CURRENT RESEARCH:

HPIS variants maintained in ECG have recently been repotted into an improved, neutral pH fritted clay growth medium. Repotting involves removing all root and shoot growth, transplanting only stolons and crowns. Transplanting insures vigorous root - shoot growth and decreases ubiquitous greenhouse pathogens on turf.

Screening variants for enhanced resistance to *R. solani* is an ongoing process. Similar experimental procedures will be followed as previously described.

Laboratory work initiating and maintaining callus for future HPIS screening is ongoing along with recovering bentgrass germplasm from resistant calli. Selected plantlets are vegetatively increased and maintained in tissue culture boxes prior to whole plant disease screening.

FIELD EVALUATION:

Selected enhanced resistant bentgrass variants will be planted in an experimental putting green located at Old Waverly Golf Course, West Point, MS. This affords a unique situation for creeping bentgrass research at Mississippi State University because a professional golf course superintendent with bentgrass management experience will be supervising the management of the green. Anticipated completion of the experimental green is November 1994.

Variants will be evaluated *in situ* primarily for disease resistance and overall turf performance. Variants will be planted in the experimental green as enhanced lines are identified.

Clones of selected lines will be included in a clonal repository at Pure Seed Testing, Danby, Oregon to maintain the integrity of the plants and evaluate for seed production characteristics.

SUMMARY:

A great deal of knowledge and experience has been gained in creeping bentgrass pathology as this research has been focused on developing a valid,

1994 Research Progress Report
Executive Summary

Recovery of *Rhizoctonia solani* Resistant Creeping Bentgrass Germplasm using the Host-Pathogen Interaction System

Maria Tomaso-Peterson and Dr. Jeffrey Krans
Mississippi State University

Research efforts in 1994 have been focused on developing a valid, quantifiable procedure at the whole plant level to verify resistance to *Rhizoctonia solani* exhibited by HPIS derived creeping bentgrass variants. Several studies were conducted to address inoculation techniques, optimum environmental factors for disease, and evaluating pathogenicity of *R. solani* isolates.

Four creeping bentgrass lines have been identified with enhanced resistance to *R. solani.* This study was conducted using HPIS derived bentgrass variants maintained in pots (7 cm dia) in an environment controlled greenhouse. Under ideal environmental disease conditions for brown patch, HPIS variants were inoculated with a highly pathogenic isolate of *R. solani*. Average diameter of diseased turf (mm) for lines displaying enhanced resistance were 21.6, 25, 27.5, and 28.3. These values represent a significant improvement compared to Penncross with an average 50.0 mm of diseased turf.

This phase of research has shown that the Host-Pathogen Interaction System is a valid *in vitro* cell selection technique for selecting creeping bentgrass germplasm with enhanced resistance to *Rhizoctonia solani*. Prior to field evaluations, selected creeping bentgrass lines have undergone three levels of exposure to *R. solani.* A cycle for selecting germplasm with enhanced resistant has been accomplished beginning at the cellular level, affirmed at the plantlet level (*in vitro*), and confirmed at the whole plant level.

Selected lines will be evaluated in the field under golf course conditions. As additional resistant lines are obtained they too will be included in field evaluations. Continued progress in this research project will lead to parental lines of creeping bentgrass that exhibit enhanced resistance to *Rhizoctonia solani*, the causal organism of brown patch.

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Recovery of *Rhizoctonia solani* Resistant Creeping Bentgrass Germplasm using the Host-Pathogen Interaction System

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The second research grant year for "Recovery of *Rhizoctonia solani* resistant creeping bentgrass germplasm using the Host-Pathogen Interaction System" has focused on developing a valid, quantifiable procedure at the whole plant level for verification of resistance to *R. solani* exhibited by HPIS derived creeping bentgrass variants. To accomplish this goal, three important factors must be addressed. First, define an inoculum technique. Rye grain, hyphal plugs of actively growing *R. solani*, and sclerotia are common sources of inocula for promoting *R. solani* infection. An effective inoculum from a highly pathogenic isolate must be identified to assure valid results in this research. Second, define optimum conditions for promoting infection. Warm temperatures coupled with high humidity have been defined for *R. solani*, but other factors such as light, infection period, and nutritional values of the turf must be considered. Third, retain surviving variants. A valid disease-screening procedure must result in only enhanced resistant variants surviving. The ultimate goal is to retain those few variants that come through disease-screening studies with minor disease symptoms.

HPIS variants are grown in fritted clay, pH 6.5. Maximum radial turf is 7 cm per pot. Variants are maintained in an environment controlled greenhouse (ECG). Turf is trimmed twice a week to maintain a short, dense, growth habit. HPIS variants are replicated up to 24 times for adequate replication in disease screening studies.

Six preliminary studies have been conducted to address these factors mentioned above and determine the best avenue for research at Mississippi State University.

RYE GRAIN INOCULUM STUDY:

This study was conducted to determine the effectiveness of *R. solani* - infected rye grain as an inoculum source on HPIS variants.

Five MT variants originating from HPIS and HPIS chamber selection studies were replicated 24 times. They were transplanted to pots containing fritted clay. When the plants matured and radial growth equaled 7 cm dia., they were ready for inoculation. This study was repeated 3 times along with 1 uninoculated control group.

A tent-style mist chamber (enclosed with clear plastic) was set up using a humidifier to create a humid environment.

The day/night temperatures were maintained between 82-86°F or 28-30°C.

Rye grain infected with *Rhizoctonia solani* (isolate RVPI) was used as the source of inoculum. It was prepared according to Gugel et al. 1987. The particle size was 2 mm and 22 mg of inoculum was applied to the center of each rep.

RESULTS:

Mycelium production was evident after 48 h in 100% humidity. However, it was not profuse and dried while being maintained under periodic misting. There were no obvious symptoms of *R. solani* infection five days following inoculation. Based on the results from the second and third inoculated run, the fourth inoculated run was maintained for 72 h in the mist tent. The extended time enhanced mycelial growth, but again it dried upon removal from the mist. MT variants displayed signs of stress from the saturated conditions of continual misting. There were no distinct areas of diseased turf among the inoculated variants. However, lesions were scattered throughout some of the reps. Lesions were collected and identified as *R. solani*.

CONCLUSIONS:

Germination of *R. solani* from the rye grain was slow and ineffective for complete infection of the turf. Variants became water-logged following 48 h of 100% humidity. Secondary infections by other pathogens such as *Curvularia spp.* and *Bipolaris sorokiniana* occurred because of the high background levels

of those fungi and the prolonged growth under hot, humid conditions. Inadequate soil fertility (pH < 5) among variants contributed to the lack of infection by *R. solani* but promoted infection of *Curvularia sp.*

HYPHAL PLUG - ISOLATE RVPI INOCULATION STUDY:

Hyphal plugs were used as the inoculum source to determine if an actively growing culture of *R. solani* could initiate more effective infection than other inoculum sources.

Original MT variants, micropropagated in 1993, were employed in this study. This included 25 MT variants recovered from the HPIS and one Penncross. They were replicated 12 times.

Variants were fertilized weekly with 20-20-20 all purpose fertilizer to enhance soil fertility and plant vigor.

The inoculum was applied to the center of each variant as a 3 mm hyphal plug of *R. solani* (isolate RVPI) grown 10 days on potato dextrose agar.

Day/night temperatures ranged from 28 - 31C.

Four successive inoculation runs were conducted with the first being an uninoculated control group. There were three replicates of each variant per run. This was carried out in the ECG with all environmental conditions being the same as the rye grain inoculum study. However, the level of humidity was decreased from 100% to 90% in the mist tent for the third and fourth run due to super-saturated conditions experienced by variants in prior runs.

RESULTS:

Uninoculated control and the first inoculated groups of variants suffered water-logged conditions in the mist tent. Mycelial growth was profuse (1 - 2 cm radial growth) for all inoculated variants and remained moist under periodic misting on the greenhouse bench.

CONCLUSIONS:

Hyphal plug inoculum initiated mycelial growth within 24 h and seemed to be effective for inciting disease on some of the variants. Over-watered conditions created in the mist tent stressed control and inoculated variants. Initially the leaves were dark bluish-gray then turned yellow. Slight infection was noticeable in the third and fourth successive inoculated runs. A few variants in the fourth run displayed symptoms of disease. Excessive days of hot, humid conditions increased pathogenic activity of secondary pathogens ubiquitous in a greenhouse environment. This, coupled with prolonged heat stress (14 days above 28C), created a difficult situation for quantifying diseased turf caused by a specific pathogen.

R. solani isolate (RVPI) was found to be slightly pathogenic (23 out of 100%) based on pathogenicity ratings conducted by Dr. Phil Colbaugh in 1993.

Increasing the soil fertility of variants stimulated some *R. solani* infection.

HYPHAL PLUG - USGA R31 INOCULATION STUDY:

R. solani isolate R31 was employed in this study based on its pathogenicity to creeping bentgrass. R31, the most pathogenic isolate in the USGA *Rhizoctonia* collection, was supplied by Dr. Phil Colbaugh.

Inoculation-incubation was conducted in a growth chamber.

Temperatures were maintained at 28C, with a 12h day/night.

MT variants were replicated three times with one uninoculated control included in the experiment.

Hyphal plugs (3 mm dia) of R31 growing on potato dextrose agar 7 days was the inoculum. One plug/rep was placed in the center of the turf and then covered with clear plastic cups to maintain high humidity.

Variants were trimmed prior to inoculation and misted with 2% V8 solution. R31 inoculum (1 plug/rep) was placed in the center of the turf. The variants were covered with clear plastic cups or pouches.

susceptible. A susceptible check was not included; therefore, the level of susceptibility/resistance could not be determined. Eighty percent of the inoculum remained viable following inoculation.

HYPHAL PLUG R31 - 32/26C TEMPERATURE STUDY:

Growth chamber temperatures were increased during a 16 h day (32C) and lowered at night (26C) to determine if the increase in daytime temperatures stimulates pathogenicity of R31. According to Dr. L. Burpee, University of Georgia and Dr. H. Wilkinson, University of Illinois, (personal communication) *R. solani* usually requires temperatures of 32 - 33C to aggressively infect creeping bentgrass.

MT variants were inoculated with 3 mm hyphal plugs of 3-day-old R31. Two percent V8 mist was applied to the turf and then was covered.

RESULTS:

Twenty-four hours following inoculation R31 plugs were browned and dried. No disease occurred on inoculated MT variants.

CONCLUSIONS:

Heat build up within the canopy of MT variants inhibited mycelial growth of R31.

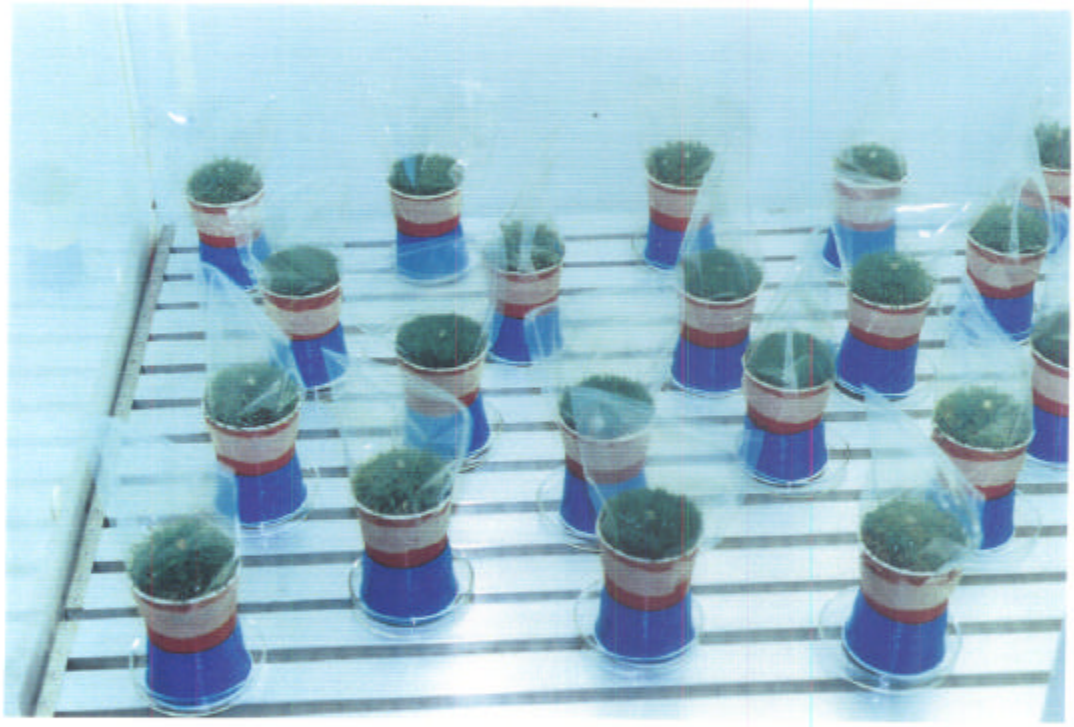
R. SOLANI ISOLATE PATHOGENICITY STUDY:

Eight isolates *of R. solani* were evaluated to determine their level of pathogenicity. Penncross and HPIS variant MT 329 were employed as host material.

R. solani isolates MO-90-45B, MO-90-137B, MO-90-22B, MO-90-58B, and MO-90-75A were donated from Dr. D. A. Sleper's collection at Univ. of Missouri - Columbia. Isolates NCCB-11 and NCTF-4 were isolated from brown patch-infected creeping bentgrass and tall fescue, respectively, originating from diseased turf collected at Pure Seed Testing, Inc., Rolesville,

North Carolina. Isolate R31 from the USGA *Rhizoctonia solani* collection, was also included.

One day prior to inoculation, the host material was trimmed and fertilized with 20-20-20. Turf was misted with 2% V-8 solution and inoculated with 3mm hyphal plugs from 3 day old PDA cultures of the eight *R. solani* isolates. Plastic bags were sealed over the turf to maintain humidity. Inoculated reps and uninoculated controls incubated 4 days in a growth chamber. The temperature was 30 C, 12 h days, relative humidity > 98%, light intensity < 72 $\mu Em^{-2}s^{-1}$.

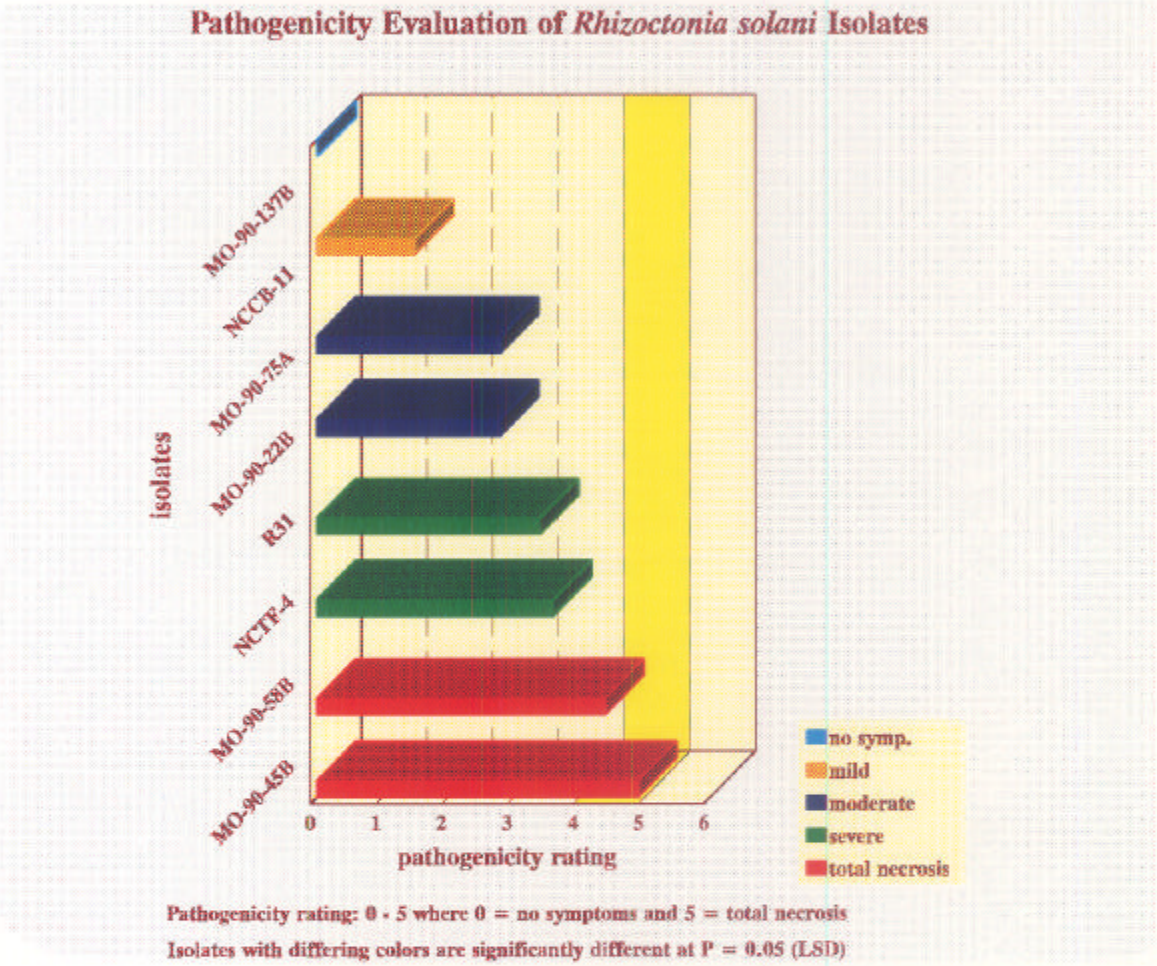


Inoculated HPIS variants incubating in the growth chamber.

RESULTS:

Penncross and MT 329 were evaluated for disease symptomology based on a visual rating where 0 = no symptoms and 5 total necrosis of turf.

Isolates MO-90-45B and MO-90-58B killed the majority of turf. NCTF-4 and R31 were severely pathogenic while other isolates were not as pathogenic.



CONCLUSIONS:

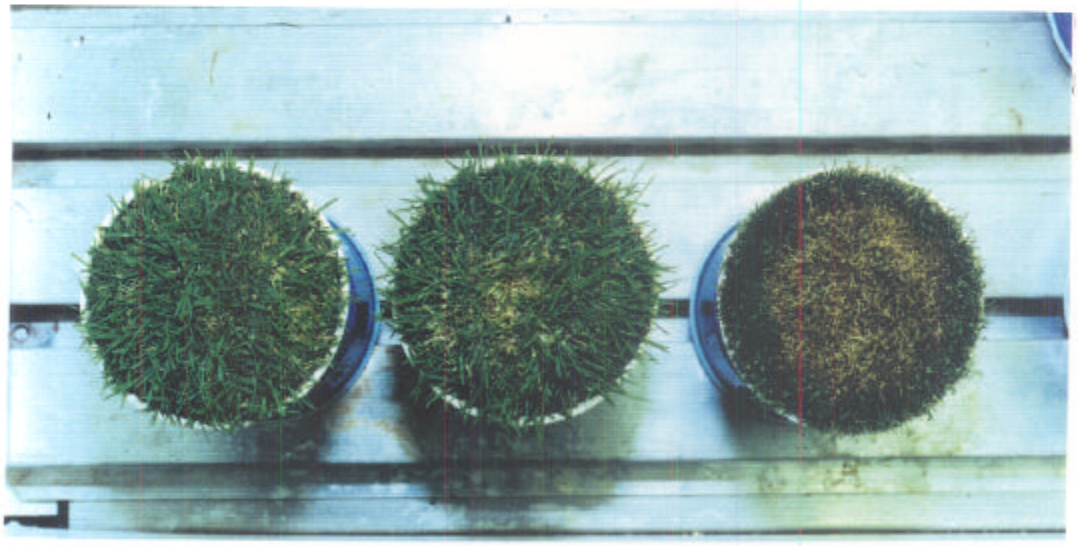
R. solani isolates MO-90-45B and MO-90-58B were highly pathogenic on Penncross and MT 329. Future disease-screening studies will employ these pathogenic isolates.

HPIS VARIANT SELECTION STUDY:

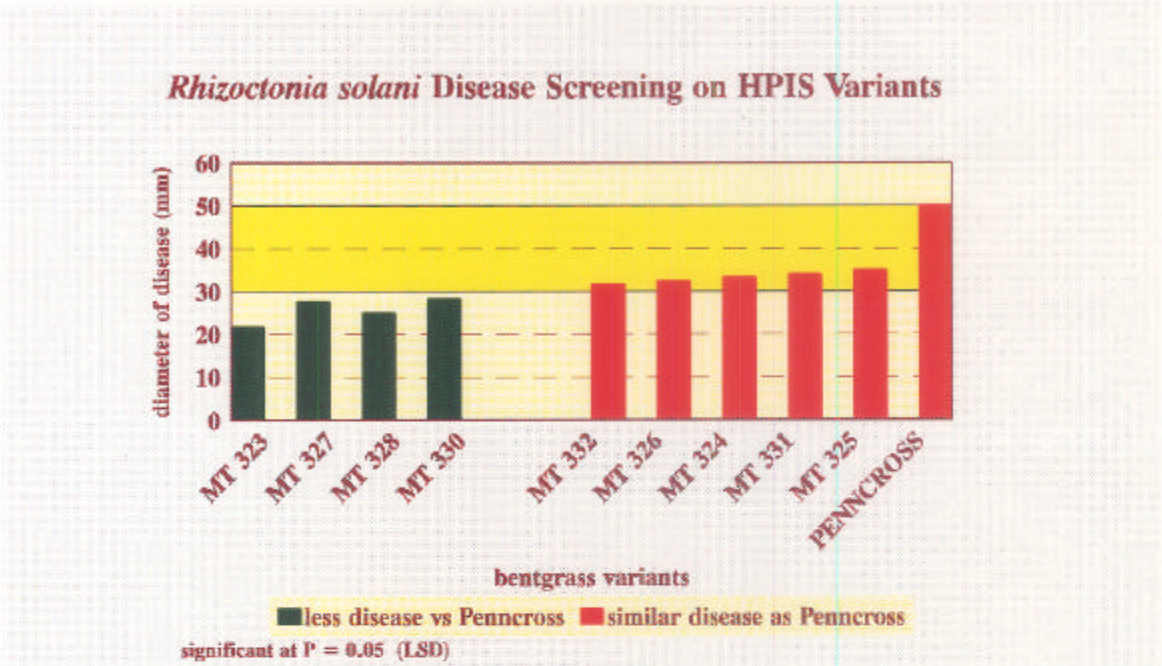
Nine HPIS variants were screened for enhanced resistance to *R. solani*. Variants were replicated eight times including six inoculated and two uninoculated reps / variant. Penncross was the susceptible check. Hyphal plugs from young cultures of isolate MO-90-45B were the inoculum source. Plastic bags covered the turf to maintain humidity throughout the incubation period. Host-pathogen interaction was carried out in a growth chamber under similar environmental conditions previously described in the pathogenicity study. Following 42 h of incubation, variants were transferred to an environment optimum for creeping bentgrass. Disease progress was halted by decreasing temperature and humidity and increasing light intensity. Precise measurements of Disease severity among inoculated variants was determined by measuring the diameter of necrotic grass in respective replicates.

RESULTS:

The diameter (mm) of disease symptoms within inoculated turf was determined at 24 and 96 h following incubation for each variant. Four of the nine HPIS variants displayed less disease symptomology than Penncross. MT 328 recovered the quickest in terms of healthy vegetative growth.



Inoculated MT 323 (center) displayed 21.6mm diseased turf vs Penncross (right) with 50.0mm. Uninoculated control MT 323 on the left.



CONCLUSIONS:

MT variants 323, 327, 328, and 330 displayed significantly less disease symptomology than Penncross. These variants are the first *R. solani* enhanced resistant variants verified at the whole plant level. They have been exposed to *R. solani* at 3 physiological levels; cellular, plantlet, and whole plant, and have exhibited resistance at each level.

The criteria for successful enhanced disease screening was met in this study. A highly pathogenic *R. solani* isolate along with a vigorous inoculum source was employed. Prior to inoculation, bentgrass variants were exposed to above optimum temperatures. Environmental conditions favorable for brown patch were met during the incubation period. Disease symptoms among replicated variants was uniform and quantifiable.

RESULTS:

Infection did not occur on the inoculated variants. The hyphal plugs dried up and R31 was inactive following 24 h in the growth chamber.

CONCLUSIONS:

Potato dextrose agar cultures of R31 were too old to promote high levels of infection. According to Metz and Colbaugh (personal communication) 3-day-old cultures of *R. solani* initiate infection within creeping bentgrass.

HYPHAL PLUG - ISOLATE R31 INOCULATION STUDY:

Isolate R31 was cultured 3 days on PDA. Hyphal plugs (3 mm dia) were used as the inoculum.

MT variants were trimmed prior to inoculation then misted with 2% V8 solution and covered with plastic cups. They were maintained in the growth chamber at 28C, 12 h day/night for 5 days.

RESULTS:

Profuse mycelial growth was visible on MT variants within 24 h of inoculation. Disease symptoms were also evident. As mycelium invaded the turf canopy, individual leaves turned bluish-gray then eventually became necrotic. Lesions were present on some of the infected leaves throughout all variants. The area of radial hyphal growth and leaf necrosis was similar for most of the inoculated variants. The area of disease averaged 14 mm diameter from the point of inoculation. One variant (MT302) suffered 20 - 22 mm diseased turf.

CONCLUSIONS:

Inoculation with 3-day-old hyphal plugs of R31 seems to be the most successful inoculation/screening procedure thus far. According to Metz and Colbaugh, R31 causes major disease on some creeping bentgrass varieties included in the National Turfgrass Evaluation Program.

Disease was visible on MT variants with actively growing R31. However, the area of disease was not as extensive as expected if the variants were

quantifiable procedure for screening HPIS variants at the whole plant level for enhanced resistance to *R. solani*. HPIS variants are successfully being identified and screened for enhanced resistance to *R. solani* at the whole plant level. The selection cycle is now complete with regard to cellular, plantlet, and whole plant selections. This phase of research has positively verified HPIS as an *in vitro* cell selection technique for recovering enhanced resistant germplasm. A manuscript describing HPIS selection technique and its validation through selected enhanced resistant creeping bentgrass germplasm can be published.

Research objectives outlined in "Recovery of *Rhizoctonia solani* resistant creeping bentgrass germplasm using HPIS" are being achieved in a promising manner. Creeping bentgrass germplasm has been successfully recovered with enhanced resistance to *R. solani* via our HPIS technique. Germplasm has been screened at the cellular, plantlet and whole plant levels to verify resistance. A clonal repository has been established with enhanced lines being added as they are selected. Selected germplasm will be evaluated under golf course conditions at Mississippi State University and Pure Seed Testing, Rolesville, NC.

Continuation of this research will ultimately lead to parental lines of creeping bentgrass that exhibit enhanced resistance to *Rhizoctonia solani*, causal organism of brown patch.