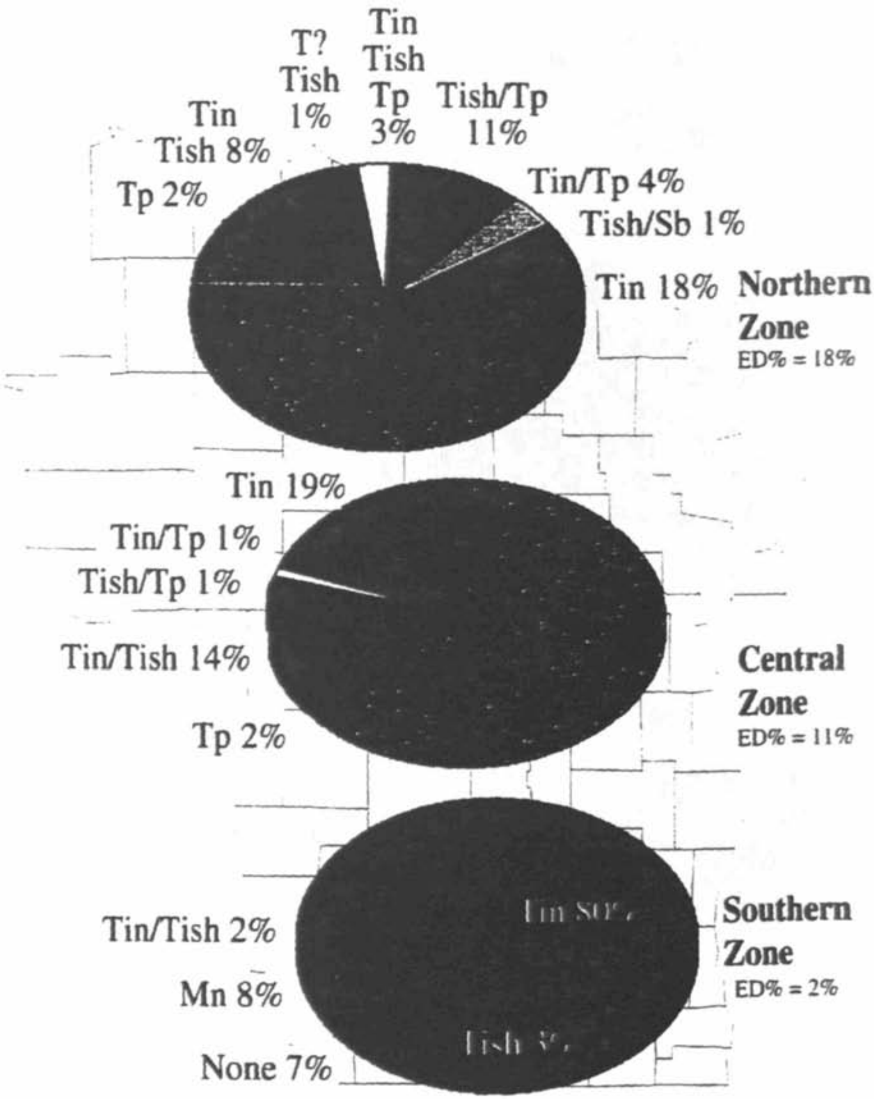


Figure 2.11. Percentage of snow mold fungi collected from Wisconsin golf courses. Tin = *T. incarnata*, Tish = *T. ishikariensis*, Tp = *T. phacorrhiza*, Mn= *Microdochium nivale*, Sb= *Sclerotinia borealis* and None= no pathogens found. ED%= estimated percent disease.

the central zone (16%). *Typhula phacorrhiza* was collected either alone or in combination with other snow molds in 20% of the samples.

The number of *Typhula* spp. collected from the symptomless samples are listed in Table 2.2. Only one symptomless sample from the southern zone contained sclerotia of *T. incarnata*. Symptomless samples from the central and northern zones contained sclerotia of *T. incarnata*, *T. ishikariensis* and *T. phacorrhiza*.

Table 2.2 Number of fungi collected from symptomless samples taken from the southern, central and northern zones of Wisconsin.

Zone of Wisconsin	None	Sclerotia present	Fungi collected[a] Tin	Tish	Tp
Southern	27	1	1	0	0
Central	20	8	4	3	3
Northern	20	8	2	2	3

[a] Tin = *Typhula incarnata*, Tish = *T. ishikariensis* and Tp = *T. phacorrhiza*.

DISCUSSION

Typhulaincarnata was the most frequently collected fungus in the southern zone while *T. ishikariensis* was the most frequently collected fungus in northern two-thirds of the State. Based on occurrence of *T. ishikariensis* in the southern zone, the first hypothesis should be rejected. In general, the snow mold pressure in the winter of 1996/1997 was mild to moderate in the southern zone and moderate to severe in the central and northern zones. *Typhula ishikariensis* was found as far south as Meadow Springs Golf Course (Jefferson) in the southern zone. *Typhula ishikariensis* was the dominant species in the central and northern zones. *Typhula phacorrhiza* was found as far south as Waupaca in the central zone. Complexes were common in the central and northern zones but not in southern zone. *Typhula phacorrhiza* was found more frequently in the northern zone than in the central but not in the southern zone.

The winter of 1996-1997 in Madison, Wisconsin, was "a strange but nearly normal winter" (Miller, 1997): "Freeze/thaw repeated five times. Lots of snow falls, yet never much on the ground. Not very cold, at least for any extended periods. Snow at inopportune times...but within a few inches of the average. Statistics will say that it was a "typical" winter....In northern Wisconsin, by mid-March, Hurley had recorded almost 260 inches of snow!" The mean temperature, the total precipitation and the total snowfall for three locations, similar to the three zones of Wisconsin, during the winter of 1996 to 1997 are presented in Table 2.3 (Miller, 1997). In general, the Wisconsin winter of 1996-1997 had normal temperatures, but areas in the north received more total snow fall than normal.

The above-normal amount of total snow fall in the northern regions could explain the greater amount of estimated disease in the northern zone (18% estimated disease for the northern zone, compared to 11% for the central zone and 2% for the southern zone). An explanation for this could be that the longer the duration of snow cover the weaker the turfgrass plants becomes and therefore provides a greater window of opportunity for pathogenesis.

Table 2.3. Snowfall, mean temperature and total precipitation for three locations near the three zones of Wisconsin during the winter of 1996 to 1997 (Miller, 1997).

Location	**Mean temperature** degrees Celsius (degrees Fahrenheit)	**Total precipitation** water equivalent centimeters (inches)	**Total snowfall** centimeters (inches)
Madison	-1° +0.4° dfn[a] (30.2° +0.8° dfn)	27.48 +1.0 dfn (10.82 +0.40 dfn)	110.24 +4.83 dfn (43.4 +1.9 dfn)
Green Bay	-2.1° +0.3° dfn (28.3° +0.6° dfn)	27.31 +1.53 dfn (10.75 +0.60 dfn)	194.31 +82.30 dfn (76.5 +32.4 dfn)
Duluth	-6.3° -0.4° dfn (20.6° -0.8° dfn)	26.9 +1.98 dfn (10.59 + 0.78 dfn)	320.04 +137.16 dfn (126 +54.0 dfn)

[a] dfn = departure from fifty year normal.

The *T. ishikariensis* isolates collected in this survey were used in dikaryon-monokaryon experiments (Chapter 3). It was noticed while processing the cultures for this experiment that there are two different types of *T. ishikariensis* based on their *in vitro* characteristics. In the dikaryon-monokaryon experiments, the *T. ishikariensis* isolates collected in this survey were separated into two groups: Wisconsin group 1 and Wisconsin group 2. The *Typhula* spp. collected were also characterized genetically by describing the specific regions of their DNA (Chapter 4). Finally, the *Typhula* species collected in this survey were assayed for their aggressiveness towards bentgrass.

The turfgrass extension program has become one beneficiary of this survey. Golf course superintendents now have a better understanding of the distribution of the Typhula snow molds (Millett, 1997). Furthermore, this survey is a beneficial reference point for extension/outreach research, since the University of Wisconsin's fungicide trials are now being conducted at 42°, 44° and 46° N latitude in Wisconsin, in correspondence to the three zones in this survey (Table. 2.4).

Further research is needed in describing the biogeographical distribution of Typhula snow molds in Wisconsin. This survey could be repeated to determine how much the distribution of Typhula snow molds in Wisconsin varies from year to year. Future surveys could also investigate the world-wide occurrence of Typhula snow molds. Investigations into the world-wide distribution patterns could center on surveying regions in the southern hemisphere between 35° and 65° south latitude and areas between Moscow, Russia and North Korea. Potential southern hemisphere survey areas include Chile, Argentina, South Sandwich Islands, Tasmania, South New Zealand and the Antarctic peninsula and potential northern hemisphere areas include Latvia, Lithuania, Belarus, Turkey, Armenia, Georgia, Ukraine, Kazakhistan and Mongolia (Fig. 2.1). Investigations into the differences between species from different geographical areas would aid in our understanding the world-wide evolution of the *Typhula* species.

Table 2.4. Location of Typhula snow mold fungicide trials conducted by personnel from the University of Wisconsin, Department of Plant Pathology from 1997 to 1998[a].

EXPERIMENT			PATHOGEN
Year	**Location**	**Latitude**	**Genus species [b]**
1997	Land O'Lakes	46° 10′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Sayner	46° 03′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Stevens Point	44° 31′	*T. incarnata* *T. ishikariensis*
	Menomenee Falls	43° 04′	*T. incarnata* *M. nivale*
	Verona	42° 59′	*T. incarnata* *M. nivale*
1998	Land O'Lakes	46° 10′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Sayner	46° 03′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Superior	46° 43′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Somerset	44° 59′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Stevens Point	44° 31′	*T. incarnata* *T. ishikariensis* *M. nivale*
	Verona	42° 59′	*T. incarnata* *M. nivale*

[a] Gregos, 1997; Gregos, 1998. There were no published fungicide trials for 1994 to 1996.
[b] Genus-species as stated by the authors.

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