

***Typhula* Snow Molds of Wisconsin Golf Courses**

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Goals:

Describe the biogeographical distribution of *Typhula* snow molds of Wisconsin golf courses
Genetically characterize the Wisconsin *Typhula* isolates by DNA sequencing
Phenotypically characterize the Wisconsin *Typhula* isolates by measuring their relative aggressiveness on bentgrass in a growth chamber assay

A systematic random sampling technique was used to estimate the distribution of *Typhula* snow molds in Wisconsin golf courses. The sampling frame divided the State into three climate zones. Within these zones, seven golf courses that are within a 70 kilometer radius of Madison (southern), Stevens Point (central) and Woodruff (northern) were randomly selected to survey. Samples were air dried, crushed and sieved to collect sclerotia. The sclerotia were identified as either *Typhula incarnata* (TIN), *T. ishikariensis* complex (TISH) or *T. phacorrhiza* (TP). TIN was the most frequently collected species in the southern zone and TISH was the most frequent in the central and northern zones. Also, TP was found associated with distinctive patches in the central and northern zones. The DNA sequence of the complete internal transcribed spacer region (CITS) of the nuclear ribosomal DNA (rDNA) was used to genetically characterize the three *Typhula* species. Also, the relative aggressiveness of TIN, TISH and TP on creeping bentgrass was also determined using a growth chamber assay.

Results

Survey: In general, the snow mold 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. *T. ishikariensis* was found as far south as Christmas Mountain Resort, Wisconsin Dells in the southern zone. *T. ishikariensis* was the dominant species in the central and northern zones. *T. 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. *T. phacorrhiza* was found more frequently in the northern zone than in the central but not in the southern zone. The survey results are illustrated in Table 1.

Characterization of rDNA ITS regions: The sequences of selected *Typhula ishikariensis*, *T. incarnata* and *T. phacorrhiza* isolates were analyzed for percent identity of the pairwise comparisons of the CITS and are presented in Table 2.

Aggressiveness assay: The relative aggressiveness of Wisconsin isolates of TIN, TISH and TP on creeping bentgrass was determined in a growth chamber assay at 5 and 10 C. The average aggressiveness ratings taken 21 days after inoculation are presented in Table 3.

Discussion: *Typhula incarnata* was the most frequently collected fungus in southern zone while *T. ishikariensis* was the most frequently collected fungus in northern two-thirds of the State. The CITS regions of the Wisconsin isolates *T. incarnata*, *T. ishikariensis* and *T. phacorrhiza* are different enough to continue exploring the molecular differences between and within these species. Wisconsin isolates will be tested for mating reactions by di-mon pairings with tester isolates provided by N. Matsumoto and ATCC. The percent similarities of the CITS regions will then be compared with the di-mon pairings.

Proposed research schedule:

Half of the dikaryon-monokaryon mating experiments were completed in Japan but the data is not presented here. The remaining half of the pairings will be finished by the end of 1998. The results of the di-mon pairings will be compared with the CITS sequence data. The CITS sequence analysis will be completed by March, 1999. As soon as the U.S.G.A. funding is presented, the PCNB *in vitro* experiments will be initiated. There are two aggressiveness characterization experiments currently be conducted. The first one will be complete in two weeks while the last experiment is more than a month away from termination. At the completion of the experiments, two articles will be written for publication in *Plant Disease* and a second Research Report will then be submitted to the U.S.G.A.

	TIN	TISH	TP	TIN/TISH	TIN/TISH/TP	TIN/TP	TISH/TP	unknown
Southern	94	4	0	2	0	0	0	0
Central	26	56	6	11	0	1	0	0
Northern	8	56	7	15	3	1	9	2

Table 1. Percentage of *Typhula* snow mold fungi collected from Wisconsin golf courses. TIN=*T. incarnata*, TISH=*T. ishikariensis* and TP=*T. phacorrhiza*.

CITS % identity	TIN 100B dikaryon	TISH 100A dikaryon	TISH 105 dikaryon	TISH 105.1 monokaryon	TISH 105.2 monokaryon	TP 7 dikaryon
TIN 100B dikaryon	100	70	70	70	70	61
TISH 100A dikaryon	x	100	100	100	99	66
TISH 105 dikaryon	x	x	100	100	99	66
TISH 105.1 monokaryon	x	x	x	100	100	66
TISH 105.2 monokaryon	x	x	x	x	100	66
TP 7 dikaryon	x	x	x	x	x	100

Table 2. Percent identity of the pairwise comparisons of the complete internal transcribed spacer regions (CITS) of Wisconsin *Typhula ishikariensis*, *T. incarnata* and *T. phacorrhiza* isolates.

	TIN 1.31	TIN 1.35	TIN 2.100	TIN 3.113	TISH 2.97	TISH 2.105	TISH 3.124	TISH 3.114	TISH 3.122	TP 3.1	TP 3.117	TP 3.120
5 C	3.0	3.1	3.0	4.0	3.0	3.1	3.6	4.0	3.6	1.0	1.0	0.6
10 C	2.9	2.7	3.4	4.0	2.9	2.1	3.2	4.0	3.9	1.0	1.9	0.8

Table 3. Relative aggressiveness of Wisconsin *Typhula* isolates on creeping bentgrass at 21 days after inoculation. Rating scale used: 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100% of turf diseased.