took the prize money. The other winners were a group of sluggers that Mike Lee sent over from the Kohler golf courses.

The day could not have been more enjoyable. The only aspect that could have been improved is the number of attendees. If anyone has suggestions to increase attendance or make the outing better, or if you would like to volunteer a golf course for next year, please call the Noer Facility at 608-845-6536. Wisconsin's turf industry remains healthy and growing because of your dedication and support.

The WTA would like to thank everyone that contributed to this year's event, especially the $100 hole sponsors that may or may not have also participated in the golf event. The hole sponsors are listed below in alphabetical order.

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Better late then never: WTA director Jerry Kershasky arrives with four holes left to play. Says he had to work late?

"I know there's a fairway here somewhere."

Most important person on the course - the beer cart lady!

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Kidd

By Monroe S. Miller, *Golf Course Superintendent, Blackhawk Country Club*

He was as black as coal and showed up at our shop one morning in early June. Although he had an attitude, he was friendly with everybody on the crew. We thought that surely somebody was looking for their pet cat.

He was kind of scrawny and skinny and looked like he'd been in more than his share of fights, even though it was equally obvious he was a young cat. His fur was roughed up, matted and dirty, certain clues he was not as domesticated as the cats some of us had at home for pets. We still believed that somehow we would find his owner - a phone call from a neighbor, an inquiry from somebody on the course perimeter looking for him, or a "lost pet" notice in the paper. We even called the Humane Society to see if people looked there for lost pets.

No luck. We made him feel welcome, feeding him table scraps and a little milk. Dave even stopped at the Farmers' Coop on the way home one night and bought five pounds of Purina Cat Chow, thinking the dry food diet would be better for him. We poured some Oil-Dri in an oil pan to serve as a scratch box for him. He hung around the shop, getting plenty of sleep. A pile of drop cloths in the loft became his favorite nap place. He also liked window sills when the warm sun was shining in.

The guys on the crew got to calling him Kiddo, so when it got to the point when we could tell he was going to stay around for a while, they formally named him Kidd, adding a "d" for distinction.

He didn't eat much, and as we observed him over those first couple of months, it was easy to know why. He was a great hunter. We never had mice in the big shop, but the lower buildings and the fertilizer shed definitely had those rodent inhabitants. Until Kidd showed up. He elim-

inated that pest problem.

We would see him, early early in the day, on the golf course, hunting. He was back at the shop early enough to avoid players. But it was common to see him slinking around with a gopher or a chipmunk in his mouth, a warm and fresh and tasty meal for him. And, believe it or not, we were able to make him understand that in our world birds were to be listened to and enjoyed, not eaten. We only had to swat him a few times for dragging a bird back to the shop, or knock him off a tree branch as he moved with stealth toward a robin or nuthatch.

Pretty soon, he was one of us. We deferred to him shamelessly. He was arrogant and confident, and we loved it. If Kidd wanted to be scratched, he had many volunteers. He would jump up on our laps and stay as long as he wanted, and then either moved to somebody else or went for a nap. He was even accepted in Green Committee meetings if that was where he wanted to be.

At least as distinctive as his 100% pure black coat was Kidd's ability to purr. He sounded like a diesel engine idling or an old Chevy 409 slightly out of timing. It was hilarious - his purring rattled the dishes in the cupboards in the lunchroom! And he was generous with it; he was purring most of the time he was in the shop.

Kidd was a hunter, we knew that. But he was also a fighter, at night we suspected and more often than suited us. Several on our staff were farm kids, and they offered an obvious solution - neutering. "No big deal," Matt said. "I've castrated hundreds of cats in my life, and most of them lived."

He laughed. And so did Val. And Jared. Frankly, I saw it as a perfectly reasonable solution and nodded approval.

Matt insisted on doing the surgery himself. Val wandered to his toolbox and opened a new single-edged razor blade. Jared went upstairs and retrieved a pair of waders we used to install and removed intake pipes from the lake. I got the alcohol from the medicine cabinet and grabbed Kidd as he was rubbing along my legs and purring. A few scratches on his head and he was relaxed and completely unsuspecting of the drastic change about to happen.

I handed him to Jared who had turned the waders inside out down to the boot portion. He stuffed Kidd into the left boot head first. Val poured alcohol first on the razor blade and then on Kidd's most personal area. I was

smart enough to stand back.

As soon as Kidd figured out we were up to no good, from his point of view, he let out a blood curdling yowl. And he let out a strong stream of urine, too, all over the guys. That's why I was standing back! But with lightning fast moves in succession - incision, squeeze, cut and incision, squeeze and cut - Kidd went from a he to an it. He was yowling, the guys (except for Matt) were laughing. A dash of alcohol on the tiny wounds and Jared let loose of the wader boot. Kidd was off like a shot.

Matt was proud of himself. "Another delicate surgery executed successfully," he said. "Next!"

We wondered if Kidd would be back. Fact is, we didn't see him for several days,. But he came back, even though he was walking lightly at first.

The city boys were wondering if it was worth it. The surgical team, well experienced in the matter of farm cats, told them nothing was worse than a mean, old tomcat and insisted that is what Kidd would have become. "Plus," Val said, "a tomcat roams a lot, for obvious reasons, and is fighting constantly. Kidd ran a real risk from being run over by a vehicle, captured by an animal control officer or eventually killed by another tomcat. He's better off this way."

And he was. He almost always was around the shop. We cut a small trap door with a rubber flap and a 90 degree turn in the shop wall so he could come and go as he wished, even if we weren't there at work. He became a bit of a nuisance with a habit of his, even though we thought it funny. He was always hitching a ride, so to speak, onto the golf course. He like riding a golf utility car with whomever was cutting cups each morning. He still hunted, but less and less as the years went by. We never did break his habit of using the sand bunkers as his own personal, king-sized scratch boxes. They were like his own rest stations. So we watched him close so we could clean up after him.

I was flattered that he liked to ride along with me early in the morning during the season when I toured the course just after daybreak. He was a genuine pal, purring that loud and unmistakable purr.

Kids loved Kidd, especially as he grew older and more docile and a little fat. Heck, he even got gray, which showed up well against his black, shiny coat.

Kidd lived with us for nine years. When he died, we moped around for days grieving the loss of a real friend. We buried him next to the row of lilacs that are on our eastern border. When they bloom in profusion each spring, the blossoms serve as a reminder of a tough little guy who was awfully easy to love. We have never tried to replace him. He was the only shop cat we will ever have.

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ZPP-TRF-020

Application of DNA Marker Technology to Turfgrass Pathology Research

By Dr. Geunhwa Jung, *Department of Plant Pathology, University of Wisconsin-Madison*

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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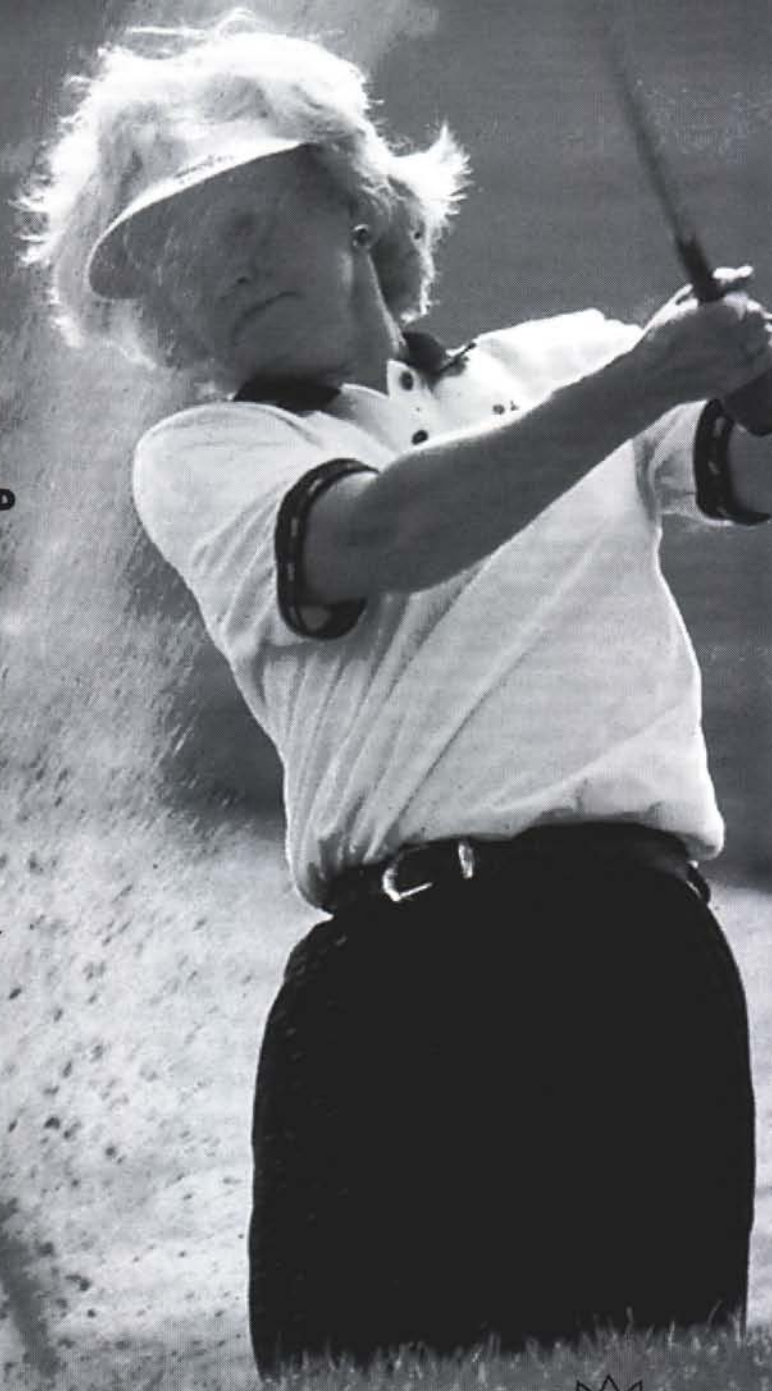

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Rich Land

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).

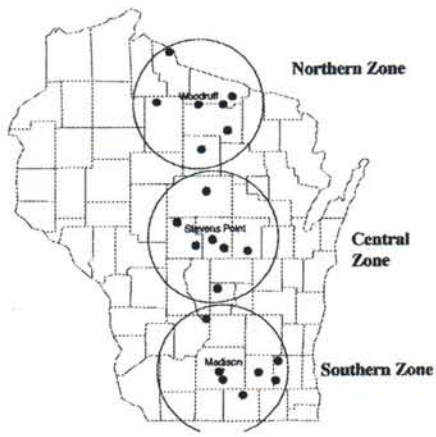

Figure 1. The distribution of locations of Wisconsin golf courses where isolates of *Typhula* species associated with *Typhula* blights were collected. Three zones were divided based on the duration of snow cover. Approximate locations of the golf courses surveyed are indicated as a black dot on Wisconsin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

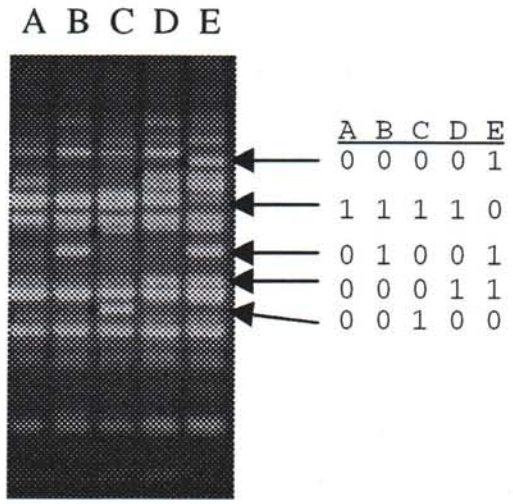

Figure 2. An example of a RAPD gel profile. Five isolates (A through E) of *Typhula ishikariensis* were ground for DNA extractions. RAPD products obtained in a PCR reaction are separated on an agarose gel. Arrows indicate polymorphic bands scored. The amplified bands were scored as either presence (1)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

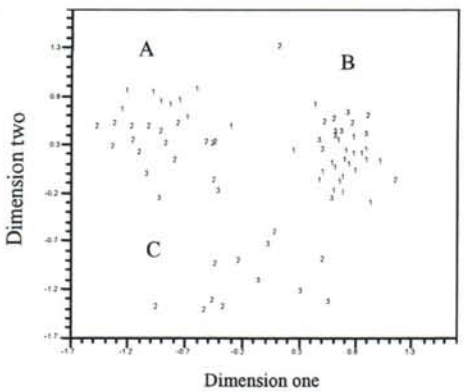

Figure 3. A multidimension scaling plot, a visual method of displaying the genetic relationship among *T. ishkeriensis* isolates, was drawn using genetic distance derived from RAPD markers. "1" indicates isolates collected from Wisconsin central zone, "2" from the northern zone, and "3" from other conturies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