Chapter 3

Dikaryon-monokaryon mating reactions of *Typhula ishikariensis* Imai complex isolates from Wisconsin golf courses with tester isolates from Japan, Norway, Russia and Canada

ABSTRACT

Speckled snow mold, caused by the *Typhula ishikariensis* (TISH) complex, is a common turfgrass disease in areas that receive extended periods of snow cover. There are two biological species (BSI and BSII) within this complex and it has been reported that they vary in their ability to be managed. BSII isolates are believed to be soil-borne and more aggressive than the BSI isolates. TISH isolates from Wisconsin golf courses were grouped according to the morphological characteristics of BSI and BSII. From mating experiments with BSI and BSII tester isolates from Japan, Norway, Canada and Russia, it was revealed that both mating groups were collected from Wisconsin. *In vitro*, Wisconsin dikaryotic isolates were paired with monokaryotic BSI and BSII tester isolates. Clamp connection formation of the tester isolate indicated a positive mating reaction. Twenty-three percent of the Wisconsin isolates were morphologically similar to, and mated with the BSI isolates, while 77% were morphologically similar to, and mated with BSII isolates. Identifying and understanding the biological differences between BSI and BSII of the TISH complex should improve Typhula blight management.

INTRODUCTION

Various methods of identification of *Typhula* species have assisted plant pathologists in diagnosing Typhula blights that cause damage to economically important plants. Early mycologists relied heavily on the characteristics of the fruiting body to identify the *Typhula* species (Persoon, 1801; Fries, 1821; Karsten, 1882). Unfortunately, the fruiting bodies of the plant pathogenic *Typhula* species are rarely found in nature and are not likely to be associated with the damaged plants. The history of *Typhula* identification and taxonomic classification starts with Persoon's first description of the genus in 1801 (Persoon, 1801). His careful descriptions of the fruiting bodies and sclerotia were the first characteristics used by mycologists and plant pathologists to identify the species of *Typhula*. However, plant pathologists diagnosing diseases in the field could only use sclerotial characteristics as identification criteria due to the lack of the fruiting bodies present on the diseased host. Sclerotia-dependent identification was often spurious as the size, color and structure of the sclerotia varied depending on the environmental conditions of the particular area (Corner, 1950; Bruehl and Cunfer, 1975). Progress on *Typhula* species identification was impeded by the lack of exchange of isolates from throughout the world (McDonald, 1961; Bruehl et al., 1975). Taxonomic confusion soon developed as researchers assigned different names to the various *Typhula* species without comparing isolates world-wide. Identification of the *Typhula* species rapidly improved when the morphological characteristics of both the fruiting bodies and the sclerotia were combined with genetic characteristics. Røed was the first to utilize genetic characteristics in identifying *Typhula* isolates by conducting interfertility experiments on three species from the Netherlands, Canada, Japan and Norway (Røed, 1969). Interfertility experiments, especially dikaryon-monokaryon pairings have since become a valuable tool in *Typhula* speciation studies and disease diagnosis.

Remsberg (1940b) summarized the confusion created in the *Typhula* literature by stating, "The student in mycology soon becomes bewildered with the current taxonomic

situation in this genus, and is usually discouraged in his study of the group." Almost sixty years later, there is still confusion concerning the *T. ishikariensis* complex (Figure 3.1). In the United States, the complex includes *T. ishikariensis* and *T. idahoensis* (Bruehl et al., 1975; Bruehl, 1988); in Canada, *T. ishikariensis* includes *T. ishikariensis* var. *ishikariensis*, *T. ishikariensis* var. *canadensis* and *T. ishikariensis* var. *idahoensis* (Årsvoll and Smith, 1978); in Japan, *T. ishikariensis* biotypes A, and B (Matsumoto et al., 1982; Matsumoto and Tajimi, 1990; Matsumoto and Tajimi, 1991); and in Norway, *T. ishikariensis* Groups I, II and III (Matsumoto et al., 1996). The most recent world-wide classification (Matsumoto, 1997) identifies two biological species within the *T. ishikariensis* complex. Regardless of whether we refer to species, varieties or biotypes, different populations exist world-wide within the *T. ishikariensis* complex.

In 1801, *Typhula* was first recognized as a distinct group of fungi by Persoon (1801). Twenty years later, Fries (1821) elevated *Typhula* to the genus level to accommodate Basidiomycetes with small clavulae in which the heads are distinct from the stems. In 1882, Karsten (Karsten, 1882) clarified the genus to confine it to only species with sclerotia. Sclerotia remained the key morphological feature of the *Typhula* genus that separated them from other genera, especially *Pistillaria* (Remsberg, 1940; Corner, 1950). Plant pathologists have since relied heavily on the morphology of the sclerotia of *Typhula* for identification, because sclerotia are often found in nature rather than the fruiting bodies (Corner, 1950).

The history of the *T. ishikariensis* complex began when Imai (1930) first described *T. ishikariensis*, collected from the Ishikari region of Sapporo on the island of Hokkaido, Japan. His description is as follows:

"***Typhula ishikariensis***. Sporophores are 0.5 to 1.5 cm high, gregarious or solitary, each springing from a sclerotium. Club 0.2-0.5 cm long, 0.5-10 mm thick, cylindrical, oblong-clavate, long filiform or rarely obovate, white or whitish, with powdery or frosted appearance, becoming light yellowish brown when dried, distinctly marked off from the stem. Stem 3-10 mm long, filiform, very slender, brownish, becoming darker when dried, slightly powdery under the lens. Basidia cylindrical-clavate, about 5.5 μ thick, 4-spored. Spores ellipsoidal or oblong, 8-10 X 4 μ, smooth. Sclerotia globose, ellipsoidal or irregular in

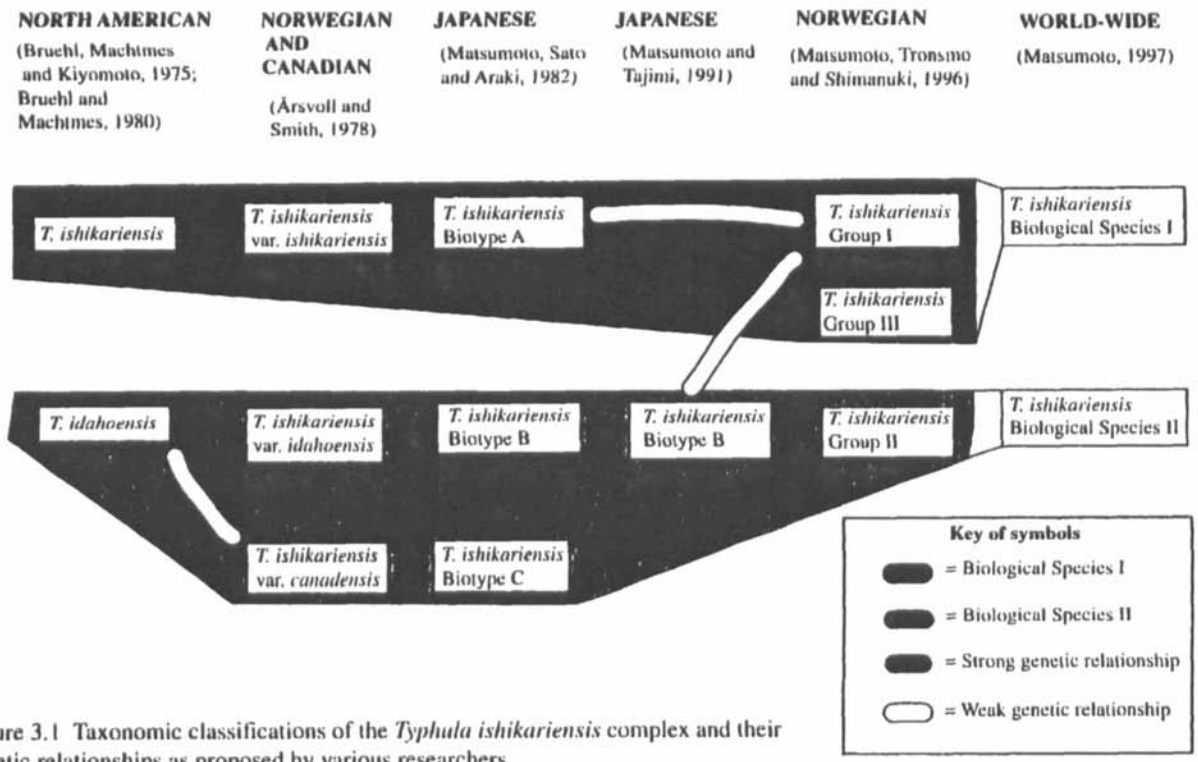

Figure 3.1 Taxonomic classifications of the *Typhula ishikariensis* complex and their genetic relationships as proposed by various researchers.

shape, often compressed, 0.5-1 mm, dark brown when wet, blackish when dry. Habitat. On the rotting stalk or petioles of *Trifolium pratense* and rarely on the rotting leaves and culms of wheat or grasses. Hokkaido: Prov. Ishikari, Sapporo (October 30, 1929, S. Imai & I. Tanaka; Nov. 6, 1929, S. Imai). Japanese name: *Ishikari-gamanohotake* . . . For the present, we wish to treat the species in question as a distinct one and named it as *T. ishikariensis* from its native locality."

Remsberg (1940a) provided an historical account of the genus *Typhula* and describes fourteen species of *Typhula*, including *T. ishikariensis* (as *T. idahoensis)*. Her description is as follows:

"Typhula ishikariensis* (as *T. idahoensis). Sclerotia. Rind a homogeneous gelatinous layer. Medulla prosoplectenchymatous throughout; cells not prominently enlarged adjacent to rind. Sclerotia always single and never coalesced. Sclerotia 0.5-2.0 mm., chestnut-brown to bone-brown; sporophores very rarely branched, fawn to wood-brown, clavate, 5-10 mm. tall. The organism grows in culture over a range of 0-18 C, with and optimum temperature of 9-12 C. Mycelial growth is abundant, fluffy and concentrically banded. Sclerotia, which appear in 5-10 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. This species causes a disease of cereals and grasses similar to that cause by *T. itoana*. Sclerotia of the two fungi are often collected in the same field but are readily distinguished by their color."

Remsberg (1940b) attempted to clarify the confusion concerning the identification of organisms causing snow molds of grains and grasses by reviewing literature and comparing the characteristics of the fruiting bodies and sclerotia of isolates from Japan, Germany and Sweden. She (1940b) concluded that the two major *Typhula* species pathogenic to grains and grasses were *T. incarnata* (as *T. itoana*) and *T. ishikariensis* (as *T. idahoensis*).

Corner (1950) assembled descriptions of the forty-two *Typhula* spp. and created two keys to aid in identification. But even Corner (1950) admitted to the lack of precision when he stated, "There are many species of uncertain microscopical character and, though I have referred some of them to particular genera on shreds of evidence, there is a tiresome remainder, and much error." His description of *T. ishikariensis* (as *T. idahoensis*) is as follows:

***"T. ishikariensis* (as *T. idahoensis*).** Sclerotium 0.5-2 X 0.5-0.9 mm, globose or subglobose, flat below, convex above, glabrous, shining then dull, erumpent or superficial, chestnut-brown to blackish, never coalescent in culture: cortex composed of a single layer of thick-walled, agglutinated hyphae: cuticle 5-20 μ thick, deep reddish brown: medulla of free

interwoven hyphae, not agglutinated, loose in the centre. Temperature-range 0-18 C, opt. 9-12 C: sclerotia after 5-10 days in culture: sterile brown sporophores abundant in culture. This species causes the same kind of disease as *T. incarnata*, but the sclerotia are easily distinguished by their colour."

T. ishikariensis received little attention until Kohei Tomiyama, from the Hokkaido National Agricultural Experiment Station in Sapporo, Japan (Tomiyama, 1951; Tomiyama, 1952) and later a Rockefeller Fellow at the Department of Plant Pathology, University of Wisconsin-Madison (Tomiyama, 1959) compared the pathogenicity on wheat of the species *T. incarnata* (as *T. itoana*) and *T. ishikariensis* and proposed control measures.

Then in 1961, McDonald (1961) placed *T. idahoensis* in synonymy with *T. ishikariensis* because, "The descriptions of the four fungi (*T. ishikariensis*, *T. idahoensis*, *T. borealis* and *T. hyperborea*) appear to be sufficiently similar to justify the belief that they are co-specific. As the name *T. ishikariensis* has priority, the other names must be placed in synonymy. . . . However, some doubts may still exist as to the correctness of placing certain names in synonymy with *T. ishikariensis*. These will not be resolved until Japanese, American, and Swedish specimens are compared culturally, morphologically, and pathogenically by one investigator." The importance of sclerotial characteristics in *Typhula* spp. identification is again emphasized when McDonald (1961) states, "Although Corner (1950) and Remsberg (1940a) described in detail the characteristics of the fruiting bodies, they state that the structure of the sclerotia are the primary diagnostic importance in the taxonomy of the genus."

Since 1801, identification of the *Typhula* species relied heavily on the characteristics of the sclerotia and fruiting bodies until Røed (1969) used interfertility experiments as a basis of species identification. The objective of Røed's study was to determine if the crossing of monokaryon cultures of *T. graminum* (from the Netherlands), *T. itoana* (from Canada) and *T. incarnata* (from Japan) resulted in dikaryotization of the cultures. The positive interfertility reaction (dikaryotization) between these three species led Røed to conclude that, "the different

cultures are regarded as the same species," and *T. incarnata* Lasch ex Fr. takes priority.

G. W. Bruehl, a 1948 graudate of the Department of Plant Pathology at the Unversity of Wisconsin-Madison, improved on this technique and used it to differentiate *T. idahoensis*, *T. ishikariensis* and *T. incarnata*. Bruehl et al. (1975) found that *Typhula* monokaryons are dikaryotized by donor dikaryons of the same species and, therefore, pairing unknown *Typhula* dikaryons with known *Typhula* monokaryons (di-mon pairings) can be useful in identifying *Typhula* isolates. The successful use of this method for rapidly identifying *Typhula* spp. without the formation of fertile sporophores laid the foundation for a di-mon (dikaryon X monokaryon) mating system of taxonomy within the genus *Typhula*. Bruehl et al. (1975) concluded that *Typhula ishikariensis* is a circumpolar species widely spread before agriculture. "Consequently," observed Bruehl, "the genetic system of *Typhula* spp. must be conservative since long separated populations, such as *T. ishikariensis* from the USA, Japan, and Finland, are compatible. Røed made similar observations on *T. incarnata* from the USA and Norway." The di-mon pairing system has substantially contributed to the clarification of the *Typhula* species identification and disease diagnosis.

Three years after Bruehl's observations, further advancements in the area of *Typhula* spp. identification were made by researchers in Norway and Canada. Årsvoll and Smith (1978) investigated the genetic relationships between an unknown *Typhula* isolate (*Typhula* FW) and other *Typhula* species pathogenic on gramineous plants. Based on results obtained from interfertility experiments including di-mon pairing experiments, Årsvoll and Smith concluded that *Typhula* FW, *T. ishikariensis* Imai and *T. idahoensis* Remsberg should be considered the same species, and, by priority, *T. ishikariensis* applied to it. Furthermore, Årsvoll and Smith used morphological characters to devise a "*T. ishikariensis* complex" that consisted of the three varieties: *T. ishikariensis* var. *ishikariensis*, *T. ishikariensis* var. *idahoensis* and *T. ishikariensis* var. *canadensis*. This "*T. ishikariensis* complex" was based on interfertility experiments and cultural and morphological characteristics. The basidia and

basidiospore characterization of *T. idahoensis* and *T. ishikariensis* "did not provide a satisfactory means of distinguishing between the species. The only reasonably reliable way of separating the latter two species (*T. idahoensis* and *T. ishikariensis*) is on sclerotial characters." The sclerotial characters that were used to separate the varieties include rind cell patterns, attachment to the host, color, size and shape *in vitro* and *in vivo*. Smith et al. (1989) provided further detailed descriptions of the *T. ishikariensis* complex as follows:

"***T. ishikariensis* Imai var. *ishikariensis* Ărsvoll and Smith.** Sclerotia erumpent, readily detached from the host, globose to subglobose or slightly flattened, light brown to almost black 0.5-1.5 mm diam., surface smooth to rough. Rind cells are fairly regular in outline, moderately lobate, rarely digitate. On agar media aerial mycelium is often sparse, sclerotia single, scattered or in concentric rings, on the surface or submerged in the agar."

"***T. ishikariensis* Imai var. *idahoensis* Ărsvoll and Smith.** Sclerotia are erumpent, less easily detached from the host than those of var. *ishikariensis*, globose to subglobose or slightly flattened, brown to almost black, 0.5-2 mm diam., surface often rough and ridged; rind cells are irregular in outline. On agar culture media, mycelium is usually sparse, but occasionally abundant and floccose in some isolates. Sclerotia are single, clustered or tending to form in concentric rings, on the surface and submerged in the agar. Var. *idahoensis* is restricted to hosts in the Gramineae."

"***T. ishikariensis* Imai var. *canadensis* Ărsvoll and Smith.** Sclerotia are readily detached from the host or from abundant wefts of mycelium spanning the leaves; they are globose to subglobose or elongate-oval, light brown to almost black 0.3-0.8 mm diam., surface smooth, often with attached hyaline hyphae when fresh, rind cells fairly regular in outline to very irregular, lobate, sometimes digitate. On agar media, aerial mycelium is abundant floccose; sclerotia are formed in the aerial mycelium, usually on the surface, but occasionally submerged in the agar, scattered or in radial rows rather than in concentric rings. The hosts are grasses, winter cereals and rarely forage legumes in northern North America. In Japan, a fungus similar morphologically to var. *canadensis* has similar host range."

Although these descriptions are very useful in identification, it is still somewhat difficult to distinguish between these three varieties based solely on the characteristics of fruiting bodies and sclerotia. Di-mon pairings are the critical experiments needed to differentiate isolates within the *T. ishikariensis* complex.

In Japan, the *T. ishikariensis* complex is composed of biotype A, biotype B and biotype C, not species or varieties (Matsumoto et al., 1982). From the results of mating experiments and cultural morphology, biotype A was identical to *T. ishikariensis* and *T. ishikariensis* var. *ishikariensis*, and biotype B was similar to *T. ishikariensis* var. *canadensis*

but not identical to *T. idahoensis* or *T. ishikariensis* var. *idahoensis*. Matsumoto et al. (1982) suggested that in Japan the process of speciation has been completed and each biotype is different enough to be regarded as a separate species. "In the U.S.A., however," Matsumoto continued, "unidentifiable isolates which mate with both *T. ishikariensis* and *T. idahoensis* exist in nature, and both species are indistinguishable in culture. Speciation there can be assumed to be still in the process." Matsumoto and Tajimi (1990, 1991) later concluded that biotypes B and C were a single biological species, with biotype C being the small sclerotial form of biotype B (biotype Bss).

In 1988, Bruehl (1988) reconsidered the *T. ishikariensis* complex in the U. S. A. and placed only *T. ishikariensis* and *T. idahoensis* in the complex. He considered *T. ishikariensis* var. *ishikariensis* to be *T. ishikariensis*; *T. ishikariensis* var. *idahoensis* to be *T. idahoensis*; and *T. ishikariensis* var. *canadensis* a different form not found in Washington. Furthermore, he proposed that the Japanese *T. ishikariensis* biotype A to be *T. ishikariensis* and biotype B to be *T. idahoensis*.

Matsumoto et al. (1996) collected and characterized *T. ishikariensis* isolates from Norway. These scientists divided the isolates into three groups according to di-mon pairings and cultural morphology. Group I isolates gave variable mating patterns with Japanese biotypes A and B, while group II isolates were compatible only with biotype B. Group III was characterized by irregular growth at 10° C and genetic incompatibility with both biotypes A and B. Cultural morphology of group III isolates resembled that of group I isolates at 0° C. They concluded that the *T. ishikariensis* complex of Norway and Japan consisted of two major subgroups which are genetically separate. "One consists of groups I and III and biotype A. The other is group II and biotype B" (Matsumoto et al., 1996; see Figure 3.1).

Tkachenko et al. (1997) investigated the di-mon mating patterns of Russian isolates of *T. ishikariensis* with isolates from Norway, Canada and Japan. According to the di-mon mating reactions, they found that the Russian subterranean tulip isolates were compatible with

Norwegian group I and Japanese biotype A isolates.

Recently, Matsumoto (1997a) divided the *T. ishikariensis* complex into two world-wide biological species (Figure 3.1). A biological species as defined by Mayr (1963): "Biological species are groups of interbreeding natural populations that are reproductively isolated from other groups." Harrington and Rizzo (1999) add that the major features of the biological species concept include ecological and morphological features that are assumed to be congruent with potential interbreeding." "Biological species I (BSI)" includes: *T. ishikariensis* var. *ishikariensis* from North America, *T. ishikariensis* biotype A from Japan, *T. ishikariensis* groups I and III from Norway and *T. ishikariensis* Russian tulip isolates. The "biological species II (BSII)" consists of *T. ishikariensis* var. *idahoensis* and var. *canadensis* from North America, *T. ishikariensis* biotype B from Japan and *T. ishikariensis* group II from Norway.

The difficulties concerning the *T. ishikariensis* complex identification was evident even in Wisconsin. Researchers in the Department of Plant Pathology of the University of Wisconsin at Madison, who have conducted snow mold fungicide experiments from 1982 to 1998, have presented variable identifications of the fungi that caused Typhula blight damage in those field experiments (Table 3.1). After discussing this problem at the 1979 Turf Disease meeting in Ohio, Gayle Worf sent three packets of *Typhula* spp. "sclerotia of different types found in Wisconsin" (Worf's letter to Smith, Appendix B, p. 234) to J. D. Smith for identification. Smith identified the sclerotia as *T. ishikariensis* Imai var. *canadensis* Smith Årsvoll, *T. incarnata* Lasch ex Fr., and *T. phacorrhiza* Reichard ex Fries (Smith's letter to Worf, Appendix B, p. 235).

Such differences of opinion in the literature and interpretation of results illustrate the importance of studying new approaches to understanding the speciation of the *T. ishikariensis* complex. This research endeavored to lay the foundation for improved molecular identification by first using previously reported techniques to place the Wisconsin *T. ishikariensis* isolates among isolates from around the world. The specific objective of this research was to

Table 3.1. Location of and pathogens present in the Typhula blight fungicide trials conducted by the University of Wisconsin, Department of Plant Pathology from 1982 to 1993[a].

EXPERIMENT		PATHOGEN
Year	**Location**	**Genus species [b]**
1982	Appelton	no disease
	Madison	no disease
1983	Wausau	unidentified *Typhula* spp.
	Waukesha	*M. nivale* only
1984	Wausau	*M. nivale* only
	Waukesha	*M. nivale* only
1985	Stevens Point	unidentified *Typhula* spp.
1986	Stevens Point	*T. ishikariensis* var. *canadensis*
1987	Stevens Point	*T. ishikariensis* var. *canadensis*
	Waukesha	no disease
1988	Stevens Point	*T. ishikariensis* var. *canadensis*
1989	Walworth	*M. nivale* only
1990	Langlade	*T. canadensis*
	Eagle River	*T. canadensis*
	Madison	no disease
1991	Langlade	*T. ishikariensis*
	Eagle River	*T. ishikariensis*
	Madison	no disease
1992	Rhinelander	unidentified *Typhula* spp.
	Plum Lake	unidentified *Typhula* spp.
1993	Sarona	unidentified *Typhula* spp.
	Madison	unidentified *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 fungicide reports from 1994 to 1996.
[b] Genus-species as stated by the authors.

genetically characterize, by dikaryon-monokaryon mating experiments, the Wisconsin *T. ishikariensis* complex isolates as compared to BSI and BSII. Specifically the hypotheses being tested were: (i) the *T. ishikariensis* complex isolates that were collected from Wisconsin golf courses can be separated into two groups based on cultural morphology and on the results of the dikaryon-monokaryon mating experiments and (ii) the Wisconsin *T. ishikariensis* complex isolates will vary in their ability to mate with BSI and BSII monokaryotic testers from Japan, Norway, Russia and Canada. The Wisconsin *T. ishikariensis* complex isolates collected in the survey were first grouped according to their morphological characteristics in culture and then paired with the monokaryon BSI and BSII testers from around the world. Clamp connection formation of the tester isolate signaled a positive mating reaction. The results of the dikaryon-monokaryon mating experiments indicate that there are at least two genetically different groups of the *T. ishikariensis* complex present in Wisconsin golf courses. The two Wisconsin groups (WIG1 and WIG2) are morphologically similar and partially genetically compatible with BSI and BSII isolates, respectively. Twenty-three percent of the Wisonsin isolates were morphologically similar to and mated with the BSI isolates, while 77% were morphologically similar to and mated with BSII isolates. Identifying and understanding the biological differences between BSI and BSII of the TISH complex should improve the efficacy of Typhula blight management tactics.

MATERIALS AND METHODS

Fungal isolates

All of the dikaryotic isolates from Wisconsin were obtained from sclerotia produced on grasses collected in the survey of Wisconsin golf courses (Chapter 2). 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.

After at least one month of growth at 10° C, three randomly selected *T. ishikariensis* isolates were selected for each of the three survey zones. The southern zone isolates were 1.31.MS, 1.83.CM and 1.93.CM. The central zone isolates were 2.160.SP, 2.189.IO and 2.109.BE. The northern zone isolates were 3.133.P, 3.257.IN and 3.285.EB. Isolate 3.133.P was not from the survey but was isolated from turfgrass, which was part of the fungicide trials at the Plum Lake Golf Club (J. Gregos, Turfgrass Disease Diagnostic Lab, University of Wisconsin-Madison).

Also, fourteen isolates were randomly selected from four fairways at Sentryworld Golf Course, Stevens Point, WI (2.96.SW, 2.97.SW, 2.99.SW, 2.102.SW, 2.103.SW, 2.104.SW, 2.105.SW, 2.105b.SW, 2.106.SW, 2.107.SW, 2.109.SW, 2.110.SW, 2.11.SW and 2.12.SW) and thirteen isolates from four fairways at Trout Lake Golf and Country Club, Woodruff, WI (3.113.TL, 3.117.TL, 3.119.TL, 3.122.TL, 3.125.TL, 3.127.TL, 3.128.TL, 3.115.TL, 3.116.TL, 3.118,TL, 3.120.TL, 3.121.TL and 3.123.TL), because they were

potential snow mold fungicide trials sites of the Turfgrass Disease Diagnostic Lab, University of Wisconsin-Madison. Furthermore, ten additional isolates from the central survey zone were chosen. These ten isolates were taken from five randomly selected central zone sites (2 isolates at each site): 2.172.GC, 2.183.GC, 2.137.W, 2.153.W, 2.161.SP, 2.165.SP, 2.212.BE, 2.222.BE, 2.227.M and 2.228.M.

In total, forty-six *T. ishikariensis* isolates were used in the dikaryon-monokaryon mating experiments. Two *T. incarnata* isolates (1.35.MS and 3.114.TL) were selected as the control and two unidentified *Typhula* isolates (3.129.TL and 3.242.TR) were selected as out groups. The forty-six *T. ishikariensis* isolates were then separated into two groups based on their culture morphology (WIG1 and WIG2).

In vitro, Wisconsin group 1 (WIG1) isolates resembled *T. ishikariensis* biotype A and *T. ishikariensis* var. *ishikariensis* , which have brown to dark brown, spherical sclerotia often produced in concentric rings (Figure 3.2). On agar media, aerial mycelium of *T. ishikariensis* var. *ishikariensis* is often sparse, with sclerotia single, scattered or in concentric rings on the surface or submerged in the agar (Årsvoll and Smith, 1978). Wisconsin group 2 (WIG2) isolates resembled *T. ishikariensis* biotype B and *T. ishikariensis* var. *canadensis*, which have abundant aerial mycelium (Figure 3.2). On agar media, aerial mycelium is abundantly floccose (cottony); sclerotia are formed in the aerial mycelium, usually on the surface, but occasionally submerged in the agar, scattered or in radial rows rather than in concentric rings (Smith et al., 1989).

Monokaryotic tester isolates of BSI and BSII were obtained from Dr. Naoyuki Matsumoto, National Institute of Agro-Environmental Sciences, Tsukuba, Japan and their descriptions are presented in Table 3.2. The presence of clamp connections and the nuclear condition of the monokaryons and dikaryons were verified by observing the mycelium stained with Safranin-O (Bandoni, 1979).

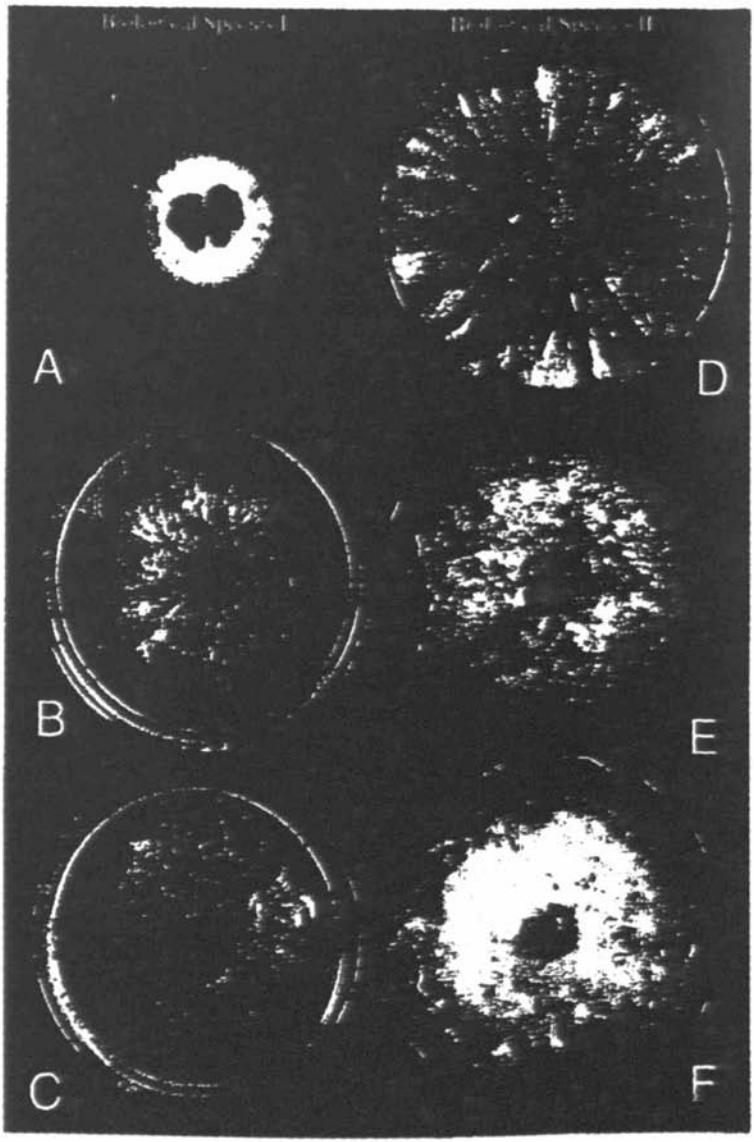

Figure 3.2. Cultural characteristics of the *Typhula ishikariensis* complex. A) *T. ishikariensis* biotype A (ATCC #56263), B) *T. ishikariensis* var.*ishikariensis* (ATCC# 38649), C) *T. ishikariensis* 3.120.TL, D) *T. ishikariensis* biotype B (ATCC# 56264), E) *T. ishikariensis* biotype C (ATCC#56259) and F) *T. ishikariensis* (2.104.SW) grown on potato dextrose agar at 10 °C for 75 days.

Table 3.2. Descriptions of the *Typhula ishikariensis* Imai complex monokaryotic tester isolates used in the dikaryon-monokaryon mating experiments.

Monokaryotic Tester [a]	Designation	Origin	Host	Reference
PR9-4-3	*T. ishikariensis* Biotype A	Hamatonbetsu, Japan	Perennial Ryegrass	Matsumoto and Tajimi, 1993
PR7-6-7	*T. ishikariensis* Biotype A	Hamatonbetsu, Japan	Perennial Ryegrass	Matsumoto and Tajimi, 1993
92-32-MI	*T. ishikariensis* var. *ishikariensis*	Moscow, Russia	Tulip bulb	Tkachenko, et al., 1997
2-5BS-1	*T. ishikariensis* Group I	Langedal, Norway	Timothy grass	Matsumoto, Tronsmo and Shimanuki, 1996
6-1BS-4	*T. ishikariensis* Group III	Fjosstykket, Sor-Varanger, Norway	Timothy grass	Matsumoto, Tronsmo and Shimanuki, 1996
35-8	*T. ishikariensis* Biotype B	Sapporo, Japan	unknown grass	Matsumoto and Tajimi, 1991
8-2	*T. ishikariensis* Biotype B	Morioka, Japan	unknown grass	Matsumoto and Tajimi, 1991
4-3,S-5	*T. ishikariensis* Group II	Kvarvet, Norway	Timothy grass	Matsumoto, Tronsmo and Shimanuki, 1996
Can 21m	*T. ishikariensis* var. *canadensis*	Canada	unknown grass	Arsvoll and Smith, 1978

[a] All monokaryotic tester isolates were obtained from Dr. Naoyuki Matsumoto, National Institute of Agro-Environmental Sciences, Tsukuba, Japan.

Dikaryon-monokaryon pairing

The dikaryon-monokaryon mating experiments, illustrated in Figure 3.3, between the dikaryotic isolates from Wisconsin and the monokaryotic BSI and BSII tester isolates from around the world were conducted as reported by Bruehl et al. (1975) and Matsumoto et al. (1996). Five-mm diameter agar discs with mycelia were cut from the margin of actively growing PDA cultures of both monokaryotic testers and dikaryotic isolates and placed approximately 2-cm apart on PDA plates. After 3 to 4 days of colony contact (ca. 14 days incubation at 10° C), a 5-mm agar disc was cut from the monokaryon colony 1-cm behind the colony junction ("clamp" piece) and transferred to the unoccupied portion of the same plate, or to a new plate, if the space was limited. The mycelia growing from the "clamp" piece was microscopically examined (100x) for the presence of clamp connections one week later. The tester's acceptance of foreign nuclei from the unknown dikaryon would cause clamp connection formation and indicated a mating compatibility reaction. All pairings were completed twice.

The possible hyphal reactions of the dikaryon-monokaryon mating experiments are presented in Figure 3.4. The symbol '+' indicates a compatible reaction with numerous clamp connections and vigorous growth from the "clamp" piece. When the growth was normal but lacked clamps (smooth hyphae, or rhizomorphs), the combination was incompatible, and the symbol '-' was given. The symbol '±' shows the combination in which the growth was poor with a few clamps or irregularly ramified hyphae. In the case where the first pairing reaction varied with the second pairing, a "/" separates the symbols (i. e., "+/-" = positive mating reaction in the first pairing and a negative mating reaction the second pairing). In the case where the growth of the dikaryon colony was inhibited and therefore did not interact with the monokaryotic tester isolate, a 'n' is given. This 'n' reaction was considered to be a particular type of negative reaction.

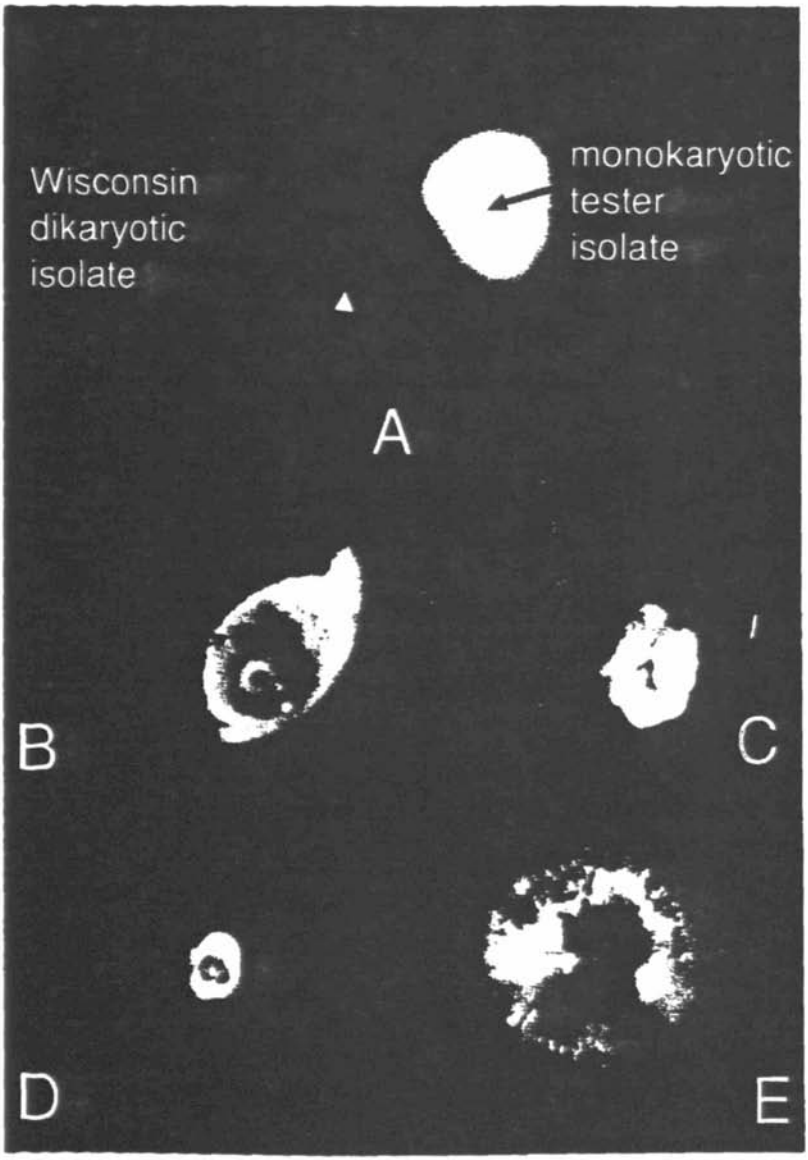

Figure 3.3. Illustration of the dikaryon -monokaryon pairing and possible results. A) Pairing with dikaryotic isolate on the left and the monokaryotic tester on the right. B) Colonies grow towards each other, arrow indicates clamp piece taken 1 cm back from the junction of the two colonies. C) Antagonism prevents colony contact. D) Negative reaction with smooth hyphae. E) Positive reaction with clamp connections, note vigorous growth and sclerotia formation.

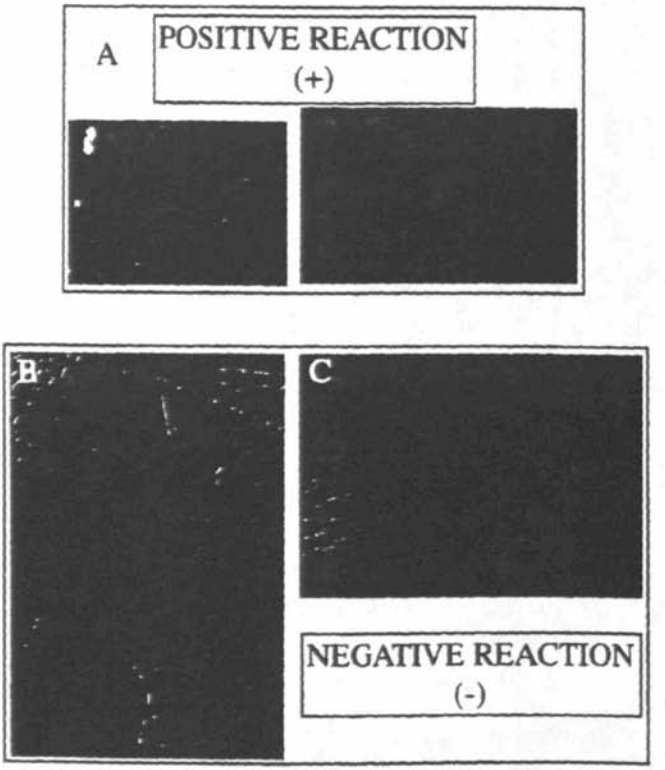

Figure 3.4. Possible hyphal reactions in the dikaryon-monokaryon mating experiments. A) Clamp connections indicate positive acceptance. B) Smooth hyphae indicate negative reaction. C) Rhizomorphs indicate negative reaction.

Statistical analysis

Contingency table analysis was used to examine the distribution of the response variable Y (monokaryotic tester response) as conditioned by the values of a factor X (ex. isolate # or Wisconsin grouping) and is presented as a mosaic chart. A mosaic chart consists of side-by-side divided bars for each level of the X variable, where each bar is divided into proportional segements that represent each discrete Y value. The width of each bar is proportional to the sample size. When the lines dividing the bars align, the response proportions are alike. When the lines are far apart, the response rates of the samples may be statistically different (Anonymous, 1995).

Contingency table mosaic charts are used to graphically portray a two-way frequency table. A mosaic chart is any chart divided into small rectangles such that the area of each rectangle is proportional to a frequency count of interest. JMP® divides the Y counts within each X level by the total of the level to compute each X, Y proportion. This estimates the response probability for each X, Y level (Hartigan & Kleiner, 1981). The proportions shown on the X axis represent the relative sizes of the *Typhula* pairing totals. The scale of the left Y axis shows response probability, with the whole axis being probability 1 (the total sample). To see how the response probabilities vary for the different levels of X, compare the heights of Y levels across the X levels. If the response divisions line up horizontally across the X levels, their response rates are the same.

Also, multi-categorical data analysis was used to calculate predicted percentages for each possible dikaryon-monokaryon mating reaction combination based on maximum likelihood estimates of the multiple category logit model (Agresti, 1996).

For the statistical analyses, the mating results of the freezing resistant *T. ishikariensis* tester isolate 6-IBS-4 (Norway group III) were excluded, because although the isolate looks morphologically similar to Norway group I isolates, it did not mate with the Norway group I, Japan biotype A or Japan biotype B tester isolates (see Figure 3.1, Matsumoto et al., 1996).

The Norway group III isolates are regarded as a freezing resistant ecotype adapted to colder temperatures and are more resistant to freezing temperatures than other *T. ishikariensis* isolates (Hoshino et al., 1997a; Hoshino et al., 1997b; and Hoshino et al., 1998). Because of the uniqueness and incompatibility of tester isolate 6-IBS-4 with other *T. ishikariensis* isolates within BSI and BSII, the mating results from this tester isolate were not used in the statistical analysis. The results of the dikaryon-monokaryon mating experiments are also summarized by frequency counts.

RESULTS

Ten *T. ishikariensis* WIG1 isolates were paired with four monokaryotic tester isolates of the *T. ishikariensis* BSI; four monokaryotic tester isolates of *T. ishikariensis* BSII; and with one tester isolate of the freezing resistant ecotype Norway group III, 6-IBS-4 (Table 3.3). The pairing of the WIG1 isolates with the BSI isolates produced 27 positive reactions (+) for both pairings, 10 pairings gave negative reactions (-) for both pairings, and three were inconclusive (different results from the first and second experiment). All of the 10 WIG1 isolates mated positively with at least one of the four BSI isolates. The freezing resistant ecotype *T. ishikariensis* Norway group III tester isolate 6-IBS-4 was not compatible with any of the WIG1 isolates. Four WIG1 isolates (2.172.GC, 2.183.GC, 3.118.TL and 3.120.TL) gave compatible reactions for both experiments with all the BSI testers (Table 3.3). The pairing of WIG1 isolates with the BSII isolates produced 36 negative reactions for both pairings, three were inconclusive, one was not determined for both pairings; and there were no positive reactions nor intermediate reactions for both pairings. The tester isolates Japan biotype A (PR9-4-3), Japan biotype A (PR7-6-7), Russia (92-32-MI), and Norway group I (2-5BS-1) were compatible with the WIG1 isolates in 12 out of 20 (60%), 15 out of 20 (75%), 14 out of 20 (70%) and 15 out of 20 (75%) of the pairings, respectively (Table 3.3).

An alternative graphical analysis of the dikaryon-monokaryon experiments is presented as a mosaic chart of the contingency table analysis (Figure 3.5). Results of the individual dikaryon-monokaryon experiments, except the Norway group III 6-IBS-4 isolate, were used in the contingency table analysis. For example, an inconclusive result of (+/-) was separated into two individual units of data (one (+) and one (-) reaction). Also, a result of (+) was separated into two individual units of data (two (+) reactions), and so on. So, for each individual WIG1 dikaryon isolate x BSI group pairing there are a total of eight reactions. Figure 3.5A visually highlights that WIG1 isolates are compatible with BSI and that all WIG1 isolates had at least two compatible reactions with the BSI tester isolates. Figure 3.5B illustrates that WIG1

Table 3.3. Dikaryon-monokaryon mating reactions of dikaryotic Wisconsin *Typhula ishikariensis* Group I isolates with monkaryotic tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Group I Isolates	Biological Species I Monokaryotic Tester Isolates [a]				Biological Species II Monokaryotic Tester Isolates [a]				Freezing Resistant
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	var. *ishikariensis* 92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *canadensis* Can 21m	Norway Group III 6-1BS-4
1.93.CM	+[b]	±/+	+	+	-	-	-	-	-
2.172.GC	+	+	+	+	-	-	-	n	-
2.183.GC	+	+	+	+	-	-	-	-	-
3.115.TL	-	+	±/-	-	-	-	-	-	-
3.116.TL	-	+	-	+	-	-	-	+/-	-
3.118.TL	+	+	+	+	-	-	-	-	-
3.120.TL	+	+	+	+	-	-	-	±/-	±
3.121.TL	-	-	+	-/+	-	-	-	±/n	+/±
3.123.TL	-	-	+	-	-	-	-	-	-
3.285.EB	+	+	-	+	-	-	-	-	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clamp connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monkaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments.

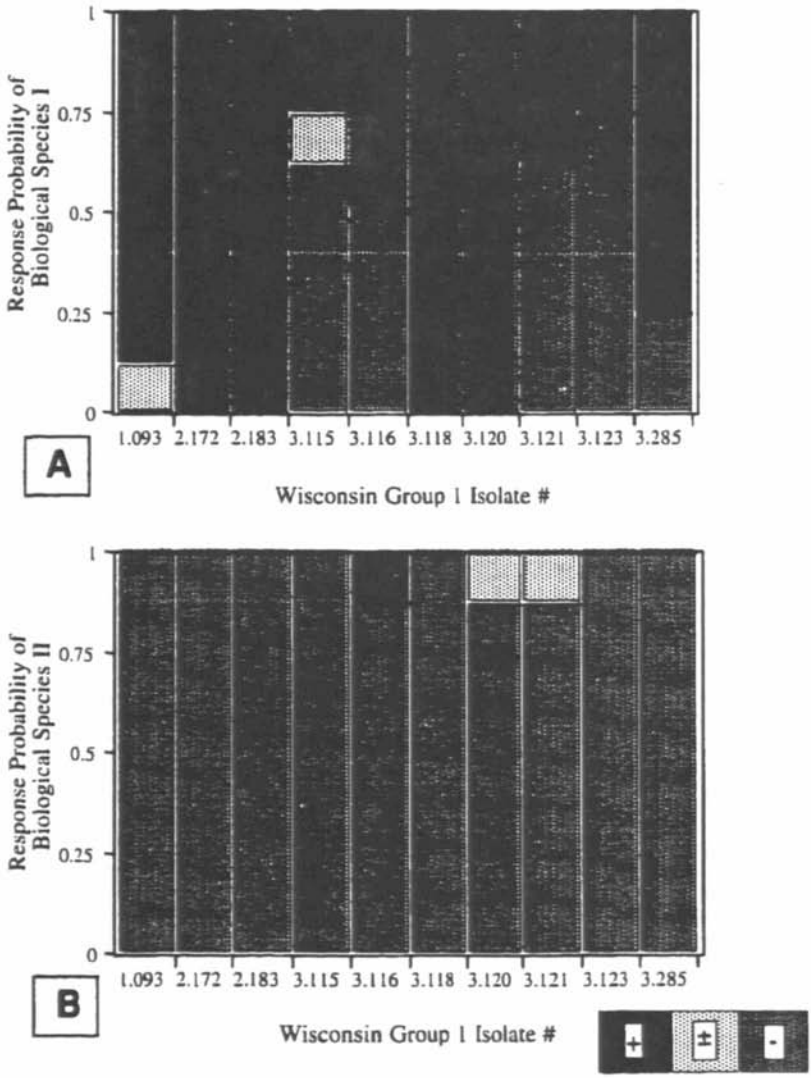

Figure 3.5 Mosaic chart of contingency table analysis of the dikaryon-monokaryon mating experiment results of Wisconsin group I isolates versus A) biological species I reaction and B) biological species II reaction. Red = (-) reaction, green = (±) reaction and blue = posititve reaction (+).

isolates are not compatible with BSII and there was only one compatible reaction with BSII tester isolates. From Figure 3.5 and Table 3.3, it is clear that the Wisconsin dikaryotic isolates that were separated into WIG1 based on morphological characteristics were more compatible with BSI than with BSII.

Thirty-six *T. ishikariensis* WIG2 isolates were paired with four monokaryotic tester isolates of the *T. ishikariensis* BSI; four monokaryotic tester isolates of *T. ishikariensis* BSII; and with one tester isolate of the freezing resistant ecotype Norway group III, 6-IBS-4 (Table 3.4). The pairing of the WIG2 isolates with the BSI isolates produced no positive reactions (+) for both pairings, 139 pairings gave negative reactions (-) for both pairings, and 5 were inconclusive (different results from the first and second experiment). None of the thirty-six WIG2 isolates mated positively with any of the four BSI tester isolates. WIG2 isolate 3.128.TL was the only isolate that was compatible with the freezing resistant ecotype *T. ishikariensis* Norway group III tester isolate 6-IBS-4. The pairing of WIG2 isolates with the BSII isolates produced 67 positive reactions for both pairings, 62 negative reactions for both pairings and 15 were inconclusive. None of the WIG2 isolates gave compatible reactions for both experiments with all the BSII testers (Table 3.4). The tester isolates Japan biotype B (35-8), Japan biotype B (8-2), Norway group II (4-3,S-5) and Canada *T. ishikariensis* var. *ishikariensis* (Can 21m) were compatible with the WIG2 isolates in 17 out of 72 (24%), 41 out of 72 (57%), 21 out of 72 (29%) and 62 out of 72 (86%) of the pairings, respectively (Table 3.3). The monokaryotic BSI tester isolate, *T. ishikariensis* var. *ishikariensis* Can 21m, was more compatible (86% compatible) with the WIG2 isolates than the other BSI tester isolates.

Again, an alternative graphical analysis of the dikaryon-monokaryon experiments is presented as a mosaic chart of the contingency table analysis (Figures 3.6-1 to 3.6-3). Results of the individual dikaryon-monokaryon experiments, except the Norway group III 6-IBS-4 isolate, were used in the contingency table analysis. Figures 3.6-1B, 3.8-6B and 3.6-3B

Table 3.4. Dikaryon-monokaryon mating reactions of dikaryotic Wisconsin *Typhula ishikariensis* group 2 isolates with monokaryotic tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Group 2 isolates	Biological Species I Monokaryotic Tester Isolates[a]				Biological Species II Monokaryotic Tester Isolates[a]				Freezing Resistant Ecotype
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	var. *ishikariensis* 92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *canadensis* Can 21m	Norway Group III 6-IBS-4
1.31.MS	-[b]	-	-	-	-	+	-	+	-
1.83.CM	-	-	-	-	-	-	-	+	-
2.96.SW	-	-	-	-	±/-	-	-	+	-
2.97.SW	-	-	-	-	±/+	-/+	-	+	-
2.99.SW	-	-	-	-	-/+	+	+	+	-
2.102.SW	-	-	±/-	-	±/-	+	-	+	-
2.103.SW	-	-	-	-	-	-	-	+	±/-
2.104.SW	-	-	-	-	+	+	±/-	+	-
2.105.SW	-	-	-	-	-	+	+	+	-
2.105b.SW	-	-	-	-	-	+	+	+	-
2.106.SW	-	-	-	-	+/-	+	+	+	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clamp connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monokaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments.

Table 3.4. (continued) Dikaryon-monokaryon mating reactions of dikaryotic Wisconsin *Typhula ishikariensis* group 2 isolates with monkaryotic tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Group 2 isolates	Biological Species I Monokaryotic Tester Isolates[a]				Biological Species II Monokaryotic Tester Isolates[a]				Freezing Resistant Ecotype
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	var. *ishikariensis* 92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *canadensis* Can 21m	Norway Group III 6-IBS-4
2.107.SW	-[b]	-	-	-	+	+	-	+	-
2.109SW	-	-	-	-	+	-	-	+	-
2.110.SW	-	-	-	-	+	-	-	+	-
2.111.SW	-	-	-	-	-	-	-	+	-
2.112.SW	-	-	-	-	-	+	-	+	-
2.137.W	-	-	-	-	-	-	-	+	-
2.153.W	-	-	±/-	-	-	-	-	+	-
2.160.SP	-	-	-	-	-	+	-	+	-
2.161.SP	-	-	-	-	-/±	-	-	+	-
2.165.SP	-	-	+/-	-	-	-	-	+	-
2.189.IO	-	+/-	-	-	-	+	+	-	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clamp connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monokaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments.

Table 3.4. (continued) Dikaryon-monokaryon mating reactions of dikaryotic Wisconsin *Typhula ishikariensis* group 2 isolates with monkaryotic tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Group 2 isolates	Biological Species I Monokaryotic Tester Isolates[a]				Biological Species II Monokaryotic Tester Isolates[a]				Freezing Resistant Ecotype
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	var. *ishikariensis* 92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *canadensis* Can 21m	Norway Group III 6-IBS-4
2.209.BE	-[b]	-	-	-	+	+	+	-	+
2.212.BE	-	-	-	-	-	+	-	+	-
2.222.BE	-	-	-	-	+	-	+	+	-
2.227.M	-	-	-	-	±/-	+	-	+	-
2.228.M	-	-	-	-	±/-	±/-	-	+	-
3.113.TL	-	-	-	-	±/-	-	-/+	+	-
3.117.TL	-	-	-	-	-	+	-/+	+	-
3.119.TL	-	-	-	-	-	-	-	+	-
3.122.TL	-	-	-	-	+	-	-/+	+	+/±
3.125.TL	-	-	-	-	-	+	-	-	-
3.127.TL	-	-	-	-	-	+	-	-	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clamp connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monokaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments.

Table 3.4. (continued) Dikaryon-monokaryon mating reactions of dikaryotic Wisconsin *Typhula ishikariensis* group 2 isolates with monkaryotic tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Group 2 isolates	Biological Species I Monokaryotic Tester Isolates[a]				Biological Species II Monokaryotic Tester Isolates[a]				Freezing Resistant Ecotype
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	var. *ishikariensis* 92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *canadensis* Can 21m	Norway Group III 6-IBS-4
3.128.TL	-[b]	-	-	-	-	+	+	+	+
3.133.P	-	-	-	-	-	+	-	+	-
3.257.IN	-	-	±/+	-	-	+	+	-	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clump connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monokaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments.

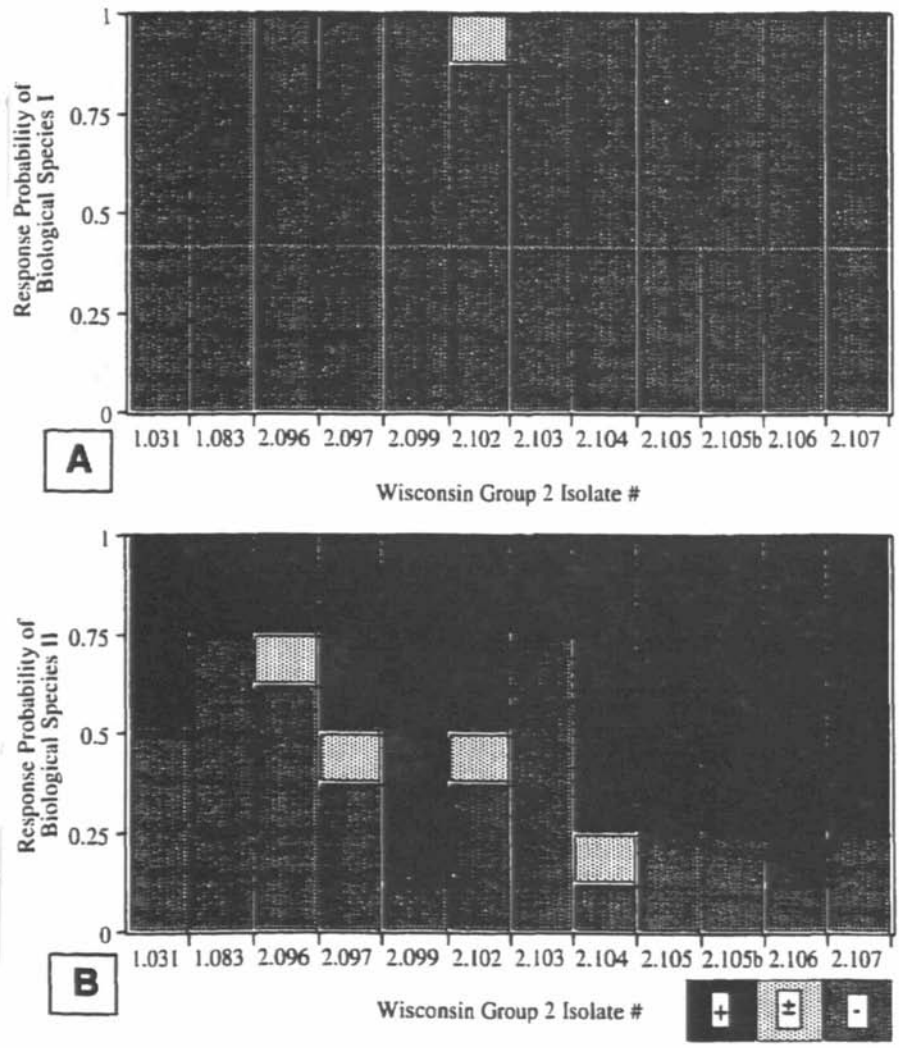

Figure 3.6-1. Mosaic chart of contingency table analysis of the dikaryon-monokaryon mating experiment results of Wisconsin group 2 isolates (1.031 to 2.109) versus A) biological species I isolates and B) biological species II isolates. Red = negative reaction (-), green = intermediate reaction (±) and blue = positive reaction (+).

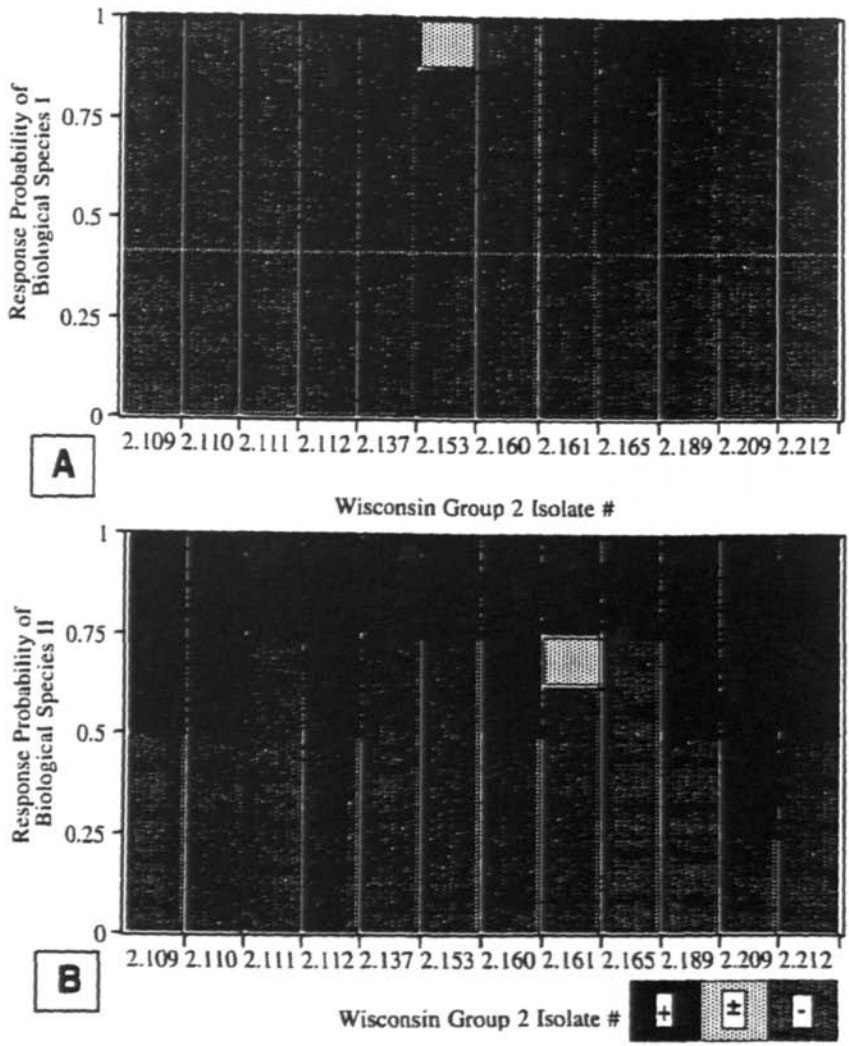

Figure 3.6-2. Mosaic chart of contingency table analysis of the dikaryon-monokaryon mating experiment results of Wisconsin group 2 isolates (2.110 to 2.222) versus A) biological species I isolates and B) biological species II isolates. Red = negative reaction (-), green = intermediate reaction (±) and blue = positive reaction (+).

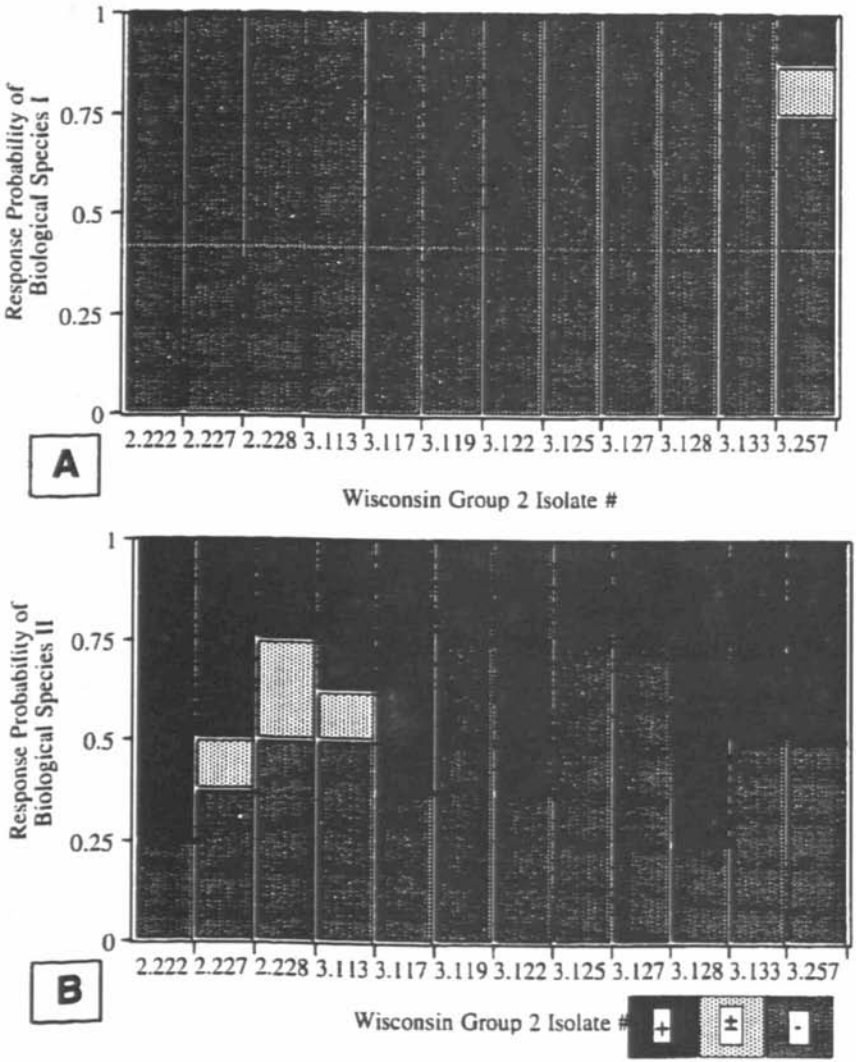

Figure 3.6-3. Mosaic chart of contingency table analysis of the dikaryon-monokaryon mating experiment results of Wisconsin group 2 isolates (2.227 to 3.257) versus A) biological species I isolates and B) biological species II isolates. Red = negative reaction (-), green = intermediate reaction (±) and blue = positive reaction (+).

visually highlight that WIG2 isolates are compatible with BSII and that all WIG2 isolates had at least two compatible reactions with the BSII tester isolates. Figures 3.6-1A, 3.6-2A and 3.6-3A illustrate that WIG2 isolates are not compatible with BSI and there were only three compatible reactions with BSI tester isolates. From Figure 3.6-1, Figure 3.6-2, Figure 3.6-3 and Table 3.4, it is clear that the Wisconsin dikaryotic isolates that were separated into WIG2 based on morphological characteristics were more compatible with BSII than with BSI.

Of the forty-six isolates that were selected from the survey samples (Chapter 2), 22% (10 out of 46) were WIG1 isolates and 78% (36 out of 46) were WIG2 isolates (Tables 3.3 and 3.4). These results indicate that WIG2 isolates could have been the predominant group of *T. ishikariensis* collected from Wisconsin golf courses in the spring of 1997. Furthermore, these WIG2 isolates are more compatible with the BSII tester isolates than with the BSI isolates (Table 3.3 and Figure 3.5). The less frequently collected (22% versus 78% of the survey sub-sample) WIG1 isolates are more compatible with the BSI than with the BSII isolates (Table 3.4 and Figures 3.6-1, 3.6-2 and 3.6-3).

A mosaic chart summary of contingency table analysis of the dikaryon-monokaryon mating reactions of WIG1 and WIG2 versus BSI and BSII are presented in Figure 3.7, and Table 3.5 gives the frequency and percentage of total counts of the mating results. WIG1 was more compatible with BSI (70%) and WIG2 was compatible with BSII (49%). However, there were more isolates in WIG2 than WIG1. The pairing of WIG1 with BSII and the pairing of WIG2 with BSI almost always resulted in incompatible reactions.

Predicted percentages for each possible dikaryon-monokaryon mating reaction combination based on maximum likelihood estimates of the multiple category logit model (Agresti, 1996) are presented in Table 3.6. This analysis predicts that identifying *T. ishikariensis* isolates from Wisconsin as WIG1 and pairing them with BSI will result in 71.6% of the pairings being compatible and 28.4% of the reactions will not be compatible. Also, pairing WIG1 with BSII will result in 1.1% of the pairings being compatible, 1% being

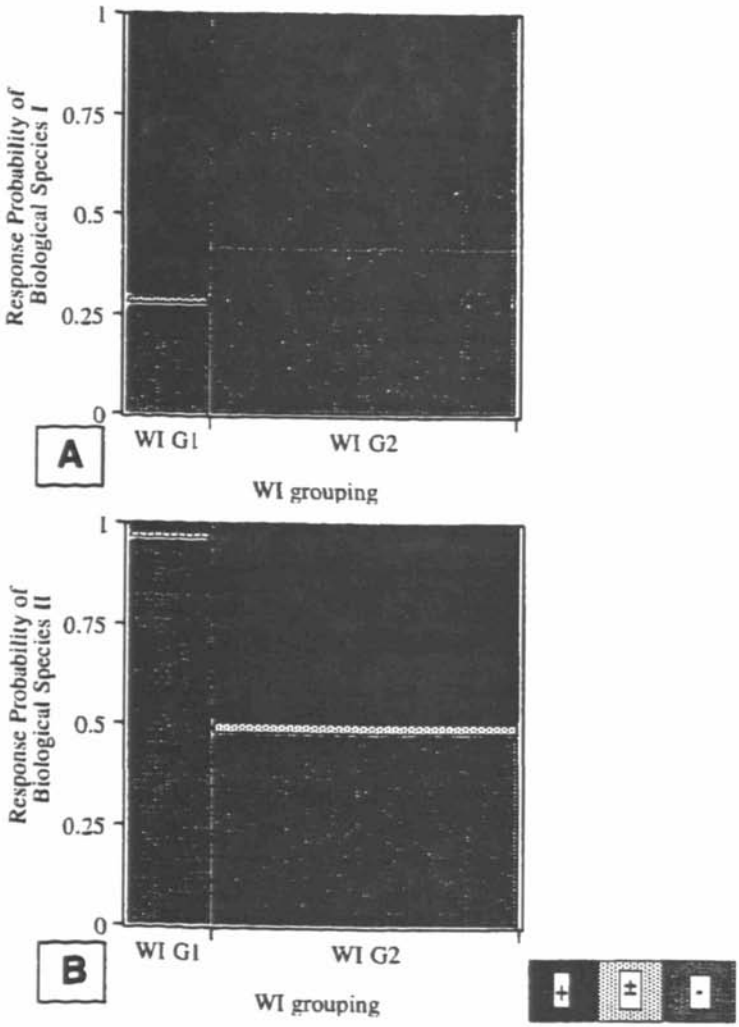

Figure 3.7. Mosaic chart of contingency analysis of the dikaryon-monokaryon mating experiment results of A) Wisconsin group 1 and Wisconsin group 2 versus biological species I and B) Wisconsin group 1 and Wisconsin group 2 versus biological species II. Red = negative mating reaction (-), green = intermediate mating reaction (±) and blue = positive mating reaction (+).

Table 3.5. Numbers of mating reactions (top number) and percentage of total pairings (bottom number) of the dikaryon-monokaryon mating experiments between Wisconsin group 1 and 2 versus biological species I and II.

Wisconsin Grouping	Biological Species I			Biological Species II		
	positive reaction (+)	intermediate reaction (±)	negative reaction (-)	positive reaction (+)	intermediate reaction (±)	negative reaction (-)
Wisconsin Group 1	56 (70%)	2 (2.5%)	22 (27.5%)	1 (1%)	2 (3%)	77 (96%)
Wisconsin Group 2	3 (1%)	3 (1%)	282 (98%)	141 (49%)	9 (3%)	138 (48%)

Table 3.6. Predicted percentages for each possible dikaryon-monokaryon mating reaction combination based on maximum likelihood estimates of the multiple category logit model (Agresti, 1996).

Dikaryon-monokaryon Mating Reaction Combinations	Wisconsin Grouping	Predicted Percentages		
		positive mating reaction	intermediate mating reaction	negative mating reaction
Biological Species I	Wisconsin Group 1	71.6	0	28.4
	Wisconsin Group 2	1.3	2.5	96.3
Biological Species II	Wisconsin Group 1	1.1	1	97.9
	Wisconsin Group 2	51.3	0.4	47.9

intermediate and 97.9% being incompatible. The pairing of WIG2 with BSII will result in 51.3% of the pairings being compatible, 0.3% being intermediate and and 47.9% being incompatible. Pairing WIG2 with BSI will result in 1.3% compatible reactions, 2.5% intermediate and 96.3% incompatible.

Table 3.7 illustrates that two unidentified *Typhula* spp. and two *T. incarnata* isolates were not compatible with BSI, BSII or the freezing resistant ecotype of *T. ishikariensis* complex.

Figures 3.8 and 3.9 are mosaic charts of the contingency table analysis of the dikaryon-monokaryon mating results of the two potential fungicide experiment sites at Sentryworld Golf Course (located in Stevens Point, central zone of Wisconsin) and Trout Lake Golf and Country Club (located in Woodruff, northern zone of Wisconsin). All of the Sentryworld *T. ishikariensis* isolates were identified as WIG1 isolates (Figure 3.9) and they mated only with the BSI isolates (Figure 3.8). On the otherhand, Trout Lake Golf and Country Club had both WIG1 and WIG2 isolates (Figure 3.9) and both partially mated with BSI and BSII, respectively (Figure 3.8).

Table 3.7. Dikaryon-monokaryon pairing reactions of two unidentified dikaryotic *Typhula* spp. isolates [c] and two *T. incarnata* isolates [d] with monokaryotic *T. ishikariensis* tester isolates from Japan, Russia, Norway and Canada.

Wisconsin Isolates	Biological Species I Monokaryotic Tester Isolates[a]				Biological Species II Monokaryotic Tester Isolates[a]				Freezing Resistant Ecotype
	Japan	Japan	Russia	Norway	Japan	Japan	Norway	Canada	Norway
	Biotype A PR9-4-3	Biotype A PR7-6-7	92-32-MI	Norway Group I 2-5BS-1	Biotype B 35-8	Biotype B 8-2	Norway Group II 4-3,S-5	var. *ishikariensis* Can 21m	Norway Group III 6-IBS-4
3.129.TL [c]	- [b]	-	-	-	-	-	-	-	-
3.242.TR [c]	-	-	-	-	-	-	-	-	-
1.35.MS [d]	-	-	-	-	-	-	-	-	-
3.114.TL [d]	-	-	-	-	-	-	-	-	-

[a] Matsumoto, 1997.

[b] (+) = Compatible reaction that is characterized by vigorous offspring hyphal growth with abundant clamp connections. (-) = Incompatible reaction that is characterized by absence of clamp connections. (±) = Intermediate reaction characterized by abnormal growth with a few clamp connections. (n) = Reaction not determined since the growth of dikaryon was inhibited so there was interaction with the monkaryon tester. All pairings were completed twice and when both experiments resulted in the same reaction, one symbol is given. Symbols divided by '/', such as '-/+', indicate different results from the first and second experiments. d) *T. incarnata* isolates.

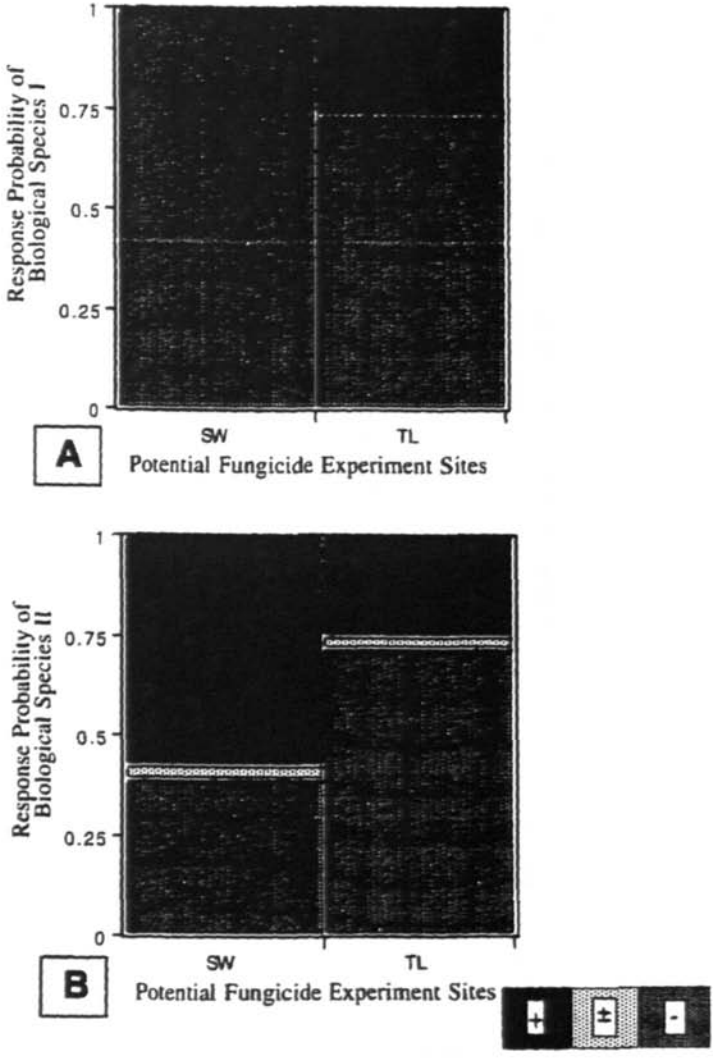

Figure 3.8. Mosaic chart of contingency table analysis of the dikaryon-monokaryon mating experiment results of two potential fungicide experiment sites (SW = Sentryworld and TL = Trout Lake Golf and Country Club. A) Potential fungicide sites versus biological species I reaction and B) potential fungicide sites versus biological species II reaction. Red = (-) reaction, green = (±) reaction and blue = posititve reaction (+).

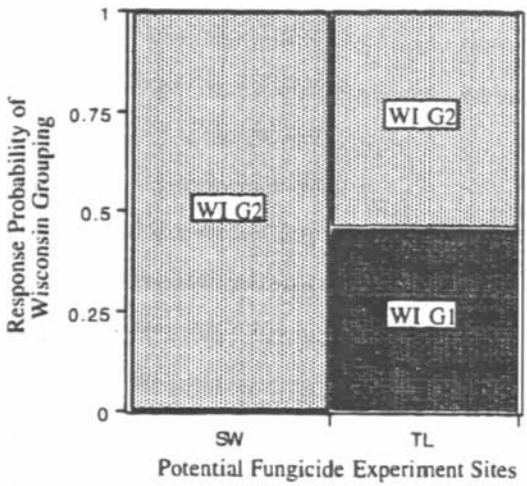

Figure 3.9. Mosaic chart of contingency table analysis of the potential fungicide experiment sites (SW = Sentryworld and TL = Trout Lake Golf and Country Club) versus the grouping of *T. ishikariensis* isolates into Wisconsin group 1 and Wisconsin group 2. Green = Wisconsin group 2 and red = Wisconsin group 1.

DISCUSSION

The dikaryon-monokaryon experiments were successful in characterizing the Wisconsin *T. ishikariensis* isolates at the subspecies level. The results indicate that there are at least two genetically different *T. ishikariensis* populations within Wisconsin golf courses. All of the dikaryotic WIG1 isolates were compatible with at least one monokaryotic tester isolate of BSI and all of the dikaryotic WIG2 isolates were compatible with at least one of the monokaryotic tester isolates of BSII. Twenty-three percent of the Wisonsin isolates were morphologically similar to and mated with the BSI isolates, while 77% were morphologically similar to and mated with BSII isolates. The results of these experiments fit into the preexisting fabric of knowledge of the *T. ishikariensis* complex as proposed by Matsumoto (1997a; Figure 3.1).

From Table 3.6, the predicted percentages for each possible dikaryon-monokaryon mating reaction combination based on maximum likelihood estimates of the multiple category logit model (Agresti, 1996), we can predict that by grouping the Wisconsin *T. ishikariensis* isolates by their morphological features (ie. color, size and location of sclerotia) we are more likely to predict a negative mating (WIG1 vs. BSII = 96%, and WIG2 vs. BSI = 98%) than a positive mating (WIG1 vs. BSI = 72%, WIG2 vs BSII = 51%).

The main significance of this work is that there are at least two different groups of *T. ishikariensis* in Wisconsin golf courses. These findings have several implications. The significance lies in the fact that other researchers have revealed that there are differences in aggressiveness, habitats and effectiveness of management practices between Japanese biotypes A (BSI isolate) and B (BSII isolate) (Mastumoto, 1992). If we assume that WIG1 is similar to Japanese biotype A and WIG2 is similar to Japanese biotype B based on the results of the dikaryon-monokaryon mating experiments, then there are inferences that can be made about management of Typhula snow molds in Wisconsin.

How can these results affect Typhula snow mold management? To illustrate this point,

I will describe a scenario that occurred in the winter of 1996-1997. In the spring of 1997, several golf course superintendents in Wisconsin reported that even though they applied a contact fungicide containing the active ingredient pentachloronitrobenzene (PCNB) in the fall of 1996, substantial damage due to Typhula blight occurred on the treated fairways (personal communication with Gary Tanko, golf course superintendent at Sentryworld Golf Course and Wayne Horman, Scotts representative). PCNB is usually effective against the Typhula blight, but in this case it failed to protect the fairway turf. The questions arose, "Are there Typhula snow molds that are resistant to PCNB? Or is there something else that caused the reduced fungicide efficacy?"

First of all, Figure 3.10 illustrates the niche separation of the *T. incarnata* and *T. ishikariensis* biotype A and B as proposed by Matsumoto and Sato (1983). Figures 3.8 and 3.9 indicate that at Sentryworld, the WIG2 isolates predominate. Based on the dikaryon-monokaryon mating experiments WIG2 is more similar to *T. ishikariensis* biotype B than to biotype A. Therefore, we can infer that WIG2 could be more aggressive than WIG1. However, the aggressiveness of WIG1 and WIG2 have not been assayed, yet, but this will be discussed in Chapter 5.

Secondly, Matsumoto (1992) discussed the position of sclerotial production on plants and stated, "the ability to produce sclerotia on underground plant parts differs according to the fungus involved; this difference is also significant in ecology and epidemiology." Matsumoto (1989a) reported that biotypes B and C were more aggressive than biotype A and produced more sclerotia on the roots of wheat plants. He later stated, "Their (biotypes B and C) ability to infect and produce sclerotia on underground plant parts has adaptive significance in its habitat, ... this nature as a soilborne pathogen appears to provide this fungus with concomitant but epidemiologically significant advantage (Matsumoto, 1989b)." In Japan, "the foliar application of fungicides is often ineffective in winter wheat fields where biotype B prevails," and turfgrass (*Agrostis stolonifera* L.) needs annual fungicide applications to protect it from

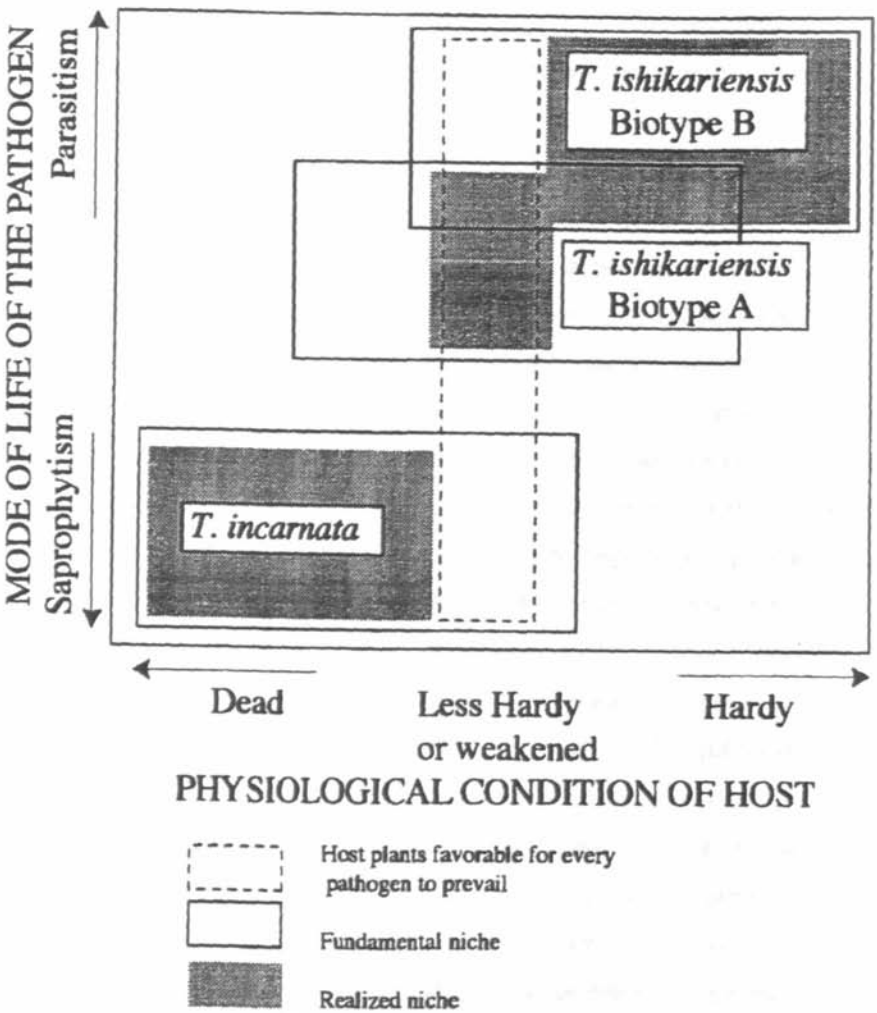

Figure 3.10. Diagrammatic presentation of niche separation of *Typhula incarnata* and *T. ishikariensis* Biotypes A and B. (Matsumoto & Sato, 1983).

Typhula blight caused by biotype B (Matsumoto, 1992).

One explanation for the fungicide failure at Sentryworld may be that the fungicide did not reach deep enough in the thatch/soil interface where the inoculum was present. This could explain why the foliar applied contact fungicide PCNB did not successfully protect the Sentryworld fairways in the winter of 1996-1997 from the *T. ishikariensis* WIG2 fungi. In fact, Figure 3.11 is a picture of sclerotia present on stolons and root of bentgrass (*Agrostis palustris* L.) that came from the same sample as *T. ishikariensis* isolate 2.104.SW (WIG2 from Sentryworld). However, further research is needed to fully understand the disease management differences between WIG1 and WIG2.

An alternative hypothesis for why the fall application of the PCNB product failed to protect Sentryworld's fairways against Typhula blight is that the product's fertilizer component (14-3-3) predisposed the turfgrass to Typhula blight. The product contained 9.3% water soluble nitrogen and 4.7% water insoluble nitrogen. The 9.3% water soluble nitrogen could have created a predisposing flush of growth just prior to the harsh winter conditions. Late season application of excessive nitrogen fertilizer can reduce turfgrass winter hardiness (Andersen, 1960; Foss, 1965; Pestalozzi, 1974; Huokuna and Hiivola, 1974; Kresge, 1974) and therefore increase snow mold susceptibility. Årsvoll and Larsen (1977) studied the effects of nitrogen, phosphorus, and potassium on the resistance to snow mold fungi and on the freezing tolerance in *Phleum pratense* L. They found that timothy grass (*P. pratense*) resistance to *T. ishikariensis* decreased significantly with increasing supply of nitrogen.

Yet another hypothesis for the fungicide failure at Sentryworld is misapplication. Applying a granular fungicide to a wet leaf surface decreases its efficacy (Couch, 1995). If the PCNB product was applied to a wet turfgrass sward, then perhaps this was a contributing factor for the fungicide failure at Sentryworld. Unfortunately, the conditions and timing of PCNB application at Sentryworld are not known.

Management practices can affect fungicide efficacy. But can management practices

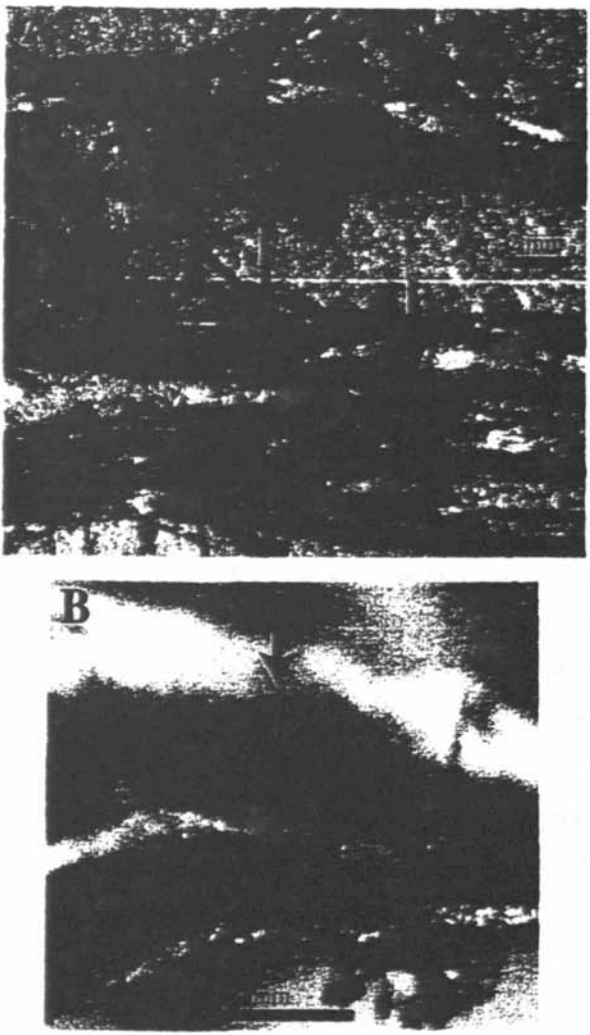

Figure 3.11. Sclerotia of *Typhula ishikariensis* embedded in A) stolons and B) attached to a root of creeping bentgrass. Wisconsin group 2 isolate 2.104.SW was taken from this same sample. Arrow indicates sclerotium.

force *T. ishikariensis* isolates to adapt to a more soil-borne group? Matsumoto (1997b) states, "Frequent mowing of lawn and foliar application of fungicides favor the soil-borne form of (*T. ishikariensis*) biotype B. Frequent existence of the (soil-borne) form in golf courses indicates (that) intensive cultural practices," have caused this form to adapt to these frequent disturbances. The effects of cultural practices on *T. ishikariensis* groups warrant further investigations.

Also, Figures 3.8 and 3.9 indicate that at Sentryworld, the WIG2 isolates predominate and that at Trout Lake Golf and Country Club both WIG1 and WIG2 isolates are present. These findings offer the possibility to compare fungicide efficacies where there are different populations of Typhula snow molds. Perhaps the WIG1 and WIG2 isolates have different levels of sensitivity to fungicides or that one has developed a fungicide escape mechanism. Contrasts and comparisons of management practices on these two courses could improve our understanding of these fungi and also bring about a site specific fungicide prescription that would improve management efficacy, savings in time and money as well as reduction of fungicide inputs.

It is interesting to note that the tester isolate Can 21m of BSII was more frequently compatible with WIG2 isolates than the other BSII tester isolates (Table 3.4). Tester isolate Can 21m is of Canadian origin and perhaps its greater frequency of positive mating with WIG2 is related to it being from the same continent as compared to the other non-North American BSII tester isolates.

Further advancements in identification of the *T. ishikariensis* complex will aid golf course superintendents in identifying which pathogen(s) are present on their course and therefore, could be a valuable tool in disease management improvement. Eventually, this could lead to a fungicide prescription that would fit the golf course's particular pathogen population. Efforts to expedite and improve accuracy of identification by molecular methods will be discussed in the next chapter.

FUTURE WORK

Future work on the *T. ishikariensis* dikaryon-monokaryon mating classification system should center on the creation of monokaryotic tester isolates from Wisconsin. This should be done by using either the ox-gall treatment (Matsumoto et al., 1995), basidiospore production (Matsumoto et al., 1982) or more efficiently with protoplast formation via cell wall degrading enzymes (Matsumoto et al., 1996). Further dikaryon-monokaryon pairings should also be performed with *T. idahoensis* and the *T. ishikariensis* testers from Japan, Norway, Russia, Canada and Wisconsin.

It would also be worthwhile to test the hypothesis that the WIG2 isolates are more common in Wisconsin golf courses than the WIG1 isolates. Further phenotypic characterization of the *T. ishikariensis* complex should center on the differences between the two Wisconsin groups, particularily management differences. Contact versus penetrant fungicides can be compared within and between differing populations. Also, studies investigating the comparative effectiveness of granular and spray fungicide formulations will improve management of this disease complex. Pathogen group diversity could also play a vital role in the success of a particular management practice or fungicide application and further investigation is needed.

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