Application of DNA Marker Technology to Turfgrass Pathology Research

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DNA is an inheritable material located inside the cells of all living organisms and is passed on from one generation to the next. Since the method for DNA extraction was unexpectedly discovered, the procedure has become routine and essential as a molecular technique in most research. In 1985 an ingenious and novel technique called Polymerase Chain Reaction (PCR) was developed. PCR is sensitive enough that a single DNA molecule can be amplified and visualized as distinct bands on an agarose gel. PCR is a powerful and extremely sensitive technique with applications in fields such as molecular biology, medical diagnostics, population genetics, forensic analysis, and virtually any research related fields.

In 1990 two groups of scientists (Williams et al., 1990; Welsh and McClelland, 1990) independently described a revolutionary technique called RAPD (Random Amplified Polymorphic DNA). RAPD is a DNA polymorphism assay based on PCR amplification of unknown segments of DNA. It uses only one primer of random nucleotide sequence rather than two primers of known sequence used in typical PCR reactions.

During the last five months a Wisconsin turfgrass pathology research team has focused on the research of snow molds. There are two reasons for working with this pathogen. The first and the most important reason is the pathogen's uniqueness (its psychrophilic or "cold-loving" nature) and difficulty of management (completely dependent upon incoming winter environments). Secondly great researchers (Drs. G. Worf, D. Maxwell, M. Casler, S. Millett, J. Gregos, and people providing experiment plots) have advanced the understanding of host and parasite interactions.

One of the best methods to describe some of our new principles/concepts is to explain, using a few familiar examples. Snow mold is the most devastating winter turfgrass disease in areas where creeping bent-

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grass grows, particularly on many golf course fairways both fungicide-untreated and even treated in Northern Wisconsin. Typhula blight, collectively called snow mold disease, is caused by *Typhula incarnata* and *T. ishikariensis*. *T. incarnata* and *T. ishikariensis* are individually called speckled and gray snow mold, respectively. Since 1994, mercury fungicides found effective in controlling snow mold are no longer available for sale in the United States. Fungicides currently used for the control of snow mold in single, two and three-way mixtures include Chloroneb, Triadimefon, Quintazine, and Chlorothalonil, among others.

Chemical companies are developing more fungicides every year, adding to the confusion of adapting a control strategy for this disease. In addition, due to cost, limited terms of efficacy, developing fungicide resistance, and adverse environmental effects, the best strategy when using fungicides is to apply the least amount of chemicals that still gives satisfactory control. This can be done by applying the most effective fungicides for the appropriate snow mold pathogens, which might differ in their presence depending on their Wisconsin geographical location (the Lake Michigan lake effect, from east to west or the duration of snow cover, from north to south, or other environmental factors). Several researchers have reported variability in the **pathogenicity** of snow mold isolates in turfgrass and cereal crops. In particular, variation in *T. ishikariensis* due to adaptation to different conditions has led to taxonomic confusion. The nomenclature of different species, varieties, biotypes, or groups differs among researchers. Matsumoto and Tajimi (1993) indicate that geographical adaptations (heavy and long-lasting snowfall) and host range, create two biotypes, A and B, of *T. ishikariensis*. They conclude that within biotype B, size variation in **sclerotia** is positively correlated with the duration of snow cover, and **virulence** (capacity of a pathogen to cause a disease) is negatively correlated with duration of snow cover. Also, Millett (1999) found that *T. ishikariensis* is the most frequently collected fungus in the northern two-thirds of Wisconsin compared to *T. incarnata* These results clearly indicate that there are huge morphologic and pathogenic variations, and adaptive ability related to geographical locations among isolates between as well as within species.

Due to these variations, adaptations, and uncertainty of classification of *T. ishikariensis* species, it is very important to characterize *T. ishikariensis* isolates based on their geographical distribution, their level of virulence, and their genetic similarity. Many Typhula isolates throughout Wisconsin were collected by Millett (1999). The locations of the sampled golf courses are marked in the Wisconsin map (Fig. 1). Seventy nine isolates (subsamples of a total collection), including 14 originating from other countries, were evaluated for genetic relationship using RAPD markers which indicate an unlimited number of differences among them, as compared to conventionally used morphological markers (sclerotium color, size, and mating experiments).

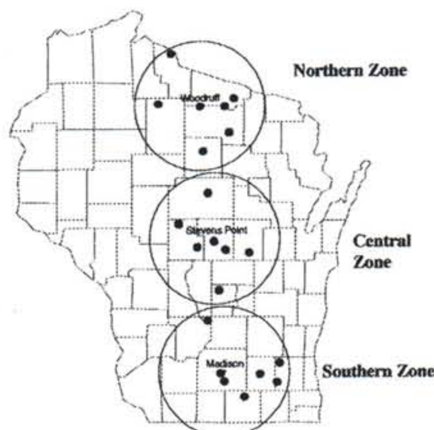

Figure 1. The distribution of locations of Wisconsin golf courses where isolates of *Typhula* species associated with *Typhula* blights were collected. Three zones were divided based on the duration of snow cover. Approximate locations of the golf courses surveyed are indicated as a black dot on Wisconsin map (Millett, 1999).

DNA markers have been used to determine if two DNA samples (or individuals) are identical and to establish a genetic relationship between a group of individuals or populations. Data generated by DNA marker studies is discrete data (presence/absence) rather than continuous. Each **locus** (amplified band) is scored as 1=presence or 0=absence and resulting a very large matrix of data exampled below. In the example data set, five individuals (A to E) were examined using one RAPD primer. The RAPD primer usually amplifies approximately 5-15 bands shown in Fig 2.

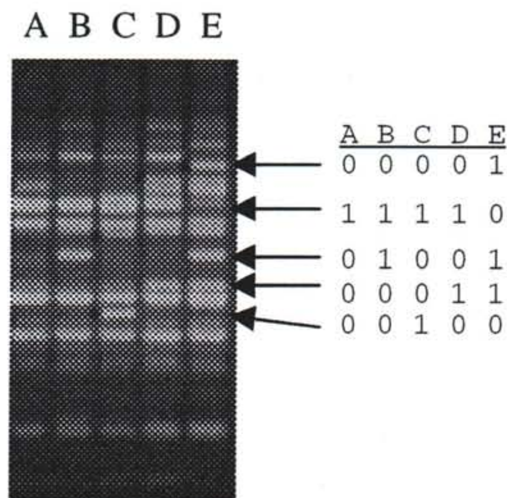

Figure 2. An example of a RAPD gel profile. Five isolates (A through E) of *Typhula ishikariensis* were ground for DNA extractions. RAPD products obtained in a PCR reaction are separated on an agarose gel. Arrows indicate polymorphic bands scored. The amplified bands were scored as either presence (1) and absence (0).

Figure 2 shows a gel picture with the amplification products in a RAPD reaction with 5 *T. ishikariensis* isolates collected from different golf courses. The top bright band indicated with an arrow is very clearly polymorphic showing amplification in lane 5 and no amplification in the other lanes. The unique and bright band distinguishes each isolate. Another example is the bottom band which shows amplification in lane 3 but no amplification in the other lanes. If you continue comparing amplification products, we soon see that these 5 samples can be uniquely determined relative to each other. The more samples you have for a comparison, the more polymorphic bands are needed in most cases.

Objective

To determine the genetic relationship among 79 turf pathogenic fungal isolates of *Typular ishikariensis* (presumably representing a wide range of geographical locations in Wisconsin) using RAPD markers.

Materials and Method

In addition to 65 *T. ishikariensis* isolates (a solid dot indicates approximate location of the golf courses sampled in Wisconsin, see Fig. 1) collected by Millett (1999), 14 isolates obtained from other sources are also included in this study. Sclorotia of 79 isolates were grown on Potato Dextrose Agar (PDA) medium for DNA extraction. Initially, 10 different RAPD primers were used, which totals 2000 reactions. This total is dependent upon the number of polymorphisms per primer. Additional primers will be tested as necessary.

RAPD data scored for presence or absence of amplified bands will be used to estimate genetic distances between *T. ishikariensis* isolates based on a simple matching coefficient. Multidimensional scaling analysis (MDS) will be performed on the data matrix of genetic distances using the statistics program (SAS). The MDS procedure is useful for viewing relationships among isolates based on DNA marker derived estimates of genetic distance.

Results and Discussion

Three genetically distinct groups were identified shown in Fig 2. Two groups (A and B) are representatives of Wisconsin Biological Species I and II, respectively which were designated based on the results of DNA sequence comparison of Internal Transcribed Sequence (ITS) region and dikaryon-monokaryon mating experiments (Millett, 1999). A third group C in Figure 3 may be another class suggested by Arsvoll and Smith (1978) and Matsumoto, et al. (1982 and 1996). Further research using more isolates is needed for confirmation. One interesting finding from our study is that any isolates collected from central and southern Wisconsin zones belong in groups A and B but not in group C. However, isolates collected from the northern Wisconsin zone fall into all three groups. Furthermore, isolates in group C came

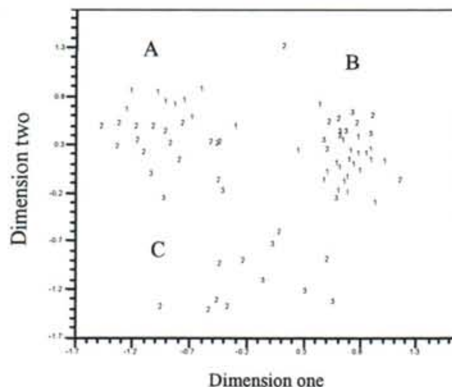

Figure 3. A multidimension scaling plot, a visual method of displaying the genetic relationship among *T. ishkeriensis* isolates, was drawn using genetic distance derived from RAPD markers. "1" indicates isolates collected from Wisconsin central zone, "2" from the northern zone, and "3" from other conturies shown in Fig. 1.

from only two golf courses in northern Wisconsin zone. Results presented here are based on the number of isolates tested and the locations of golf courses sampled. Future research using isolates from more golf courses throughout Wisconsin is required for the confirmation of our findings. Studies on the pathogenicity of three genetically distinct groups within *T. ishikariensis* species and the efficacy of fungicides in controlling them are also needed both in *vitro* and in field experiment.

The future confirmed information will help golf course superintendents and other people facing snow mold problems to choose the correct fungicides in the proper amounts depending on the different levels of pathogenicity found in the *T. ishikariensis* isolates collected from different parts of Wisconsin and their geographical location.

As we can see from the research described in Figure 3, it is clearer how DNA marker techniques can be utilized to answer some difficult problems which have not been easily tackled by conventional methods. Typically DNA marker technology is more accurate and efficient than conventional methods. In the future, I will present other examples of DNA marker technology to illustrate its application.

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Glossary

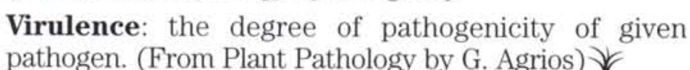
PCR: a rapid procedure for in vitro enzymatic amplification of a specific segment of DNA. DNA to be amplified is denatured by heating the sample. In the presence of DNA polymerase and excess deoxynucleotide triphosphate, oligonucleotides that hybridize specifically to the target sequence can prime new DNA synthesis. The first cycle is characterized by a product of indeterminate length; however, the second cycle produces the discrete "short product" which accumulates exponentially with each successive round of amplification. This can lead to the many million-fold amplification of the discrete fragment over the course of 20 to 30 cycles.

Locus: any genetically defined site, i.e., a gene, a part of a gene, or a DNA sequence with a regulatory role. (Glossary of Genetics by Rieger R., A. Michaelis, and M.M. Green)

Pathogenicity: the capability of a pathogen to cause disease. (From Plant Pathology by G. Agrios)

Sclerotium: a compact mass of hyphae with or without host tissue, usually with a darkened rind, and capable of surviving under unfavorable environmental conditions. (From Plant Pathology by G. Agrios)

Virulence: the degree of pathogenicity of given pathogen. (From Plant Pathology by G. Agrios)



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