SECOND ANNUAL PROGRESS REPORT

DEVELOPING BROWN PATCH AND PYTHIUM DISEASE RESISTANCE IN BENTGRASS AND ZOYSIAGRASS

submitted by

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USGA TURFGRASS PATHOLOGY RESEARCH

SECOND ANNUAL PROGRESS REPORT 1988

INDEX

Executive Summary

Second Annual Progress Report 1 November, 1988

Developing *Rhizoctonia* Brown Patch and *Pythium* Disease Resistance in Bentgrass and Zoysiagrass

Principal Investigator: Dr. Phillip F. Colbaugh

Research Period of this report: 1 May 1988 to 1 Nov. 1988

This is the second annual U.S.G.A. report; the TAES-Dallas plant pathology research program for bentgrass and zoysiagrass is now completing a second year of study.

Progress during the period from May to November 1988 has followed several lines of investigation. The fungal culture collections for *Rhizoctonia* spp. and *Pythium* spp. attacking turfgrasses now number 70 and 41 isolates, respectively. The most virulent strains of these fungi are currently being used for disease resistance screening studies with experimental lines of bentgrass and zoysiagrass. Cultures for both of the fungal collections were placed in long-term storage vials which are being maintained at temperatures favoring their

extended survival.

The past six months of pathology investigations have focused on inoculation studies with members of the elite collection of bentgrass germplasm lines located at Dallas. Results of these investigations have been very worthwhile. Inoculations with Pythium spp. were conducted in the field, greenhouse and laboratory utilizing four of the most virulent isolates from the USGA culture collection. Weather conditions were not favorable for successful field inoculations; however, both laboratory and greenhouse trials with these pathogens demonstrated experimental germplasm lines with high degrees of tolerance to foliar blighting, when compared to the commercial variety 'Penncross' which was easily killed.

Results of inoculation trials with four virulent isolates of Rhizoctonia spp. on a similar collection of bentgrass experimental lines also demonstrated resistance to foliar blighting compared to the variety 'Penncross'. Rhizoctonia isolates used in the test were very aggressive foliar blighting pathogens. Less than 5% of the experimental germplasm lines tested demonstrated resistance to all four pathogen isolates.

Field observations of diseases on zoysiagrass during the past summer and fall included the dollar spot and Rhizoctonia brown patch diseases. The reaction of varieties in field plantings to the dollar spot disease were generally the same as those noted during 1987. The fine-leaved zoysiagrass varieties were more susceptibile to dollar spot. Observations of Rhizoctonia brown patch on replicated nursery plantings

of ten zoysiagrass selections also indicated that one experimental line, and the commercial variety 'Meyer', were more susceptible to the disease than the other experimental lines under evaluation.

I. **Introduction:**

On 17 February, 1987, the Texas Agricultural Experiment Station and the Texas A&M University 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 project with the turfgrass breeding and development efforts for both grasses, under the direction of Dr. Milton C. Engelke also at the Texas A&M University Research and Extension Center at Dallas, Texas. This second annual research annual research report is for the period 1 May 1988 to 1 November 1988, and represents the past six months of active research on the cooperative disease assessment project.

II. **Project Personnel:**

Mr. Robert Markley, who was formerly assisting in the project, has been replaced by Mrs. Ann Heidel. Mrs. Heidel has previously been employed by this research department and has experience in laboratory and greenhouse research. Ann has also had some valuable computer training in obtaining the M.S. Degree in Agricultural Economics at Texas A & M University. She is presently employed as a part-time assistant.

III. *Pythium* DISEASES - ONGOING RESEARCH

1. Isolate Collection and Storage:

The USGA isolate collection of *Pythium* spp. has been assembled for long-term preservation through encapsulation of the cultures and storage at a temperature known to insure their survival (Table 1). Initially, routine transfers of *Pythium* spp. cultures on agar slants soon became contaminated by saprophytic bacteria which necessitated repeated transfers to keep the cultures viable. After conversations with Dr. A. F. Schmitthenner (OARDC, The Ohio State University) and Dr. O. K. Ribiero, (Microbiotica Inc., Bainbridge, WA) I was able to find a solution to the problem. Subsequently, all *Pythium* spp. isolates were grown on Difco water agar and 0.5 cm dia discs from the periphery of the colonies were transferred to perfume bottle vials (3 dram) containing 4 ml of sterile distilled water. The labeled and sealed vials are now being maintained at 11 C for long-term preservation of the cultures. At this writing, the vials have been in storage for six months and they appear to be viable.

2. Pathogenicity Studies - *Pythium* spp. on Bentgrass

a) Field studies *in situ*:

The possibility of inoculating bentgrass germplasm lines in the field nursery would overcome many logistical problems of digging, labeling and transporting plants to the study

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

Isol #	Pathogen I.D.	Colony Charact.	*Sporangial Type	Radial Growth Rate on Agar (mm/hr)	Pathogenicity on Bentgrass**
1.	*Pythium aphanidermatum*	White/Fluf	lobate	–	– ***
2.	*Pythium* sp.	WhtBrn/Appr	spherical	0.5	0
3.	*Pythium* sp.	White/Fluf	lobate	1.5	1.0
4.	*Pythium* sp.	WhtBrn/Appr	spherical	1.4	2.5
5.	*Pythium aphanidermatum*	White/Fluf	spherical	1.3	1.0
6.	*Pythium* sp.	White/Fluf	-	-	-
7.	*Pythium* sp.	White/Fluf	-	-	0
8.	*Pythium* sp.	White/Fluf	-	-	-
9.	*Pythium* sp.	White/Fluf	-	-	-
10.	*Pythium* sp.	White/Fluf	-	-	-
11.	*Pythium* sp.	White/Fluf	-	-	-
12.	*Pythium* sp.	White/Fluf	-	-	-
13.	*Pythium* sp.	White/Fluf	-	-	-
14.	*Pythium* sp.	White/Fluf	-	-	-
15.	*Pythium* sp.	White/Fluf	-	-	-
16.	*Pythium* sp.	WhtBrn/Appr	spherical	0.6	0
17.	*Pythium* sp.	Brn/Appr	-	-	-
18.	*Pythium* sp.	White/Fluf	spherical	1.3	2.0
19.	*Pythium aphanidermatum*	White/Fluf	lobate	-	-
20.	*Pythium* sp.	White/Fluf	spherical	0.2	0
21.	*Pythium* sp.	White/Appr	lobate	1.2	2.5
22.	*Pythium* sp.	White/Fluf	spherical	1.3	3.5
23.	*Pythium* sp.	White/Appr	-	-	-
24.	*Pythium* sp.	White/Fluf	lobate	1.0	2.5
25.	*Pythium* sp.	White/Fluf	lobate	1.0	4.0
26.	*Pythium* sp.	White/Fluf	-	-	-
27.	*Pythium* sp.	White/Fluf	-	-	-
28.	*Pythium* sp.	White/Fluf	-	-	-
29.	*Pythium* sp.	White/Appr	lobate	0.7	1.0
30.	*Pythium* sp.	White/Appr	spherical	0.5	1.5
31.	*Pythium* sp.	White/Appr	lobate	0.5	0
32.	*Pythium* sp.	White/Appr	lobate	1.1	0
33.	*Pythium* sp.	White/Appr	lobate	0.5	0
34.	*Pythium* sp.	White/Appr	lobate	0.5	2.5
35.	*Pythium* sp.	White/Appr	spherical	1.3	0
36.	*Pythium* sp.	White/Appr	spherical	1.3	4.0
37.	*Pythium* sp.	White/Appr	spherical	1.3	3.5
38.	*Pythium* sp.	White/Appr	spherical	0.7	1.0
39.	*Pythium aphanidermatum*	White/Appr	lobate	0.6	3.0
40.	*Pythium aphanidermatum*	White/Appr	lobate	0.6	3.0

* Colony characteristics, Appr = appressed; Fluf = fluffy growth habit.

** Pathogenicity ratings 0-4 where 4 = maximum pathogen activity. Isolates maintained on respective hosts under humidity chambers for 72 hr at 28 C.

*** (-) = Data not determined

area. Although late May was rather advanced in the year to perform field inoculation studies with Pythium species, it was decided to proceed with the experiment because the weather conditions were somewhat cooperative. On May 30, 1988, one cm dia discs from the advancing growth edge of Pythium spp. growing on water agar were used for inoculation of the elite bentgrass nursery on the old green at TAMU-Dallas. Agar discs of virulent isolates #5, 18, 24, and 36 were aseptically cut in the laboratory using a flamed and cooled cork-borer. In the field, the agar discs of the four cultures were oriented face up in a clockwise manner on 7.5 cm squares of glassine weighing paper, such that when the paper was inverted and pressed onto the surface of the turfgrass, the four discs and their contained Pythium cultures were simultaneously placed onto the turfgrass leaf canopy.

One hundred three samples of elite bentgrass genotypes planted on a USGA sand base green with two replications of each were inoculated in situ in the above manner, after which a light sprinkling of water was applied to the green. The grasses were subsequently watered throughout the day and night on regular timed cycle, both to promote the growth of the Pythium and to keep the agar discs hydrated, and to keep the grass cool. Both Pythium inoculated and uninoculated Penncross controls were included in the study. For the duration of this experiment, the weather conditions were overcast and cooler than normal.

The turf area had previously been maintained at a height

of 5 mm (3/16 inch) but was left unmowed for 7 days in order to increase the availability of leaf tissue for disease activity. The bentgrass nursery was also fertilized with sulphur coated urea (21-4-12) every six weeks and last received a fertilizer treatment one month prior to the fungal inoculations.

Although the individual bentgrass genotypes were closely observed for a period of two weeks, no evidence of Pythium infection was detected. Finally, the favorable weather turned typically hot for the season and the experiment was terminated.

In spite of seemingly favorable conditions for Pythium disease activity in the field nursery and the use of all the important variables known to encourage the disease, no activity of the pathogens was detected. There may be several reasons for the failure of this experiment. The weather may have been less favorable for the development of fungal infection than was realized. The results do point to the difficulty in conducting field experiments for the evaluation of germplasm disease resistance with this pathogen.

b) **Greenhouse Studies:**

On 5 May, 1988, triplicate core samples (10.5 cm dia) of 20 selected bentgrass experimental lines were cut, removed from their respective growing areas, and placed in large, flat plastic dishes on raised benches in the greenhouse.

The next day, each of two experimental cores was inoculated, in a clockwise direction in quadrant sectors around the periphery of the core, with four strains (#5, 18,

24, and 36) of actively growing Pythium on water agar; a plastic marker stuck in each core was used to orient the inocula. The inocula consisted of 1 cm dia water agar discs of the fungal growing edges which were stamped out asceptically with a cork-borer and placed individually on their assigned quadrants of the the grass leaf canopy of each core. One set of each of the turfgrass genotype cores was kept as uninoculated comparative controls. Penncross cores were used as infection controls.

The agar discs were placed fungus side down on the grasses and leaf canopies were misted with sterile distilled water. A large glass petri dish cover (12.5 cm dia) was then placed over each turfgrass core to retain moisture and to keep the agar discs in intimate contact with the grasses. The petri dishes were marked so that they were oriented with the plastic marker in the turfgrass core to identify each Pythium culture used in the study. Periodically the petri dishes were removed in order to make observations on any developing fungal infections and to mist the grasses.

Thirty six days passed before unequivocal Pythium disease was observed and confirmed by microscopic observation (Table 2). Some of the bentgrass genotypes were affected by only one of the four Pythium strains inoculated, eg., 1009, 1101, 1111, 1405, 1510, 1602, whereas some genotypes were affected by all four of the Pythium strains, e.g. 905, 1109, 1110, 1111, 1209, and 1504. A large number of the duplicates of a pair of genotypes did not evidence infection. Four

Table 2. Estimated percentage of bentgrass foliar canopies infected by Pythium spp. 28, 36 and 40 days after inoculation with four virulent isolates.

Bentgrass Genotype		Isolate #5	Isolate #18	Isolate #24	Isolate #P58
Penncross	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	-
903	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	+20%	-	+20%
905	28d	-	-	-	-
	36d	+100%	+100%	+100%	+100%
	40d	-	-	-	-
910	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	-
1009	28d	-	-	-	-
	36d	+25%	-	-	-
	40d	-	-	-	-
1101	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	+10%
1109	28d	-	-	-	-
	36d	+80%	+80%	+80%	+80%
	48d	-	-	-	-
1110	28d	-	-	-	-
	36d	+50%	+50%	+50%	+50%
	40d	+20%	-	-	+20%
1111	28d	-	-	-	-
	36d	+100%	+100%	+100%	+100%
	40d	-	-	+20%	-
1201	28d	-	-	-	-
	36d	-	-	-	-
	40d	+20%	-	+20%	-
1209	28d	-	-	-	-
	36d	+90%	+90%	+90%	+90%
	40d	-	-	-	-
1309	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	+10%	+10%
1405	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	+10%
1504	28d	-	-	-	-
	36d	+80%	+80%	+80%	+80%
	36d	+100%	+100%	+100%	+100%
	40d	-	-	-	-
1505	28d	-	-	-	+ 5%
	36d	-	-	-	+30%
	40d	-	-	-	-
1507	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	-
1508	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	-
1510	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	+10%	-
1602	28d	-	-	-	-
	36d	-	-	+20%	-
	40d	-	-	-	-
2104	28d	-	-	-	-
	36d	-	-	-	-
	40d	-	-	-	-

* Turfgrass germplasm lines maintained in six-inch dia pots where turfgrass canopy was covered with an inverted 12.5 cm diam petri dish lid.

** Inoculum supplied by four virulent strains of Pythium spp. on agar under an inverted petri dish lid.

*** (+) = disease reaction in percentage area blighted, (-) = no observable disease symptoms.

experimental lines, 910, 1507, 1508, 2104 and Penncross which is known to be susceptible, did not show fungal infection.

The unexpectedly long delay before uncontroversial symptoms of Pythium disease were evident could be due to problems with fungal outgrowth from the petri dish lid. Saprophytic bacteria were observed in high numbers in films of condensation water on the surface of the glass and these bacteria could have prevented infection by the fungus. There may have also been a heating influence of sunlight on the glass or limited gas exchange that could have also curtailed infection.

c) **Laboratory studies**

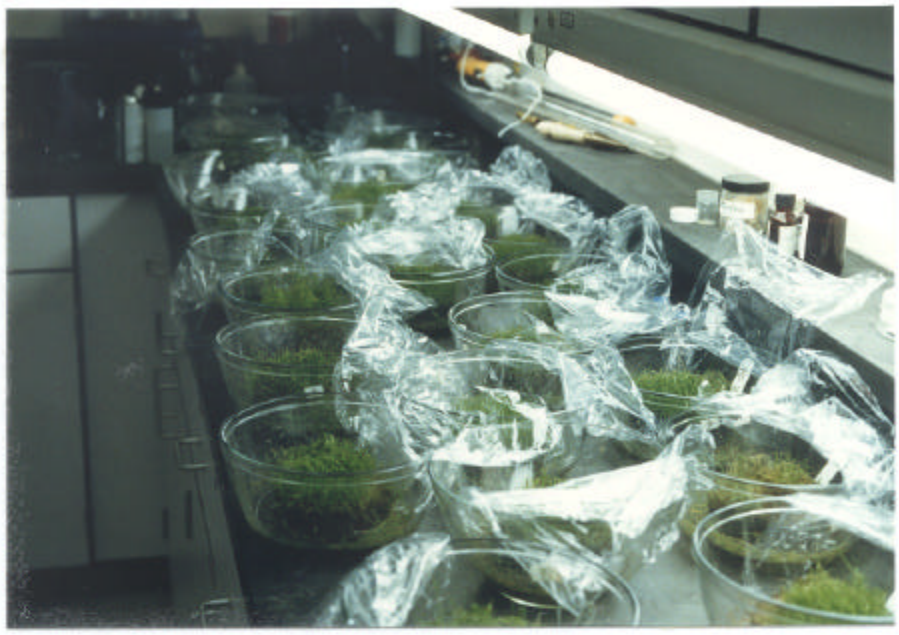
On 26 July, 1988, triplicate samples of each of 20 bentgrass genotypes were cut from their field sites and transferred to flat plastic dishes in the greenhouse. They were fertilized four times at three day intervals with Peters 20-20-20 fertilizer (100 ppm N) and watered regularly. After 12 days, the samples were removed from the greenhouse to the laboratory, where they were each placed in a glass mixing bowl (2.5 qt), watered and misted with sterile distilled water, and the tips of the bowls sealed with plastic wrap to maintain free moisture on the grass leaves. The bowls were placed near fluorescent lights and exposed to 250 fc light (Fig. 1A).

The next day, 7 July, 1988 the grasses were misted with water and each turfgrass core was inoculated with active water

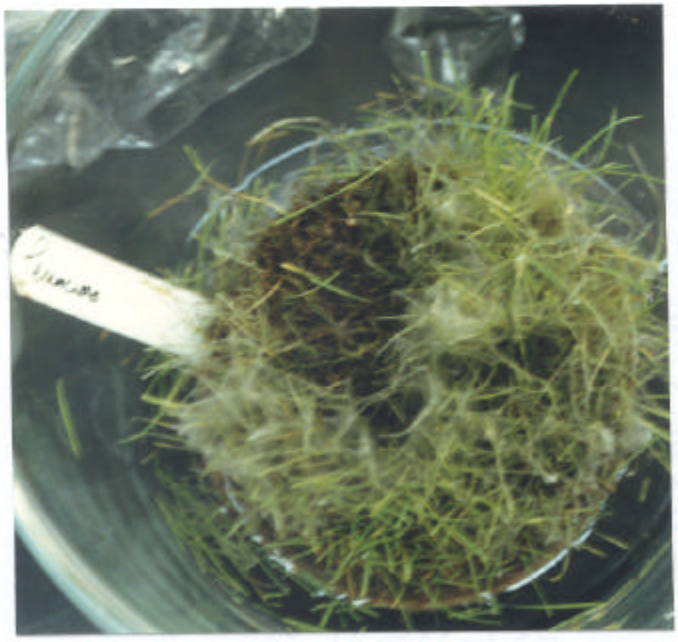
agar cultures of Pythium strains #5, 18, 24, and 36. The inocula were 1 cm diameter agar discs taken aseptically from the leading growth edge of the fungal cultures and placed face down on the grasses. Plastic markers were placed in the cores to orient the inocula in a clockwise direction. One set of each of the cores was used as uninoculated controls. Penncross was included as a control on the growth of fungi.

Nine days following the inoculation of the bentgrasses severe foliar disease was observed on several experimental bentgrass lines and on the Pythium susceptible control Penncross (Fig. 1 B,C). Pythium induced foliar blighting could be observed on both inoculated test plants near the origins of the inoculum (Table 3) and on the uninoculated controls for many of the plants used in the study. The presence of Pythium spp. was verified by direct microscopic examinations of diseased leaf blades and by isolation of the fungus on water agar which produced growth typical of Pythium spp.

The rapidity of the spread of the Pythium disease indicates that the causative organism was exceptionally virulent. It may also indicate that the artificial, isolated growth conditions of the samples, taken from the field to the laboratory, allowed the infection to occur, since disease was not initially obvious in the larger field plots from which the samples were taken. Note: two weeks after the sample cores were removed, disease was obvious on the Penncross genotype in the field plots, from whence the core samples had been taken.



A



B

C

Fig 1 (A,B,C). A. Laboratory inoculation area for Pythium spp. on bentgrass experimental lines.
B,C. Pythium disease activity on 'Penncross' versus disease tolerant germplasm #2305.

Table 3. Estimated percentage of bentgrass leaf canopy infected by Pythium spp. nine days following inoculation with four virulent isolates 15 August 1988.

Bentgrass germplasm	Uninoculated * Control	Disease Reaction (% infected) Isolate #5	Isolate #18	Isolate #24	Isolate #P58
Penncross	+80%	+25%	+25%	+25%	+15% **
		-	+20%	+25%	-
1701	-	+25%	-	+20%	+10%
		-	-	-	-
1709	+20%	-	-	-	-
		-	-	-	-
1710	-	-	-	-	-
		-	-	-	-
1801	-	-	-	-	-
		-	-	-	-
1810	+20%	-	-	-	-
		-	-	-	-
2104	-	-	-	-	-
		-	-	-	-
2111 ***	+20%	-	-	-	-
2205	-	+20%	-	+30%	-
		-	-	-	-
2206	-	-	-	+15%	-
		-	-	-	-
2305	+60%	-	-	+20%	-
		-	-	-	-
2310	+20%	-	-	-	-
		-	-	-	-
2404	-	-	-	-	-
		-	-	-	-
2405	-	-	-	-	-
		-	-	-	-
2410	+10%	-	-	+10%	-
		-	-	-	-
2411	+10%	-	-	+25%	+30%
		-	-	+30%	-
2506 ***	-	-	-	-	-
2508	-	-	-	-	-
		-	-	-	-
2510	+25%	-	-	-	-
		-	-	-	-
2511	+30%	+20%	+20%	+20%	+20%
		-	-	-	-

* Each Bentgrass variety had two replications inoculated with each Pythium strain. Control had one replication.

** + = infected, - = uninfected

*** One sample used for this variety.

Inoculation studies in the laboratory were necessary to distinguish differences in Pythium foliar disease activity among experimental bentgrass lines (Table 4). In this study, five of the experimental lines did not show signs of blight activity and six others were judged to be more tolerant to Pythium blight than the commercial variety 'Penncross'.

II. Rhizoctonia Diseases Ongoing Research

1. USGA Turfgrass Rhizoctonia Isolate Collection:

Turfgrass Rhizoctonia isolates are now being maintained in long term storage in small perscription vials (3 drams) on PDA at a temperature of 20 C. The collection is the larger of the two being maintained for the research program. Some of the Rhizoctonia spp. isolates (Table 5) appear to develop a leathery crust on the surface of the culture which is very difficult to obtain subcultures for use in inoculation studies. For this reason, transfers from the isolate collection will be made at 6 month intervals to insure survival of isolates in the Rhizoctonia collection.

2. Field Observations

A field fertility study with Meyer and Emerald Zoysiagrass was in progress during the summer months at TAES Dallas and this provided an opportunity to see the occurrence of Rhizoctonia brown patch with varying fertility levels. These studies utilized blocks 4 x 4 feet and fertility varying from 0 - 5 pounds/1000

Table 4. Infection progress of elite bentgrass germplasm lines nine days following inoculation with four Pythium spp. isolates under a covered dish on a laboratory bench. August 1988

Percent Diseased Foliage *

	not inoculated		inoculated		inoculated **			
	Rep A		Rep B		Rep C		Mean %	Resistance
Expervar	8-8	15-8	8-8	15-8	8-8	15-8	Disease	Classification ***
Penncross	100	100	100	100	100	100	100	Susceptible
1701	78	85	85	90	80	100	86	Susceptible
1709	18	25	0	0	0	0	7	Tolerant
1710	0	0	0	0	0	0	0	Resistant
1801	5	10	0	40	0	30	14	Susceptible
1810	90	90	0	10	0	0	32	Tolerant
2104	0	0	0	0	0	0	0	Resistant
2111	90	100	0	0	0	0	31	Tolerant
2205	0	0	100	100	100	100	67	Susceptible
2206	5	5	5	30	10	80	22	Susceptible
2305	100	100	0	0	55	55	52	Tolerant
2310	50	100	0	0	60	80	48	Susceptible
2404	0	0	0	0	0	0	0	Resistant
2405	0	60	0	0	0	0	10	Resistant
2410	12	40	4	60	0	0	19	Tolerant
2411	100	100	100	100	85	0	81	Susceptible
2506	0	0	0	0	0	0	0	Resistant
2508	23	50	80	90	0	0	40	Susceptible
2510	100	100	70	30	55	55	68	Susceptible
2511	55	75	2	40	2	0	29	Tolerant

* Inoculated and not inoculated treatments incubated in a covered dish; test plants received daily misting with sterile water for nine days.

** Inoculated with Pythium spp. isolates #5, 18, 24 and P58

*** Resistance classification based on Pythium foliar blighting on inoculated and noninoculated treatment plants after nine days.

Table 5. *Rhizoctonia* spp. and *Rhizoctonia*-like fungal isolates maintained in the USGA *Rhizoctonia* isolate collection TAMU-Dallas.

Isol #	* Pathogen I.D.	** Colony Charact.	Radial Growth Rate on Agar	*** Pathogenicity on Bentgrass
1.	*Rhizoctonia solani*	Buff Brown	0.3	0
2.	*R. solani*	Buff Brn/Fluf	0.3	0.3
3.	*R. solani*	Lt Brn/Fluf	0.5	0
4.	*R. zeae*	Pink/Brown	-	-
5.	Binucleate RLF	Lt Pink	1.1	0
6.	*R. cerealis*	White/Brown	0.1	0
7.	*R. oryzae*	Pink	0	2.5
8.	*R. solani*	Lt Brown	0.4	0
9.	*R. solani*	Dk Brn/Fluf	0.6	0
10.	*R. solani*	Dk Brn/Fluf	0.5	1.5
11.	*R. solani*	Lt. Brown	0.4	1.0
12.	*R. solani*	Lt. Brown	0.2	0
13.	*R. solani*	Dk Brn/Fluf	0.2	1.0
14.	*R. solani*	Dk Brn/Fluf	0.2	0
15.	*R. solani*	Brown Fluf	-	-
16.	*R. solani*	Lt Brn Fluf	0.5	0
17.	*R. solani*	Lt Brn Fluf	0.5	2.5
18.	RLF	White Fluf	1.1	-
19.	RLF	White Fluf	-	-
20.	*R. solani*	Lt. Brown	-	0
21.	*R. solani*	Lt. Brown	0.6	2.0
22.	*R. cerealis*	White/Appr	0.2	2.0
23.	*R. solani*	Lt.Brn/Appr	0.3	0
24.	*R. solani*	Lt.Brn/Fluf	0.5	0
25.	*R. solani*	White/Appr	0.7	0.7
26.	RLF	Dk Brn/Appr	0.3	0.2
27.	RLF	Dk Brn/Appr	0.6	0
28.	RLF	Dk Brn/Appr	-	-
29.	RLF	White/Appr	0.6	2.5
30.	*R. solani*	Lt Brn/Appr	0.5	0
31.	*R. solani*	Dk Brn/Appr	-	0.5
32.	*R. zeae*	Lt Brn/Appr	0.9	0
33.	*R. solani*	Lt Brn/Appr	0.8	0
34.	*R. zeae*	Lt.Pink/Appr	1.0	0
35.	*R. zeae*	Lt Pink/Appr	0.8	1.8
36.	RLF	WhteLtBrn/Apr	0.5	0
37.	RLF	Lt Brn/Appr	-	0
38.	RLF	Lt Brn/Appr	0.4	1.0
39.	*R. solani*	Lt Brn/Appr	0.3	-
40.	RLF	WhiteLtBrn/Fluf	0.3	1.5
41.	RLF	Lt Brn/Appr	0.5	-
42.	RLF	White	0.7	0
43.	*R. zeae*	Lt Brn/Fluf	0.6	3.5
44.	RLF	Lt Brn/Fluf	0.04	0
45.	*R. zeae*	PinkLtBrn/Fluf	0.8	0
46.	*R. solani*	Dk Brn/Appr	0.1	0
47.	RLF	LtBrn-Wht/Appr	-	-
48.	*R. zeae*	White/Appr	0.8	0
49.	RLF	Brn/Fluf	0.8	1.0
50.	*R. zeae*	White/Appr	0.4	2.5
51.	RLF	Brn/Appr	0.6	0
52.	RLF	White	0.6	0
53.	RLF	White/Appr	0.3	0
54.	RLF	Brn/Appr	0.3	0
55.	RLF	White/Appr	0.6	1.5
56.	*R. zeae*	White/Pnk/Appr	0.8	0
57.	*R. zeae*	White/Pnk/Appr	0.7	0
58.	*R. zeae*	White/Pnk/Appr	0.8	0
59.	*R. zeae*	White/Pnk/Appr	0.8	0
60.	*R. zeae*	White/Pnk/Appr	1.0	0
61.	*R. solani*	White/Appr	0.5	3.0
62.	*R. solani*	LtBrn/Appr	0.6	0
63.	*R. solani*	Brown	0.2	0
64.	*R. solani*	DkBrn/Fluf	0.5	2.5
65.	*R. solani*	White/Fluf	0.6	0.3
66.	*R. solani*	White/Fluf	0.5	0
67.	*R. solani*	Brn/Appr	0.6	0
68.	*R. solani*	White/Appr	0.5	3.0
69.	*R. solani*	White/Appr	0.5	0
70.	*R. solani*	LtBrn/Appr	0.6	0

*RLF = *Rhizoctonia*-like fungus

** Br = brown; lt = light; dk = dark; appr = appressed; fluf = fluffy growth habit.

*** Pathogenicity ratings 0-4 whwere 4 = maximum pathogen activity. Isolates maintained on Penncross bentgrass under humidity chambers for 72 hr at 28 C.

(-) = data not determined

feet square.

Observations made during the month of October indicated the occurrence of brown patch was directly related to areas fertilized at a rate of 1.3 kg of N or higher when the turf was maintained at a height of 2.0 cm. Severe brown patch symptoms were observed on Emerald zoysia. The symptoms of the disease were diagnosed by microscopic examination and with serological kits with specific antigens for Rhizoctonia spp. This fertility study will be continued during the next year and allow us to further explore the effects of fertilization on Rhizoctonia disease activity.

A second study for the field evaluation and production of zoysiagrasses was initiated during the winter 1987/88 where plant material was increased in the greenhouse to accommodate a 5.5 sq m area for each cultivar. Observations of brown patch were made in October to study disease activity on transplants. Brown patch disease was noted to be heavier on 'Meyer' in this study (Table 6). Future observations will be made on this block to determine the incidence and severity of disease on the blocks maintained under three fertility levels and one cutting height.

3. **Greenhouse Inoculation Studies with Rhizoctonias on Bentgrass**

On 11 October 1988 two replicate field cores of 53 experimental bentgrass lines were removed from the old field nursery at TAMU-Dallas for pathogen resistance

Table 6. Field ratings of Rhizoctonia brown patch and Dollar Spot Diseases on members of Elite Zoysiagrass Collection planted in cultural study blocks at TAMU-Dallas

Plant ID	Dollar Spot (% Disease on 209 block planting)	Brown Patch (% Disease on 209 block planting)
EMERALD	44	0
TAES3372	0	0
DALZ8516	0	11
BELAIR	0	0
MEYER	0	1
EL TORO	0	0
DALZ8516	0	3
TAES3477	0	0
TAES3477	0	3
DALZ8508	0	0
DALZ8501	0	0
DALZ8516	0	31
DALZ8508	0	0
EL TORO	0	0
DALZ8501	0	0
TAES3372	0	0
DALZ8508	0	0
TAES3477	100	0
DALZ8502	7	0
BELAIR	0	0
EL TORO	0	9
MEYER	0	100
TAES3372	0	0
EMERALD	7	0
DALZ8501	16	0
MEYER	0	20
EMERALD	7	0
DALZ8502	3	0
DALZ8502	2	0
BELAIR	0	0

DATA SUMMARY

VARIETY	DOLLAR SPOT			MEAN %	**	BROWN PATCH			MEAN %	**
DALZ8501	0	0	16	5.3	a	0	0	0	0	b
MEYER	0	0	0	0	a	1	100	20	40.3	a
EMERALD	44	7	7	19.3	a	0	0	0	0	b
DALZ8502	7	3	2	4.0	a	0	0	0	0	b
BELAIR	0	0	0	0	a	0	0	0	0	b
EL TORO	0	0	0	0	a	0	0	9	3	b
TAES3372	0	0	0	0	a	0	0	0	0	b
DALZ8508	0	0	0	0	a	0	0	0	0	b
TAES3477	0	0	100	33.0	a	0	3	0	1	ab

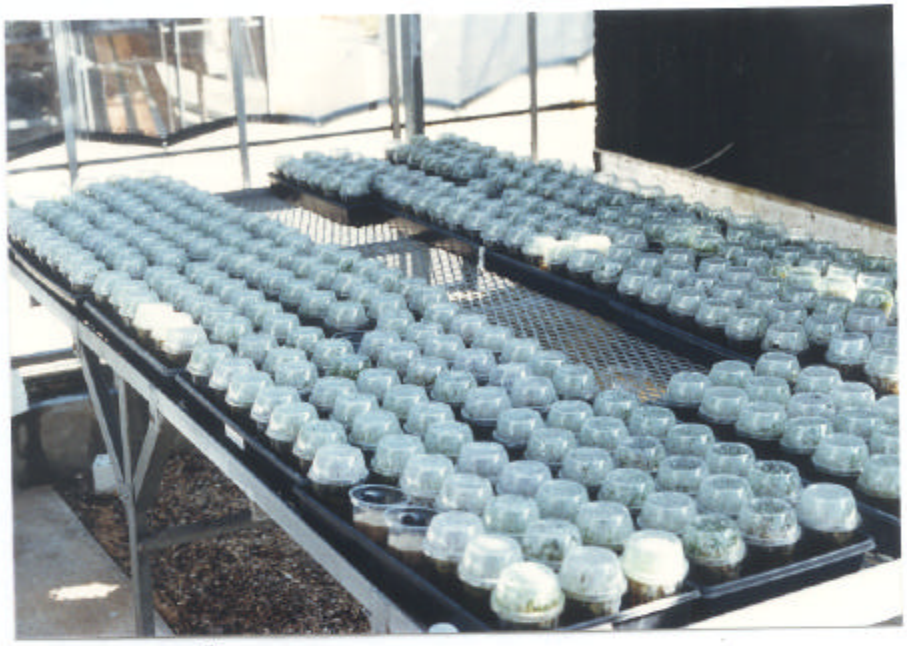
* Percentage disease estimates from number of diseased plugs in a 209 block planting for each germplasm line

** Values followed by the same letter do not differ significantly at p=0.05 according to Waller Duncan's Multiple Range Test

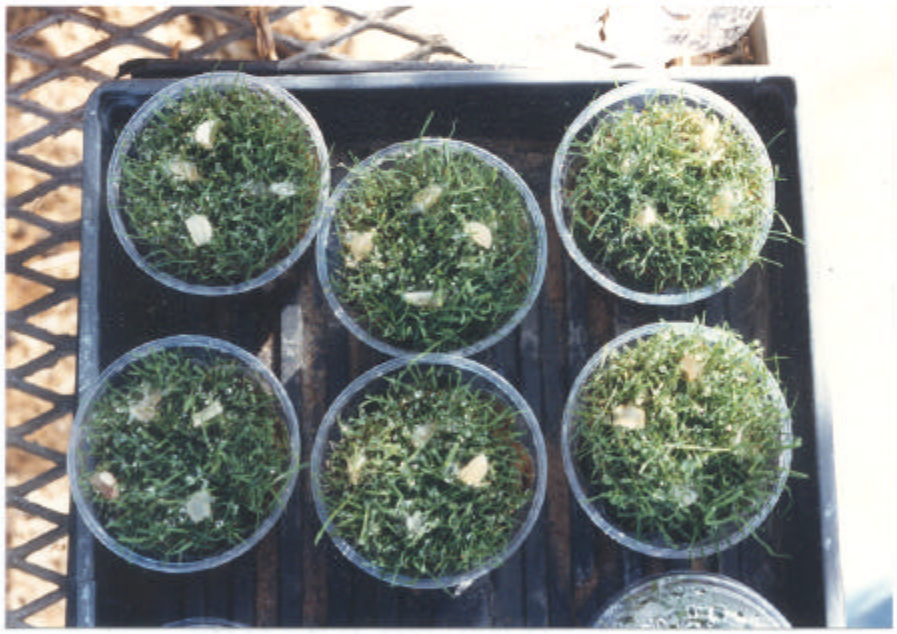
tests. The cores were 8 cm dia and were immediately placed in plastic cups obtained from the McDonalds Corporation (Fig. 2A). Cups containing the plants were placed on a greenhouse bench for 12 days with fertilizer supplimented irrigation water. Rhizoctonia spp. inoculation was performed as previously described for Pythium spp. using isolates 43, 50, 61 and 68 from the U.S.G.A. Rhizoctonia isolate collection. Inoculated leaf blades were misted with sterile water using a hand antomizer and caps were placed on the top of the cups to insure high humidity.

Fungal outgrowth could be observed 24 hours after inoculation of the foliar canopy of the grasses (Fig. 2B). Three days after inoculation the test plants were clipped to a height of 0.5 cm and the clippings were allowed to fall onto the plug. Disease symptoms were obvious on the inoculated turfgrasses 10 days after inoculation and observations of foliar blighting by the test fungi were recorded.

Results of the greenhouse inoculation study with Rhizoctonia spp. were similar to those previously reported for Pythium spp. The disease reaction was rated by the use of a disease index (0 - 5) where 5 resulted in complete death of the test plants. Of the 53 experimental lines tested for Rhizoctonia blight susceptibility almost one half of the population was severely blighted by one or more isolates used for inoculum (Table 7). Disease ratings for susceptible lines appeared to be fairly uniform among



A



B

Fig 2 (A,B,C). A. Greenhouse bench showning plastic cup inoculation chambers for each germplasm line.
B. Rhizoctonia spp. inoculation discs from four virulent isolates on bentgrass germplasm line 12 hrs.

the two replicate cores tested. Only four of the experimental lines were considered to have a disease resistant reaction with both replicate pots showing resistance.

Table 7. *Rhizoctonia* spp. foliar blighting on bentgrass germplasm lines following Inoculation with four virulent turfgrass strains on leaf canopies under covered plastic cups

Cup #	Germplasm #	*Rhizoctonia* * Foliar Blight	Cup#	Germplasm#	*Rhizoctonia* Foliar Blight
103	2768	1, 2	508	1251	3, 3
108	1247	2, 3	602	2886	2, 2
112	1250	3, 3	608	1252	3, 3
114	2895	2, 3	615	1254	3, 3
117	2752	0, 2	618	2739	0, 1
118	2735	1, 2	702	2887	3, 3
200	PENNCROSS	2, 3	708	1253	1, 1
202	2770	1, 2	715	2897	3, 3
203	2767	1, 2	716	2747	3, 3
204	2748	1, 3	717	1252	3, 3
208	1248	2, 3	802	2888	3, 3
210	2758	3, 3	808	1254	3, 3
211	1198	1, 2	814	2558	0, 2
214	1258	3, 3	817	2740	1, -
216	2771	3, 3	818	2563	1, 1
217	1248	3, 3	902	2889	0, 1
218	1499	0, 1	908	1255	1, 1
303	2766	2, 2	910	1247	2, 3
304	2749	1, 3	917	2770	1, 3
305	2741	3, 3	918	2768	1, 1
310	2761	1, 1	1017	2888	1, 3
312	2764	3, 3	1105	2734	1, 3
406	2380	0, 2	1110	1199	1, 3
408	1250	3, 3	1111	2737	0, 2
416	2767	1, 3	1115	1255	3, 3
418	2741	0, 0	1116	2886	0, 3
503	2764	3, 3			

* *Rhizoctonia* foliar blight disease index (0-5, where 5 = complete death) Disease assessments of two replicate 8 cm dia pots inoculated with four virulent isolates of *Rhizoctonia* spp. attacking turfgrasses after a 7-day period.