Polymorphism (RFLP) to determine population composition. This method can be used to determine the relative percentages of two (or more) varieties in bulk tissue samples (clippings).

The following fall treatments, applied each September, are being evaluated for their effect on bentgrass conversion:

1. Control, no interseeding
2. Broadcast interseeding with *L93* and *A4*
3. Cultivation with JobSaver tines plus broadcast interseeding
4. Verticutting plus broadcast interseeding
5. Primo plus JobSaver cultivation plus broadcast interseeding
6. Primo plus verticutting plus broadcast interseeding

Primo was applied at a rate of 0.3 ounces per 1000 ft^2 three days before interseeding. Success of the treatments are being evaluated annually for at least three years by sampling clippings from each plot and subjecting them to molecular genetic analysis.

Leaf tissue was sampled from the plots in late August. DNA was extracted and Southern analysis was conducted using a RFLP probe which distinguishes *A4* from *PENNCROSS*.

Computer imagery and data analysis indicates that conversion from *PENNCROSS* to *A4* during the first year occurred to the greatest extent with the JobSaver plus Primo treatments (Table 4). Conversion was approximately 20 percent. The least effective treatments were verticutting and verticutting plus Primo. These results led us to cultivate the plots more aggressively in year two, hoping to open-up the canopy more and provide a more favorable environment for the seedlings.

The data indicate that conversion from *PENNCROSS* is probably feasible, but that it will take a number of years. Further, it seems likely that complete conversion, in which *PENNCROSS* is completely eliminated, may not be possible.

Table 4. Conversion of *PENNCROSS* to *A4* bentgrass.

Treatment	Conversion to A4
	--------- % --------
Control, no interseeding	0.0 c
Broadcast seeding	13.8 abc
JobSaver Tines	16.2 ab
JobSaver Tines + Primo	21.2 a
Verticutting	2.5 bc
Verticutting + Primo	2.5 bc

Values followed by the same letter are not significantly different at P = 0.10.

The Distribution, Characterization and Management of Gray (*Typhula incarnata*), Speckled (*T. ishikariensis* complex) and *T. phacorrhiza* snow molds of Wisconsin Golf Courses

University of Wisconsin

Steve Millett

Start Date: 1998
Number of Years: 1
Total Funding: $18,225

Objectives:

1. *Determine the distribution and population structure of T. incarnata, the T. ishikariensis complex and T. phacorrhiza in Wisconsin golf courses.*
2. *To investigate the genetic variation within the nuclear ribosomal DNA (rDNA) among isolates of T. incarnata, the T. ishikariensis complex and T. phacorrhiza.*
3. *To determine the relative aggressiveness of T. incarnata, the T. ishikariensis complex and T. phacorrhiza on PENNCROSS creeping bentgrass.*
4. *To determine if fungicides and alternative tactics have different efficacies for control of gray (T. incarnata), speckled (T. ishikariensis complex) and T. phacorrhiza snow molds.*
5. *To determine the in vitro sensitivity of Typhula incarnata, the T. ishikariensis complex and T. phaxorrhiza to standard fungicides.*

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.

Survey Results. 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 5.

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 6.

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 7.

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

Table 5. Percentage of Typhula snow mold fungi collected from Wisconsin golf courses.

	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

TIN=*T. incarnata*, TISH=*T. ishikariensis* and TP=*T. phacorrhiza.*

Table 6. Percent identity of the pairwise comparisons of the complete internal transcribed spacer regions (CITS) of Wisconsin Typhula *ishikariensis*, *T. incarnata* and *T. pharcorrhiza* isolates.

	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 7. Relative aggressiveness of Wisconsin *Typhula* isolates on creeping bentgrass at 21 days after inoculation.

	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

Rating scale used: 0=0%, 1=1.25%, 2=26-50%, 3=51-75%, 4=76-100% of turf.

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 from Japan, Russia, Norway, Canada and the United States of America. The percent similarities of the CITS regions will then be compared with the di-mon pairings. I

Increasing the Nitrogen Use Efficiency of Cool-Season Turfgrasses by Regulating Nitrate Metabolism

University of Rhode Island

Richard J. Hull

Start Date: 1998
Number of Years: 3
Total Funding: $75,000

Objectives:

1. *To quantify each step in nitrate metabolism for ten Kentucky bluegrass (Poa pratensis L.) and five creeping bentgrass (Agrostis palustris Huds.) genotypes.*
2. *To determine which of these steps correlates best with nitrogen use efficiency under field conditions.*
3. *To assess the potential for increasing nitrogen use efficiency by optimizing the activity and location of those steps which are limiting.*

A long-standing paradox confronting turfgrass managers is the simple fact that high quality turf cannot be maintained without annual applications of nitrogen even when clippings are retained on the turf and no nitrogen is removed. This paradox is made all the more intriguing by recent research that shows very little nitrogen is lost from turf through nitrate leaching, ammonia volatilization or denitrification (usually less than 15% of that applied as fertilizer). Our research has shown that more than 2000 lbs. of nitrogen per acre can be recovered within the turf-soil ecosystem of long established turf. With that much nitrogen present in the turf environment, it would appear unlikely that additional applications are unnecessary.

The answer to this paradox lies in the fact that in the spring when nitrogen is most needed by turfgrasses, soils are too cold to mineralize much of the organic nitrogen available. During the summer, when the soil is warm and nitrogen becomes available, turfgrass roots are starved for energy because of high respiratory demands by the shoots due to elevated air temperatures. Warm-season turfgrasses do not experience this problem because their leaf respiration does not increase as much during hot weather.

This research project is intended to find means for making cool-season turf more efficient in recovering soil nitrogen. One obvious approach would be to promote greater root development and less shoot growth. This would make the grass better able to absorb nitrate from a larger volume of soil while less energy is committed to rapid shoot growth. Plant growth regulators have been used to achieve this goal but their action is not long lasting and while they inhibit shoot growth they often fail to stimulate root development. We believe the location of nitrogen metabolism in turfgrasses may be the key to this problem.

Most soil nitrogen is available to turfgrass roots as nitrate and is readily absorbed in that form. However, before nitrate-nitrogen can be assimilated into amino acids and proteins it first must be reduced to ammonium. This reduction of nitrate occurs in two steps or reactions: nitrate reduction (NR) and nitrite reduction (NiR). In roots, the eight electrons (e^-) required for these two reactions come from the reduction of sugars produced during photosynthesis and translocated to the roots. In leaves, most of the eight electrons come directly from photosynthetic reactions. The ammonium (NH_4^+) is assimilated directly into the amide-nitrogen of glutamine, which is a five carbon amino acid.

$$NO_3^- + 2H^+ + 2e^- \text{----------NR---------->} NO_2^- + H_2O$$
$$NO_2^- + 8H^+ + 6e^- \text{----------NiR----------->} NH_4^- + 2H_2O$$

The first reaction in this process is catalyzed by the enzyme nitrate reductase and this has been determined to be the rate limiting step in the chain of reactions leading to nitrogen assimilation. If nitrate is reduced in the roots, amino acids are produced there and root growth is promoted. If roots are unable to reduce nitrate as rapidly as it is absorbed from the soil, the nitrate can be transported to leaves where it will be reduced, assimilated into amino acids and stimulate shoot growth. When nitrate is reduced in leaves, photosynthetic products are diverted to shoot growth and away from roots. This lowered carbon flow to roots makes them even less able to reduce nitrate so more is transported to leaves and shoot growth is further stimulated and roots are not. This is what normally happens when turf receives nitrate from the soil or fertilizers.

The research conducted in this project will determine if the nitrate stimulation of shoot growth can be minimized by promoting nitrate reductase activity (NRA) within roots. We have found that in all Kentucky bluegrass cultivars studied, NRA is often ten times more active in the leaves than it is in roots. However, some cultivars (*LIBERTY*) did exhibit significantly greater NRA in their roots. We will determine if such cultivars produce greater root growth and if this contributes to more efficient nitrogen use. Currently we are extending this investigation to include diverse cultivars of perennial ryegrass and creeping bentgrass. Similar comparisons will be made and we will determine if greater root NRA correlates well with increased nitrogen use efficiency and field performance. We are also examining management practices that promote greater root growth (higher mowing heights, lower nitrogen fertilization, infrequent but thorough irrigation, etc.) to determine if they contribute to greater root NRA.

If this relationship between NRA in turfgrass roots and increased root growth is substantiated, efforts will be made to alter turfgrasses genetically to produce cultivars that have a more active nitrate reductase enzyme in their roots. This may produce turfgrasses that will utilize soil nitrate so efficiently that