#### FIRST ANNUAL PROGRESS REPORT

# DEVELOPING BROWN PATCH AND PYTHIUM DISEASE RESISTANCE IN BENTGRASS AND ZOYSIAGRASS

## submitted by

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### Executive Summary

## First Annual Progress Report

Developing <u>Rhizoctonia</u> Brown Patch and <u>Pythium</u> Disease

Resistance in Bentgrass and Zoysiagrass

Principal Investigator: Dr. Phillip F. Colbaugh

Research Period of this report: 1 May 1987 to
1 November 1987

Funds for the disease resistance project were received 1 March, 1987: and the project was initiated one month later on 1 April, 1987. The period from 1 April to 1 November has been spent assembling isolates of Rhizoctonia spp. and Pythium spp for pathogenicity studies on the Breeding and Genetics Project germplasm lines. Our efforts in this area have yielded some 40 isolates of Rhizoctonia spp. and 28 isolates of Pythium spp. from various grasses and environmental regimens throughout the U.S. A few isolates of Rhizoctonia and several isolates of Pythium spp. are due to arrive from other turfgrass pathology laboratories for a full representation of fungal species in each group. With the exception of a few fungal isolates from other University Laboratories, the pathogenic potential of isolates within the collection largely remains to be determined. Pathogenic isolates of Pythium spp. and Rhizoctonia spp. will be identified in laboratory and greenhouse inoculation studies during the upcomming winter

months (November - February). Modified greenhouse benches with bentgrass and zoysiagrass and some incubation dishes containing plugs of field-grown turf will be used to assess pathogenicity of all isolates we have collected. Construction of special greenhouse benches is in progress for support of the pathology project with bentgrass and zoysiagrass. Some preliminary greenhouse inoculation studies we conducted with Rhizoctonia spp. isolates collected during the spring varied considerably in pathogenicity on Raleigh St. Augustinegrass.

Other diseases noted on bentgrass and zoysiagrass during the past spring, summer and fall seasons included an unidentified soil-borne fungal disease of zoysiagrass on the TAMU-Dallas field plots and on samples from St. Louis, Missouri. The disease appears to be very damaging during the spring and early summer and is presumed to be a Leptosphaeria type of soil-borne disease. Dollarspot disease was also noted to be very severe on the fine-leaved 'Emerald' Zoysiagrass but not on the thicker leaved variety 'Meyer'during the spring and fall growing seasons. Fungicide tests we conducted in the spring indicated the disease could be easily controlled with one or two applications of iprodione or chlorothalonil at recommended use rates. The first set of notes were taken the experimental varieties of zoysiagrass during October. These observations, although preliminary, suggest that most of the elite collection of 25 selections were apparently not dollar spot susceptible. More detailed data will be collected on these experimental lines during the 1988 growing season.

#### I. Introduction:

On 17 February, 1987, the Texas Agricultural Experiment Station and the Texas A&M Research Foundation accepted research funds as per contract agreement (FPN: 5654000) with the United States Golf Association to conduct investigations to develop Rhizoctonia Brown Patch and Pythium disease Resistance in Bentgrass and Zoysiagrass. This is a cooperative research project with the turfgrass breeding and development efforts for both grasses, under the direction of Dr. Milton C. Engelke also of the Texas A&M Research Center at Dallas Texas.

This first annual research report is for the period of 1 March to 1 November, 1987 and essentially represents eight months of active research on the disease assessment project.

Ms. Jo Ann Treat, President of the Texas Research Foundation and Mr. Charles W. Smith, Director of Administration and Services for the United States Golf Association, signed the original contract agreement effective 1 February, 1987.

This is the first annual report for the research contract and will summarize the first year of pathogen isolate collection and initial work on the project.

#### II. Project Personnel:

Funds for the disease resistance project were received in March, 1987: and the project was initiated shortly thereafter on 1 April, 1987. Ms. Gretchen Heidel who was my laboratory assistant until May (now a graduate student in Dr. R. W. Tolers laboratory at Texas A&M University) worked on

the isolation of <u>Rhizoctonia</u> spp. from typical brown patch symptoms on turfgrasses in North Central and other areas of Texas. Ms. Heidel assembled a number of <u>Rhizoctonia</u> isolates which are now held in my laboratory.

Additional fungal isolates were obtained in isolations from diseased shoots of bentgrass and zoysiagrass during the hot summer months by Ms. Laurie Blanz who maintained an active program of collecting Pythium spp. isolates through most of the summer and fall season. With the departure of both of these technicians, Mr. Robert Markley has assumed duties for the project and is now working for 19 hours per week on the USGA Project. Mr. Markley is a chemist from an area firm now being sold to another company and he has several years of laboratory research experience.

# RHIZOCTONIA DISEASES - ONGOING RESEARCH:

## 1) Isolate Collection and Assembly:

With the exception of collections of a few Rhizoctonia isolates from North Carolina and Florida, the collection of species for this fungus is nearly complete. Most of the isolate collection is from Texas St. Augustinegrasses because of their proximity and ease in identifying the diseases and collection of isolates (Table 1). Other isolates were obtained from colleaques around the country in order to get a geographical representation of pathogens. The isolates appear to vary greatly in their cultural appearance which tends to support the observed taxonomic variation within this confused genus.

Table 1. Rhizoctonia spp. and Rhizoctonia-like fungal isolates maintained in the USGA Rhizoctonia isolate collection TAMU-Dallas.

| Isol # | Pathogen I.D.      | Colony Chara  | ct. Turf/Origin  | Comments   |
|--------|--------------------|---------------|------------------|------------|
| 1.     | Rhizoctonia solani | Buff Brown    | St. Augras (Tx)  | Ag2-2      |
| 2.     | Rhizoctonia solani | Buff Brn/Fluf | St. Augras (Tx)  | Ag2-2      |
| 3.     | Rhizoctonia solani | Lt Brn/Fluf   | St. Augras (Tx)  | Ag2-2      |
| 4.     | R. zeae            | Pink/Brown    | Bentgrass (NC)   | R. Jones   |
| 5.     | Binucleate RLF     | Lt Pink       | St. Augras (Tx)  | R. Jones   |
| 6.     | R. cerealis        | White/Brown   | Unknown turf     | R. Jones   |
| 7.     | R. oryzae          | Pink          | Rice (Tx)        | R. Jones   |
| 8.     | R. solani          | Lt Brown      | St. Augras (Tx)  | Stone 4    |
| 9.     | R. solani          | Dk Brn/Fluf   | St. Augras (Tx)  | Stone 6    |
| 10.    | R. solani          | Dk Brn/Fluf   | St. Augras (Tx)  | Stone 7    |
| 11.    | R. solani          | Lt. Brown     | St. Augras (Tx)  | Stone 10   |
| 12.    | R. solani          | Lt. Brown     | St. Augras (Tx)  | Stone 11   |
| 13.    | R. solani          | Dk Brn/Fluf   | St. Augras (Tx)  | Stone      |
| 14.    | R. solani          | Dk Brn/Fluf   | St. Augras (Tx)  | Milb. 1    |
| 15.    | R. solani          | Brown Fluf    | St. Augras (Tx)  | Milb. 3    |
| 16.    | R. solani          | Lt Brn Fluf   | St. Augras (Tx)  | Milb. 5    |
| L7.    | R. solani          | Lt Brn Fluf   | St. Augras (Tx)  | Milb. 6    |
| 18.    | R. solani          | White Fluf    | St. Augras (Tx)  | Dich. 1    |
| 19.    | R. solani          | White Fluf    | St. Augras (Tx)  | Dich. 3    |
| 20.    | R. solani          | Lt. Brown     | St. Augras (Tx)  | Mata 4     |
| 21.    | R. solani          | Lt. Brown     | St. Augras (Tx)  | Mata 1     |
| 22.    | R. cerealis        | White/Appr    | Kentucky BG (Mi) | Vargas     |
| 23.    | R. solani          | Lt.Brn/Appr   | Bentgrass (Mi)   | Vargas     |
| 24.    | R. solani          | Lt.Brn/Fluf   | St. Augras (Tx)  | Milb. 4    |
| 25.    | R. solani          | White/Appr    | St. Augras (Tx)  | Stone 9    |
| 26.    | R. solani          | Dk Brn/Appr   | Bentgrass (Pen)  | Couch 63G  |
| 27.    | R. solani          | Dk Brn/Appr   | Bentgrass (Va)   | Couch      |
| 28.    | R. solani          | Dk Brn/Appr   | Bentgrass (Pen)  | Couch 63F  |
| 29.    | R. solani          | White/Appr    | Tall Fescu (Va)  | Couch 45   |
| 30.    | R. solani          | Lt Brn/Appr   | Tall Fescu (Va)  | Couch 44   |
| 31.    | R. solani          | Dk Brn/Appr   | Tall Fescu (Md)  | Couch 63H  |
| 32.    | R. solani          | Lt Brn/Appr   | TAMU Dal (Tx)    | Colbaugh   |
| 33.    | R. solani          | Lt Brn/Appr   | Bentgrass (Tx)   | Lakewood   |
| 34.    | Rhizoc sp.         | Lt.Pink/Appr  | Bentgrass (Tx)   | El Dorado  |
| 35.    | Rhizoc. sp.        | Lt Pink/Appr  | Bentgrass (Tx)   | Mira Vista |
| 36.    | Rhizoc. sp.        | WhteLtBrn/Apr | Bentgrass (Tx)   | Lakewood   |
| 37.    | Rhizoc. sp.        | Lt Brn/Appr   | Bentgrass (Tx)   | Firewhl Lk |
| 38.    | R. solani          | Lt Brn/Appr   | St. Augras (Tx)  | TAMU Dal   |
| 39.    | R. solani          | Lt Brn/Appr   | Emer Zoysa (Tx)  | TAMU Dal   |

- 2) Field and Greenhouse Testing with <u>Rhizoctonia</u> brown patch fungal isolates:
- a) Rhizoctonia Brown Patch Fungicide Control Trials:

Field tests conducted during the fall season over two consecutive years were used to evaluate commercial and experimental fungicides for controlling Rhizoctonia blight of 'Raleigh' St. Augustinegrass. The test fungicides were evaluated for their long-term effectiveness (1 month) in controlling the brown patch disease under environmental conditions favoring heavy disease pressure. Results of investigations with 24 and 27 fungicide treatment programs during the two respective years indicate only a few of the fungicides gave protection for one month following application (Tables 2 and 3). Most of the fungicides were not effective under conditions of the study. The results of these investigations with highly susceptible St. Augustinegrass indicates the limited value of fungicides for long term control of the brown patch diseases.

Table 2. Field Fungicide Evaluations for the Control of Rhizoctonia

Brown Patch on 'Raleigh' St. Augustinegrass. Fall, 1985.

| SN-84364 50W 2.0 1.6 A  NC-28410 40SC 4.0 4.0 AB  Chipco 26019 50W 2.0 9.6 ABC  NC-28410 40SC 6.0 10.2 ABC  Duosan 60W 3.0 15.8 ABCD  MF-745 50W 1.5 18.6 ABCDE  MF-745 50W 1.0 18.8 ABCDE  SN-84364 50W 0.5 19.6 ABCDE  MF-690 50W 1.5 20.0 ABCDE  Kromad 27.5W 1.65 20.0 ABCDE  Kromad 27.5W 1.65 20.0 ABCDE  NC28410 40SC 2.0 25.6 ABCDE  NC28410 40SC 2.0 25.6 ABCDE  Fungo 50W 1.0 25.6 ABCDE  Fungo 50W 1.0 26.4 ABCDE  Duosan 60W 1.8 28.0 BCDE  PCNB 75W 12.0 28.2 BCDE  Corbel EF700 0.97 28.8 BCDE | Treatment  | Application oz a.i./1000  |        |  |                                       |
|--|--|---|--------|--|---------------------------------------|
| Fungo 50W 0.5 32.0 CDE Corbel EF700 2.91 32.2 CDE Kromad 27.5W 1.1 33.4 CDE Corbel EF700 5.8 33.6 CDE MF-654 1.0 34.2 CDEF Control Not Treated 38.4 DEF  | SN-84364 50W<br>NC-28410 40SC<br>Chipco 26019 5<br>NC-28410 40SC<br>Duosan 60W<br>MF-745 50W<br>MF-745 50W<br>MF-690 50W<br>Kromad 27.5W<br>PCNB 10G<br>NC28410 40SC<br>MF-690 50W<br>Fungo 50W<br>Duosan 60W<br>PCNB 75W<br>Corbel EF700<br>Fungo 50W<br>Corbel EF700<br>Kromad 27.5W<br>Corbel EF700 | 2.0<br>4.0<br>0W 2.0<br>6.0<br>3.0<br>1.5<br>1.0<br>0.5<br>1.65<br>1.65<br>10.94<br>2.0<br>1.0<br>1.0<br>1.8<br>12.0<br>0.97<br>0.5<br>2.91 | sq It. | 1.6 A 4.0 AB 9.6 AB 10.2 AB 15.8 AB 18.6 AB 19.6 AB 20.0 AB 20.0 AB 224.4 AB 25.6 AB 25.6 AB 25.6 AB 25.6 AB 28.0 B 28.2 B 32.0 33.4 33.6 34.2 | C C C C C C C C C C C C C C C C C C C |

a % disease represents the mean % disease of five replicates.
b Treatment values followed by same letter are not significantly different.

Table 3. Field fungicide evaluations for brown patch control on 'Raleigh' St. Augustinegrass. Fall, 1986.

| Treatment        | Application Rate oz a.i./1000 sq ft | % Disease<br>22 Days |     |
|------------------|-------------------------------------|----------------------|-----|
|                  |                                     |                      |     |
| SN84364 50W      | 1.0                                 | 2.0 A                |     |
| Corbel EF700     | 5.0/L                               | 3.3 AB               |     |
| PCNB 75W         | 12.0                                | 8.0 ABC              |     |
| FBC39865 25W     | 0.25                                | 9.3 ABCD             |     |
| SAN619 40W       | 0.1                                 | 9.3 ABCD             |     |
| MF654 50W        | 3.0                                 | 10.7 ABCDE           |     |
| SN84364 50W      | 2.0                                 | 11.3 ABCDE           |     |
| MF654 50W        | 1.5                                 | 12.0 ABCDE           |     |
| MF745 50W        | 1.5                                 | 12.0 ABCDE           |     |
| Kromad 27.5W     | 1.1                                 | 13.3 ABCDE           |     |
| SAN619 40W       | 0.05                                | 14.0 ABCDE           |     |
| Duosan 60W       | 1.8                                 | 15.0 ABCDE           |     |
| SN84364 20SC     | 2.0                                 | 17.0 ABCDEF          |     |
| SAN619 40W       | 0.8                                 | 18.0 ABCDEF          |     |
| MF745 50W        | 1.0                                 | 20.3 BCDEF           | G   |
| Fungo 50W        | 0.5                                 | 23.0 CDEF            | G   |
| Fungo 50W        | 1.0                                 | 24.3 CDEF            | G   |
| MF654 50W        | 0.5                                 | 26.7 DEF             | G   |
| Control Not Trea | ated -                              | 28.3 EF              | Ġ   |
| FBC39865         | 0.13                                | 33.0 F               | 'G  |
| Chipco 26019     | 1.0                                 | 37.0                 | G   |
| Duosan 60W       | 3.0                                 | 47.7                 | . 1 |

a % Disease represents the mean % of three replicate plots.
b Treatment values followed by the same letter are not significantly different.

b) Greenhouse Inoculation Trials/St. Augustinegrass

During the summer months one test for pathogenicity using Rhizoctonia isolate numbers 1-21 was conducted on a raised greenhouse bench containing Raleigh St. Augusinegrass The isolates were each placed into a 1.2 x 3.1 meter sod. area of sod and allowed to stand in humid conditions for 72 hours under a plastic canopy held over the bench. Differences were noted for pathogenic activity among the isolates tested. This technique will be also be used during the winter on special greenhouse benches containing Emerald zoysia and 'Pencross' bentgrass to determine the pathogenicity of isolates in the Rhizoctonia collection. Pathogenicity assessments of fungal isolates maintained in the Rhizoctonia spp. collection will help to determine which combination of isolates to use in assessing disease resistance among germplasm lines of bentgrass and zoysiagrass.

### Pythium DISEASES - ONGOING RESEARCH

# 1) Disease Periodicity:

As noted in my earlier report, the cycle of disease activity by Pythium spp. on bentgrass reaches the maximum disease potential during the summer months on a heat stressed and weakened host. A fingerprint for a typical turfgrass disease scenario for Dallas, Texas and much of the South is shown in Fig. 1. Pythium diseases are favored by excessively moist growing conditions and high rates of nitrogen fertility during peak periods of summer stress.

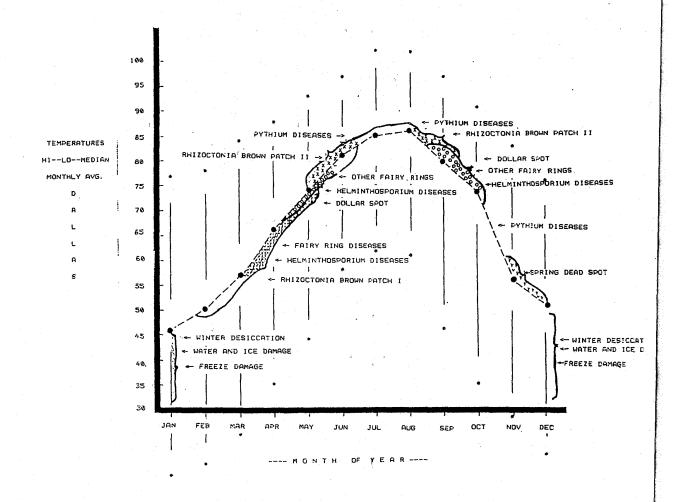


Fig. 1. Seasonal Periodicity of Turfgrass Diseases in the Area of Dallas, Texas.

## 2) The Pythium spp Strain Collection:

Our Pythium spp. isolate collection now totals some 28 fungi which have been collected on various bentgrasses on golf course greens, from seedling diseases related to damping off diseases and from scattered turfgrass samples from other areas of the Southwest U.S. (Table 4). Additional isolates will be collected during the winter from turfgrass pathology colleagues in Iowa, Florida, and Virginia, however, we have not received some of the cultures at this writing.

Difficulty in isolating Pythium fungi from infected root, crown and foliar tissue was overcome by contacting Dr. Olaf K. Ribiero who is very experienced in these matters. Dr. Ribiero suggested a recipe for a Pythium selective medium he has employed and we are now using the medium for routine isolations of Pythium from soil and plant tissue. At present we are holding some 28 isolates of Pythium spp. in our collection of water mold fungi. These isolates are now being screened on Penncross bentgrass for assessment of their pathogenicity.

Unless cultures have been received with the proper taxonomic identification, no attempt will be made to determine the <u>Pythium</u> spp. in the collection until the pathogenicity studies have been completed. At that time identification of highly pathogenic isolates on both bentgrasses and zoysiagrasses will be determined.

Table 4. Pythium spp. and Pythium-like fungal isolates maintained in the USGA Pythium isolate collection TAMU-Dallas.

| Isol | # Pathogen I.D.                   | Colony Char | act. Turf/O  | rigin | Comments    |
|------|-----------------------------------|-------------|--------------|-------|-------------|
| 1.   | Pythium aphanidermatum            | White/Fluf  | Bermudagr (H | Fla)  | P117(Fla)   |
| 2.   | P. sp.                            | WhtBrn/Appr | Bentgrass (N | Mi)   | Vargas      |
| 3.   | P. sp.                            | White/Fluf  |              | ľx)   | Fossl Creek |
| 4.   | P. sp.                            | WhtBrn/Appr | Bentgrass (1 | Tx)   | LaVista     |
| 5.   | P. aphanidermatum                 | White/Fluf  |              | ľx)   | NevCoop     |
| 6.   | P. sp.<br>P. sp.<br>P. sp.        | White/Fluf  |              | ľx)   | P-1         |
| 7.   | P. sp.                            | White/Fluf  | Unkn turf (1 | ľx)   | P-2         |
| 8.   | P. sp.                            | White/Fluf  |              | Tx)   | P-3         |
| 9.   | P. sp.                            | White/Fluf  | Unkn turf (1 | rx)   | P-4         |
| 10.  | P. sp.                            | White/Fluf  | Unkn turf (1 | ľx)   | P-5         |
| 11.  | P. sp.                            | White/Fluf  |              | ľx)   | P-6         |
| 12.  | P. sp.                            | White/Fluf  |              | ľx)   | P-7         |
| 13.  | P. sp.                            | White/Fluf  | Unkn turf (  | Tx)   | P-9         |
| 14.  | P. sp.                            | White/Fluf  | Unkn turf (  | Tx)   | P-11        |
| 15.  | P. sp.                            | White/Fluf  |              | Гх)   | P-12        |
| 16.  | P. sp.                            | WhtBrn/Appr | Bentgrass (  | Tx)   | FirWhl Lk   |
| 17.  | P. sp.                            | Brn/Appr    |              | Colo) | Brknrdg     |
| 18.  | P. sp.                            | White/Fluf  | Bentgrass (  | Tx)   | M. Allen    |
| 19.  | P. aphanidermatum P. sp.          | White/Fluf  |              | Tx)   | Dw-1        |
| 20.  | P. sp.                            | White/Fluf  | Bent/Blk Pt( | Colo) | Brknrdg     |
| 21.  | P. sp.                            | White/Appr  | Bermudagr (1 | Nev)  | NevCoop     |
| 22.  | P. sp.                            | White/Fluf  | Bentgrass (  | Tx)   | FosCrek2    |
| 23.  | P. sp.                            | White/Appr  | Zoysia Emld( | ILL)  | Engelke Col |
| 24.  | $\underline{\underline{P}}$ . sp. | White/Fluf  | Bentgrass (  | Tx)   | Prstn Tr1   |
| 25.  | P. sp.                            | White/Fluf  | Bentgrass (  | Tx)   | Prstn Tr2   |
| 26.  | P. sp.                            | White/Fluf  |              | Tx)   | P-13        |
| 27.  | P. sp.                            | White/Fluf  | Unkn turf (  | Tx)   | P-17        |
| 28.  | P. sp.                            | White/Fluf  | Unkn turf (  | Tx)   | P-1-1       |

3) Overview of <u>Pythium</u> spp. and <u>Pythium</u> Diseases from the Literature:

#### Disease Symptoms:

Grass is rapidly killed in distinct spots that form streaks on depressed areas of turf. The spots range from several inches to a foot in diameter. Under humid conditions the fungus grows profusely over the surface of affected plants and the diseased areas have a cotton-like appearance. Environmental relationships are important for determining the occurrence of <a href="Pythium">Pythium</a> blight. An abundance of moisture is required for destructive activity by <a href="Pythium">Pythium</a> spp. Blighting activity is most prevalent during rainy or overcast weather in low lying areas where air circulation is poor.

## Isolation Techniques:

Isolation of <u>Pythium</u> spp. from plant tissue is relatively easy if precautions are used to minimize bacterial contamination. Plant tissues should be washed thoroughly in running tap water or water containing wetting agents such as Tween 20 or Dreft soap prior to attempting isolation. Clorox solutions used for surface sterilization of plant tissues for other types of fungal pathogens are toxic to <u>Pythium</u> spp. and should be avoided (Schmitthenner, 1980). Allowing infected plants to incubate overnight in water containing sesame seed baits or weak amendments of V/8 juice is useful for studying outgrowths of <u>Pythium</u> spp. associated with infected plant parts. Use of nutritionally rich cultural media for isolation usually results in a proliferation of bacterial growth

which inhibits outgrowths of <u>Pythium</u> spp. from the host tissue. Water agar, cornmeal agar, and V/8 juice agar are low nutrient cultural mediums routinely used for isolating <u>Pythium</u> spp from infected plant parts. Pieces of agar are frequently placed on top of the tissue used for isolation to allow fungal growth to escape bacterial contamination (Hall, 1980).

Selective cultural media containing polyene antibiotics are also extensively employed for isolation of <u>Pythium</u> spp.

Several selective cultural mediums are available for isolating <u>Pythium</u> spp. from infected plants and decomposing turfgrass debris (Tsao, 1970; Schmitthenner, 1980). Streptomycin sulfate and rose bengal used in many selective cultural mediums are known to be toxic to some <u>Pythium</u> spp. (Waterhouse, 1967).

Hall et al. (1980) described methods of isolating Pythium spp. from soil. Soil core samples obtained from bentgrass greens could be successfully stored for up to three days at 14 C prior to isolation attempts. Isolation of Pythium spp. from soil is often more difficult because of the presence of high populations of associated bacteria on soil dilution samples (Schmitthener, 1980). Pythium populations in soil are typically low and it is necessary to place relatively large amounts of soil on isolation plates for detection. A washing procedure (Schmitthenner, 1980) is useful for physical removal of contaminating bacteria 24-48 hrs following the soil dilution procedure.

Once isolated, <u>Pythium</u> spp. are very difficult to maintain in culture for long periods of time. Bacterial contamination

often destroys Pythium cultures maintained on agar cultural plates. Maintenance of Pythium cultures is aided by the use of weak nutritional media or cultural mediums employing antibiotics which suppress bacterial growth. Rapid growth of cultures will insure mycelium free of bacterial for several days however all cultural mediums will eventually allow bacterial contamination to colonize the plate even though strongly suppressive antibiotics are initially present. Long term preservation of cultures have been possible using fresh transfers of the fungus on agar slants in test tubes with a sterile mineral oil layer added to the mycelium on the agar surface.

# Pythium Identification:

Many pathogenic and saprophytic species of Pythium are known to occur on turfgrasses (Schmitthenner, 1980; Smiley, 1983). Morphological characteristics of both sporangia and oospores are used for identification of Pythium isolates to species. Weak nutritional growth mediums and agar slurys are commonly used to induce sporulation by Pythium spp. (Schmitthenner, 1980). Grass leaf cultures recommended by Waterhouse (1967) are also very useful for identifying cultural isolates. Oospores and sporangia of most species will develop in 3-4 days. The keys to Middleton (1943) or waterhouse (1967) are primarily used for Pythium identification where the types of sporangium and relationship of oospores to oogonium are shown in detailed drawings. Other minor taxonomic characteristics are the thickness of the oospore wall and number of antheridia in

association with the oogonium. Some Pythium spp. do not produce oospores in single cultures.

Methods of Inoculation:

Inoculation with Pythium spp. is relatively easy to accomplish if precautions are used to minimize bacterial suppression of applied inoculum. Inoculation studies used by Moore et al. (1963) employed eight day old mycelial inoculum obtained from potato dextrose broth cultures. Mycelium obtained from PDB was washed in sterile distilled water cut into small pieces and spread evenly over seedlings in crocks grown in silica sand. The inoculated crocks were covered with plastic bags for 3-6 days and maintained at 26 C. Three days following the removal of plastic bags, disease severity was recorded as per cent survival of seedling stands. This inoculation method was used to assess the influence of nutrition, pH and temperature on disease severity. methods of inoculation were used by Muse et al. (1974) using a synthetic liquid medium for growth of mycelia for inoculation of bentgrass , Kentucky bluegrass, red fescue and ryegrass.

Freeman (1963) reported successful inoculation of ryegrass seedlings of varying age using a 1 cm mycelial plug taken from 6 day old cultures of P. aphanidermatum. After inoculation test seedlings were maintained under warm (25-30 C) humid conditions for a 6 day period of study. Field and greenhouse inoculation methods using 7-day old cultures of P. aphanidermatum grown on autoclaved rye grain have been

successfully used by Cole et al. (1978). Inoculation consists of spreading the infested rye kernels to achieve an inoculation density of one kernel/5.8 cm sq of turfgrass. After inoculation, turfgrass areas are maintained in a moisture chamber at high temperature not exceeding 38 C. Hall et al. (1980) used bentgrass thatch and soil from putting greens to inoculate pot plants containing seedlings of bentgrass. Inoculum was placed on the soil surface of pots with six week old plants and incubated at 30 C under flourescent lighting with a 12 hr photoperiod.

Mycelium of <u>Pythium</u> spp readily lyses and does not survive for long in soil (Loyd and Lockwood, 1966). Zoospores are also short lived (Burr and Stanghlleni 1973). Sporangia of lobulate sporangial species generally are short lived as mycelium (Stanghellini 1974) but temperatures however oospores of P graminicola and <u>P. vanterpoolii</u> have not been studied.

Mitchell (1978) has recently shown the relationship of inoculum density to disease with several Pythium spp. There are studies also of seasonal inoculum density variation of Pythium (Lumsden et al. 1976) the most pertinent to cottony blight of turfgrasses is the research of Hall (1978). He reported that P. aphanidermatum is found primarily in the thatch. Maximum colony numbers occurred in winter, presumably following a cottony blight infection in the summer that caused an increase in the number of oospores. Populations declined during spring and early summer, probably because oospores

germinated and conditions were not favorable for cottony blight. Increases in <u>P.aphanidermatum</u> populations coincided with the onset of the cottony blight season. Hall also monitored environmental conditions that were correlated with cottony blight epiphytotics. Similar information has been developed for <u>P. ultimum</u> (Ayers and Lumsden 1975; Lumsden 1973; Stanghellini and Handcock 1971; and 1971) and possibly for <u>P. myriotylum</u> (Mitchell, 1975, 1978).

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Field Observations on the Dollarspot Disease:

The occurrence of the dollar spot disease similar to the brown patch disease. Both diseases are prevailent during the transition seasons of spring and fall during periods of excessive moisture accumulation in the turfgrass foliar canopy. Although studies on the dollar spot disease are not directly tied to these investigations, the disease is important and should be commented on.

During the spring season, we conducted a fungicide efficacy test with the dollar spot disease because it was very damaging to the Emerald zoysiagrass on our field nursery plots. Results of the fungicide trials are shown in Table 5. Control of the disease was fairly good with iprodione (Chipco 26019) and with chlorothalonil (Daconil 2787) when applied at manufacturers recommended rates.

Table 5. Fungicide Effectiveness for Control of Dollarspot on Emerald Zoysiagrass Spring 1987.

| Fungicide            |              |             |                   |  |  |
|----------------------|--------------|-------------|-------------------|--|--|
| Name                 | Formula      | Lb ai/A     | Mean % Dollarspot |  |  |
| SAN619F              | 40WG         | .096        | 20                |  |  |
| SAN712F              | 83EC         | .096        | 8                 |  |  |
| SAN619F +<br>RIZOLEX | 40WG<br>75DF | .096<br>5.4 | 14                |  |  |
| CHIPCO 26            | 019 50WP     | 6.1         | 4                 |  |  |
| DACONIL 2            | 787 4.2FL    | 8.2         | 15                |  |  |
| NOT TREAT            | ED CONTROL   | _           | 100               |  |  |

a Mean % Dollarspot of four replicated field plots.

b lsd (.05) = 15, sd = 10, coeff. variability = 14

It does appear however, that fungicide spray applications on susceptible turfs will need to be repeated often, perhaps 10-14 day intervals for effective control.

In contrast to the heavy dollar spot symptoms noted on the variety Emerald, the variety Meyer had no apparent symptoms of the disease during the spring and fall seasons of 1987. In response to this observation Ms. Melinda Quick and I took some dollar spot ratings on the zoysiagrass nursery with some 25 germplasm and commercial lines of the grass (Table 6). Although the field plots are still very young and not completely filled in, we were able to detect dollar spot symptoms on several fine bladed lines of the turf including the most susceptible type which was the variety Emerald. Additional observations of this sort are scheduled for the spring and fall growing seasons during of 1988.

Table 6. Field Evaluations of Naturally occurring dollarspot on 25 zoysiagrass cultivar selections from USGA Breeding Block Nursery. October, 1987.

| Cultivar I.D.          | Dollar Spot<br>Block 1 | Ratings Mean<br>Block 2 of |      |
|------------------------|------------------------|----------------------------|------|
| Dalz 8501              | 0                      | 0                          | 0    |
| Dalz 8502              | 1.0                    | 0                          | 0.5  |
| Dalz 8503              | 0 -                    | 0                          | 0    |
| Dalz 8504              | 0                      | 0                          | 0    |
| Dalz 8505              | 0                      | 0                          | 0    |
| Dalz 8506              | 0.5                    | 0                          | 0.25 |
| Dalz 8507              | 0                      | 0                          | 0    |
| Dalz 8508              | 1.0                    | 0                          | 0.5  |
| Dalz 8510              | 1.0                    | 0                          | 0.5  |
| Dalz 8511              | 0                      | 0                          | 0    |
| Dalz 8512              | 0                      | 0                          | 0    |
| Dalz 8513              | 0                      | 0                          | 0    |
| Dalz 8514              | 0                      | 0                          | 0    |
| Dalz 8515              | 1.0                    | 0                          | 0.5  |
| Dalz 8516              | 0                      | 0                          | 0    |
| Dalz 8517<br>Dalz 8522 | Ü                      | 0                          | 0    |
| Dalz 8522<br>Dalz 8523 | 0                      | 1.0                        | 0.5  |
| Dalz 8523              | 0                      | 1.0                        | 0.5  |
| Meyer                  | 0                      | 0                          | 0.0  |
| Emerald                | 1.0                    | 2.0                        | 1.5  |
| El Toro                | 0                      | 0                          | 0    |
| Belair                 | Ô                      | Õ                          | Ŏ.   |
| FC 13521               | ŏ                      | 0                          | Ŏ    |
| Korean Common          | ŏ                      | 0                          | 0    |

a/ Dollar spot ratings from 0-3 where 1=trace, 2=moderate and 3=very severe.