Distribution of *Typhula* species and their sensitivity to fungicides *in vitro* and under field conditions in Wisconsin

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INTRODUCTION

Snow mold is the most devastating winter turfgrass disease on golf courses in northern parts of the USA. Mercury fungicides with a wide spectrum of efficacy on the various snow molds have not been available since 1994 in the United States. The fungicides currently used for the control of snow mold such as PCNB (the most commonly used), chloroneb, triadimefon, do not have wide spectrum efficacy. Chemical companies are developing new fungicides every year adding to the complexity of adapting a control strategy for this disease.

Several factors limit the control of snow mold: 1) high fungicide costs, 2) limited efficacy, 3) variability in sensitivity of the snow mold pathogens, 4) problems of chemical registration, and 5) environmental effects which reduce efficacy of the fungicides. The best strategy when using fungicides is to reduce the amount of chemicals applied, while still achieving satisfactory control. This is achieved by applying the most effective fungicides on the sensitive snow mold pathogens, a simple idea but problematic because of differential chemical efficacy due to snow mold isolate variability. Furthermore, a particular fungal isolate may differ in its sensitivity depending on its geographical location (Ex: lake effect, duration of snow cover, and elevation) and turfgrass specie.

Recently 3 genetically diverse groups of snow mold pathogens were detected among *T. ishikariensis* isolates collected throughout Wisconsin (Fig. 2). The group with the most genetic variability (group 3 in Fig. 2) represents two golf courses 15 miles apart and located in northernmost Wisconsin. More than 70% of fairways treated with fungicides on these golf courses became infected with snow mold in the two courses. Our preliminary results on *in vitro* sensitivity of 10 commonly used fungicides with 6 Typhula isolates indicated that there is a significant difference in fungal growth depending on source of the pathogen and chemical concentration. These results clearly indicated that there are huge morphologic and pathogenic variations and fungicide sensitivities among isolates as well as within species.

OBJECTIVES

1. to determine the geographical distribution and population structure of *T. incarnata* and the *T. ishikariensis* complex on golf courses in Wisconsin.
2. to investigate the genetic variation among isolates of the *T. incarnata and T. ishikariensis* complex.
3. to determine the *in vitro* sensitivity of the *T. incarnata* and *T. ishikariensis* complexes to standard fungicides
4. to determine the effectiveness of several fungicides labeled for snow mold control against Typhula blight on 3 golf courses in Wisconsin

MATERIALS AND METHODS

Objective 1: Determine the geographical distribution and population structure of *T. incarnata* and the *T. ishikariensis* complex in Wisconsin golf courses

Millett (1999) showed that *T. incarnata* isolates are the most frequent fungus in the southern zone, while *T. ishikariensis* isolates are the most frequent fungus in northern two-third of Wisconsin (Fig. 1). The greater amount of disease in the northern zone compared to the central and southern zones is linked to the above-normal amounts of snow fall in the northern regions. This supports the idea that the duration of snow cover is a key factor in disease incidence in northern turfgrass. However, the virulence of isolates might also play a role in pathogenicity.

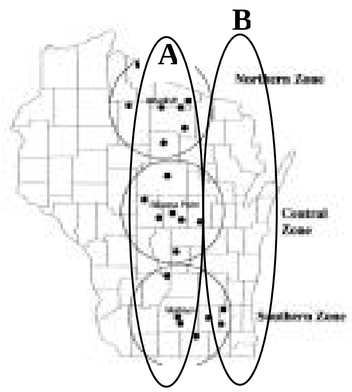

Figure 1. The distribution of locations of Wisconsin golf courses where isolates of *Typhula* species associated with *Typhula* blights were and will be collected. Approximate locations of the golf courses surveyed are indicated as a black dot on Wisconsin zone B (Millett, 1999).

The Typhula isolates (representing Wisconsin zone A, Fig 1) collected by Millett (1999) were analyzed for genetic relationships using two DNA techniques (DNA sequences of the ITS region and RAPD marker based genetic distance). Three genetically distinct groups (Fig. 2) in 88 isolates of the *T. ishikariensis* complex were detected but no distinct group was found in 53 isolates of *T. incarnata* (data not presented). Because of these results, the biogeographical distribution of Typhula species in Wisconsin zone B needs to be compared to Wisconsin zone A. Typhula isolates will be sampled from three fairways (2 sites per fairway) on each of the five golf courses within each zone in Wisconsin zone B using the same sampling techniques as Millett (1999). The total number of isolates sampled will be 90.

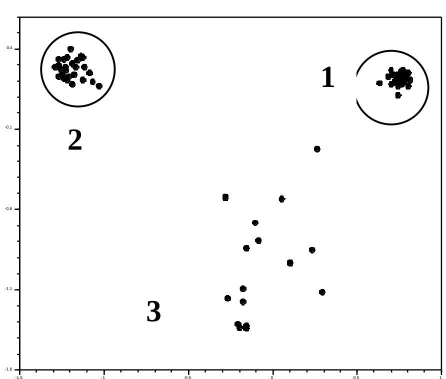

Figure 2. Multi-dimensional scaling plot of the genetic distance matrix generated by 102 RAPD data collected for 88 *Typhula ishikariensis* isolates throughout Wisconsin zone A and other countries (Fig. 1; Jung, 2000). Note the three genetically distinct groups (1, 2, and 3) in isolates of the *T. ishikariensis* complex.

Objective 2: Investigate the genetic variation of Typhula isolates based on molecular markers derived genetic distance

Genetic relationship studies (Fig. 2) raise several important questions. One is why are fewer isolates of *T. ishikariensis* falling into group 3 compared to groups 1 and 2? Furthermore, isolates in group 3 were uniquely represented by two golf courses (15 miles apart each other) located in far northern Wisconsin. More than 60 percent of the fairways treated with fungicides were infected by snow mold in the year when the isolates were sampled. Secondly, why does more genetic variability exist in the group 3 than groups 1 and 2? In order to answer these questions, more isolates need to be collected from other locations representing wider ranges in geographic and environmental factors.

Objectives 3 and 4: Determine the *in vitro* sensitivity of *T. incarnata* and *T. ishikariensis* complex to standard fungicides and determine the effectiveness of several fungicides labeled for snow mold control against Typhula blight in 3 golf courses in each of Wisconsin

From the previous *in vitro* sensitivity studies using 6 isolates of Typhula species (Fig. 3) the most effective concentrations of 10 most commonly used fungicides labeled for snow mold control were determined. As shown, the optimum concentrations to completely control fungal growth varied according to the fungicides tested.

Three representative golf courses from Wisconsin which have different ratios of snow mold species, different durations of snow cover, and evidence of genetic diversity of Typhula isolates will be selected for field fungicide efficacy experiments. Roughly 10 standard and experimental fungicides will be applied on fairways, alone or in combination, at varying rates. Individual plots (3 ft x 10 ft) will be arranged in a randomized complete block design with four replications. The percent snow mold damage will be evaluated the springs of 2002 and 2003.

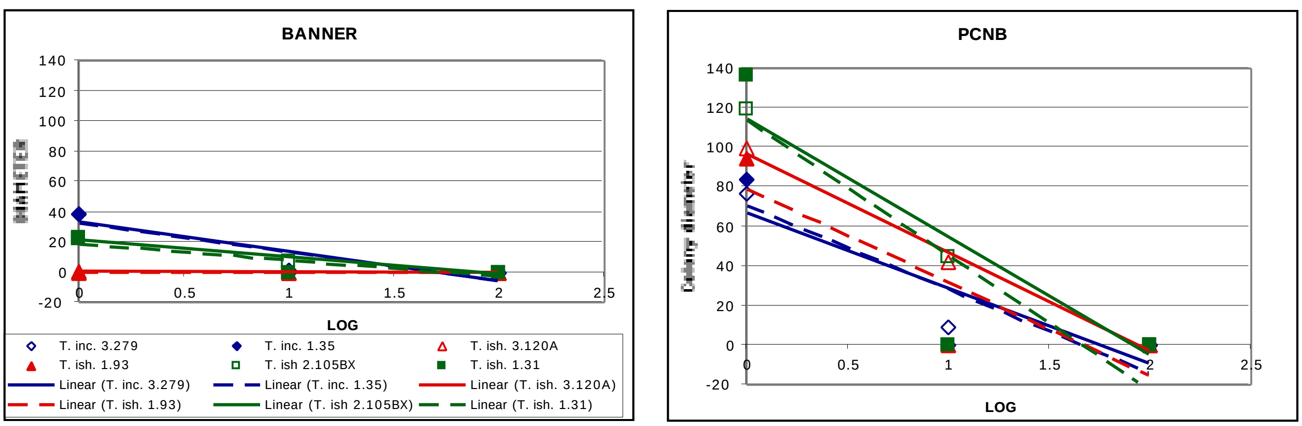

Figure 3. *In vitro* sensitivity of six *Typhula* species isolates (isolates 3.279 and 1.35 of *T. incarnata*; 3.120A and 1.93 of *T. ishikariensis* group 2; 2.105BX and 1.31 of *T. ishikariensis* group 1) to propiconazole and PCNB on a log scale (unpublished).

PRELIMINARY RESULTS

Due to the on-going nature of this research we are hesitant to disseminate any hard and fast results as of yet. This winter's (00-01) field and *in vitro* studies will be critical for robust results. At the present we are working with resistant/susceptible clones provided by M.Casler, and running trials in various conditions around the state, as well as Madison. There are however a couple of points of interest that have at this early stage become manifested.

1. There appear to be 3 distinct types of *Typhula ishikariensis complex* that occur in the state. The most efficacious fungicide treatment is dependent on what isolate of the fungus a particular location has.

2. Isolate distribution is heavily dependent on duration of snow cover. North to south collections corroborate this. In 2001 east, west (lake effect) distributions will be sampled, and a map of more utility will be produced.

3. Isolate virulence appears to rise with snow cover duration.