RECOVERY OF *RHIZOCTONIA SOLANI* RESISTANT CREEPING BENTGRASS GERMSPLASM USING THE HOST-PATHOGEN INTERACTION SYSTEM

FINAL RESEARCH PROGRESS REPORT 1995
EXECUTIVE SUMMARY

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The Host-Pathogen Interaction System (HPIS) is an *in vitro* cell selection system developed in conjunction with efforts to obtain creeping bentgrass with resistance to *Rhizoctonia solani*. The primary objective throughout our USGA research projects has been to verify HPIS as a valid *in vitro* cell selection system. With this objective achieved, we can recover bentgrass germplasm from HPIS selections and evaluate those genotypes in the field with confidence that some will segregate from the population, exhibiting enhanced disease resistance.

The first step in achieving our primary objective was to obtain disease resistant bentgrass callus via HPIS selection. HPIS refinement studies associated with our initial USGA research project confirmed selection of resistant callus. Callus mortality increased significantly as 'Penncross' calli co-cultured in HPIS with a virulent isolate of *R. solani*. Small numbers of plantlets were regenerated from resistant callus compared to high numbers of plantlets recovered from control populations. With bentgrass germplasm successfully regenerated from resistant calli, our USGA research project progressed from there to determine whether enhanced resistance could be exhibited at plantlet and whole plant levels.

Plantlets were evaluated for tolerance to *R. solani* using two *in vitro* screening techniques: 1.) HPIS Chamber - Plantlets were placed in an HPIS Chamber, exposing them to *R. solani* for two weeks. Over 33% of the plantlets did not survive. 2.) Leaf Bioassy - Leaves of plantlets were exposed to exudate produced by *R. solani*. Plantlets recovered from resistant callus displayed significantly less leaf necrosis as compared to control plantlets. Plantlets surviving both screening techniques were subsequently transferred to soil (whole plant) and maintained in a greenhouse.

Selected bentgrass plants [variant (s)] were inoculated with *R. solani* using growth chamber techniques and evaluated for disease response. Preliminary results indicated some variants expressed enhanced resistance to *R. solani*. Based on these findings, variants were established in the field under putting green conditions. Establishment and turf quality data indicate the majority of variants are similar to or better than Penncross. Concurrent with establishment, variants were rated for brown patch resistance based on natural infestation. Preliminary observations indicate enhanced resistance may exist among some variants. Several plots displayed brown patch symptoms while adjacent plots had no symptomology. Bentgrass variants will be evaluated under various stress and environmental conditions through two successive brown patch seasons (2 yr). Natural infestation and field inoculations will occur under natural putting green conditions. Results from these evaluations will provide us the opportunity to confidently identify variants exhibiting enhanced resistance to brown patch. Selected variants will be used to improve existing creeping bentgrass gene pools by incorportating genes with enhanced *R. solani* resistance.

A clonal repository of HPIS germplasm is maintained in an environment controlled greenhouse at Mississippi State University. To date, almost 200 genotypes are established under putting green conditions at locations in Mississippi and North Carolina. HPIS refinements studies have confirmed HPIS as a valid *in vitro* cell selection tool, giving us confidence that some bentgrass variants will be identified as having enhanced resistance brown patch.

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'Penncross' creeping bentgrass, (*Agrostis stoloniferous var. palustris* Huds.) is the host plant source throughout the studies. Penncross callus was obtained by induction procedures described by Krans et al. (1982). *Rhizoctonia solani* Kühn AG 1-IA) is the turfgrass pathogen. Isolates of *R. solani* are maintained in the dark at 25° C on potato dextrose agar (PDA) slants.

Plant tissue culture systems have been successfully employed as *in vitro* cell selection tools for recovering disease resistant plants (Ingram and McDonald, 1986). Phytotoxins, crude and purified culture filtrates, and propagules have been used as selection agents within cell selection systems (Pauly et al., 1987; Wilmot et al., 1989; Jang and Tainter, 1990).

Host-Pathogen Interaction System (HPIS) offers a unique approach to *in vitro* cell selection (Tomaso-Peterson and Krans, 1990). Most commonly, fungal selection agents are isolated, purified, and incorporated into tissue culture media to interact with a host plant. Within HPIS, actively growing *R. solani* naturally produces exudates, interacting with Penncross calli in a non-invasive manner. HPIS simulates naturally occurring host-pathogen interactions *in vitro*.

Callahan and Rowe (1991) demonstrated that while a fungus is actively growing in HPIS, exudates are produced and diffuse throughout the media. Interactions occur between fungal exudates and the host plant. Soussi and Kremer (1994) found HPIS to be a rapid, effective bioassay technique for screening large populations of microorganisms for their use as biological controls, delivering results similar to observations at the whole plant level.

This report provides an overview of HPIS research conducted in association with USGA funded Alternative Pest Management research projects. Highlighted are development and applications of HPIS for selecting creeping bentgrass germplasm with enhanced disease resistance.
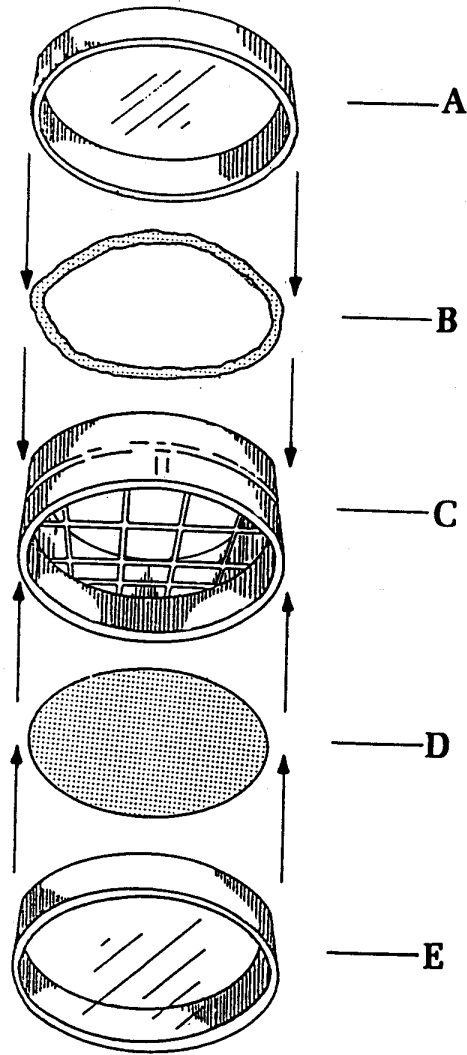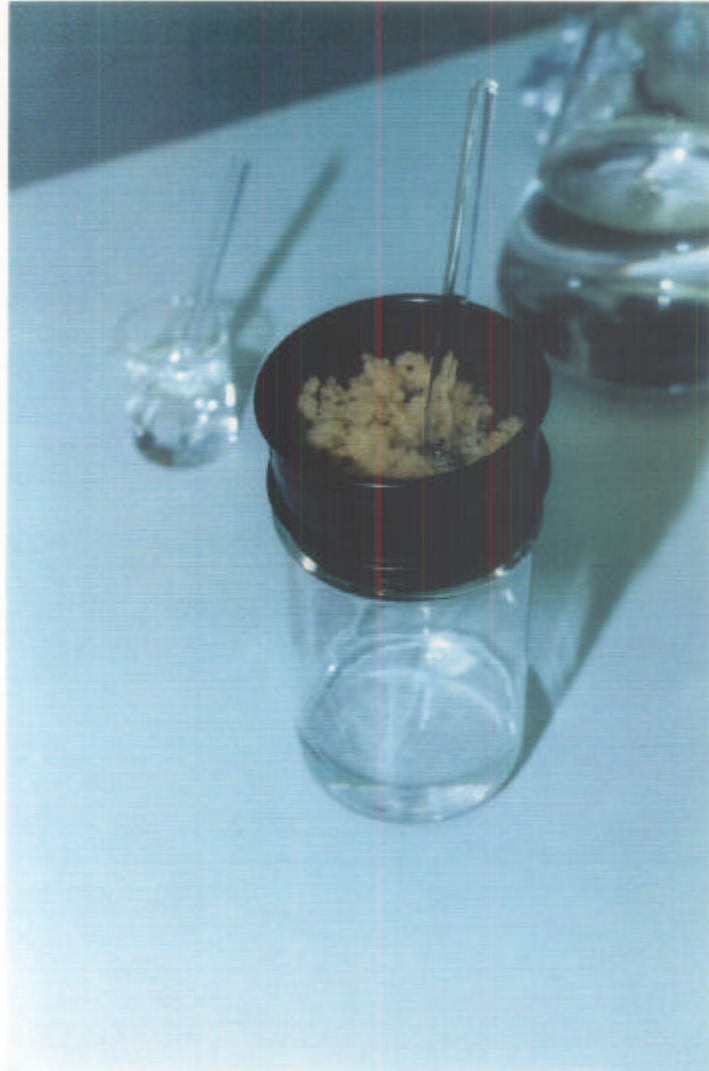
Components of HPIS: (A) Lid of fungal compartment; (B) Calking cord; (C) Main body of Lutri-Plate; (D) 0.2uM membrane; (E) Lid of tissue culture compartment.
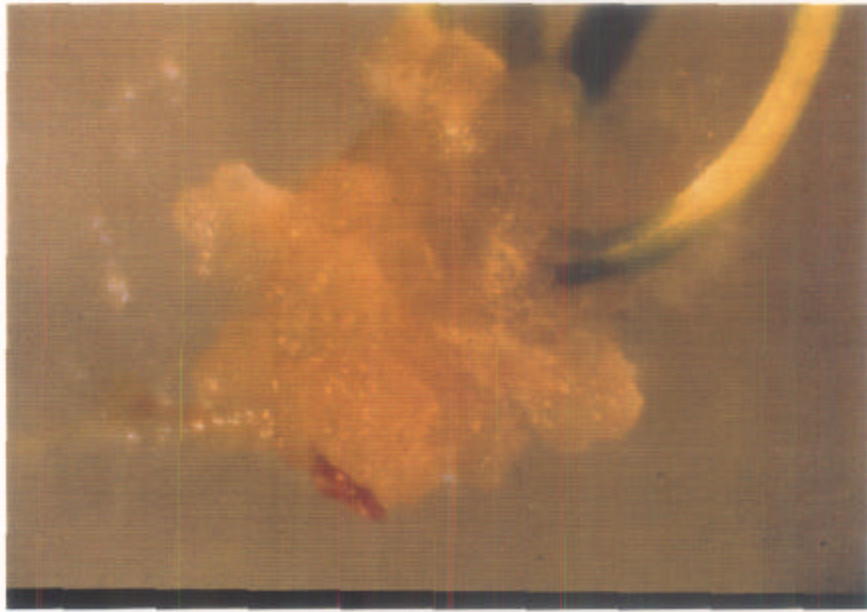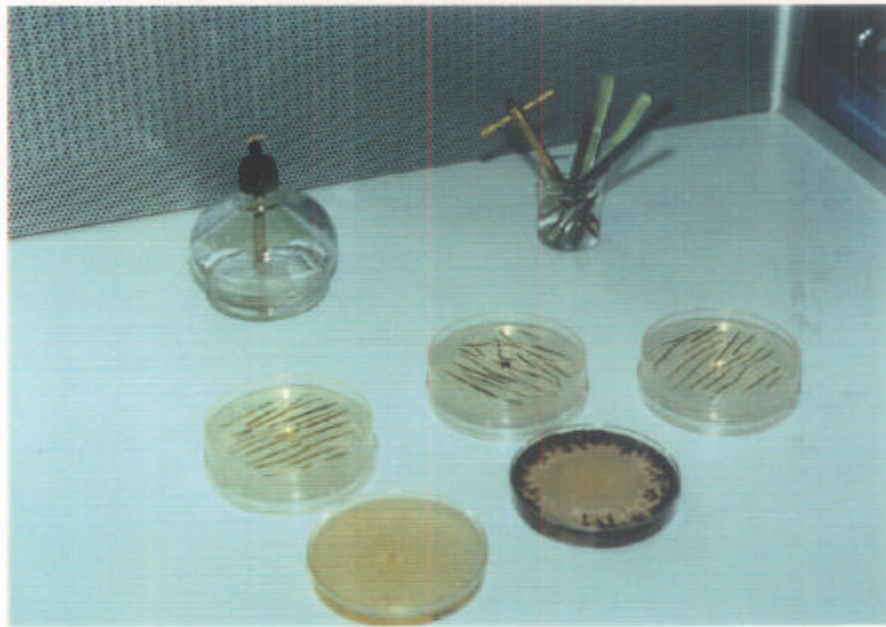
Stock callus is induced from mature Penncross caryopsis.

Aggregates of Penncross stock callus are reduced to 1 mm using a series of seives, they are washed, collected in liquid MS, plated (300 mg) onto glass fiber support discs, then transferred to 3MS within HPIS.

Preparation of HPIS fungal compartment: Water agar is poured into the fungal compartment, sterile bentgrass leaves are placed on the water agar as a natural substrate for R. solani, and HPIS is inoculated with hyphal plugs of R. solani.
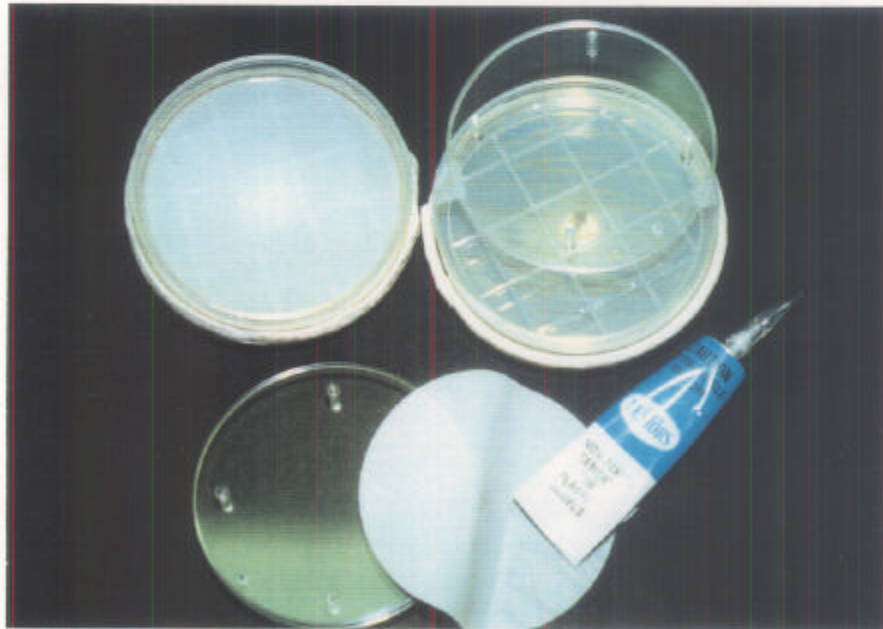

Preparation of HPIS tissue culture compartment: A false bottom is removed from under the water agar and a 0.2uM membrane is sealed into place. The membrane serves as a physical barrier, preventing the fungus from invading the tissue culture compartment while allowing enzymes, metabolites and other toxic compounds to move throughout the media. Tissue culture medium, 3MS, is poured in HPIS after the membrane is in place.
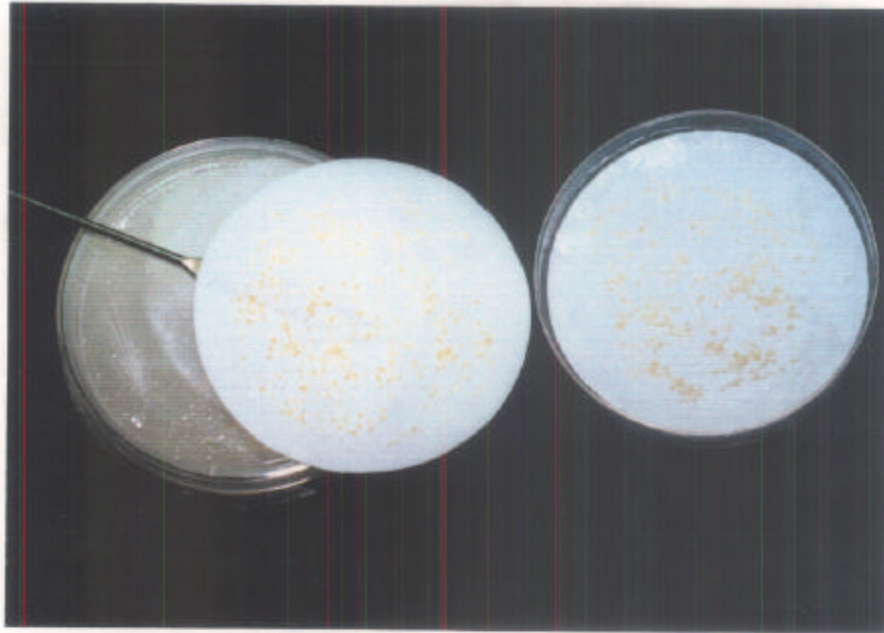
Following callus / R. solani co-culture in HPIS, callus populations are evaluated for callus mortality or transferred via glass fiber support disc to regeneration medium for plantlet recovery.

58%
CALLUS MORTALITY

0.6%
CALLUS MORTALITY
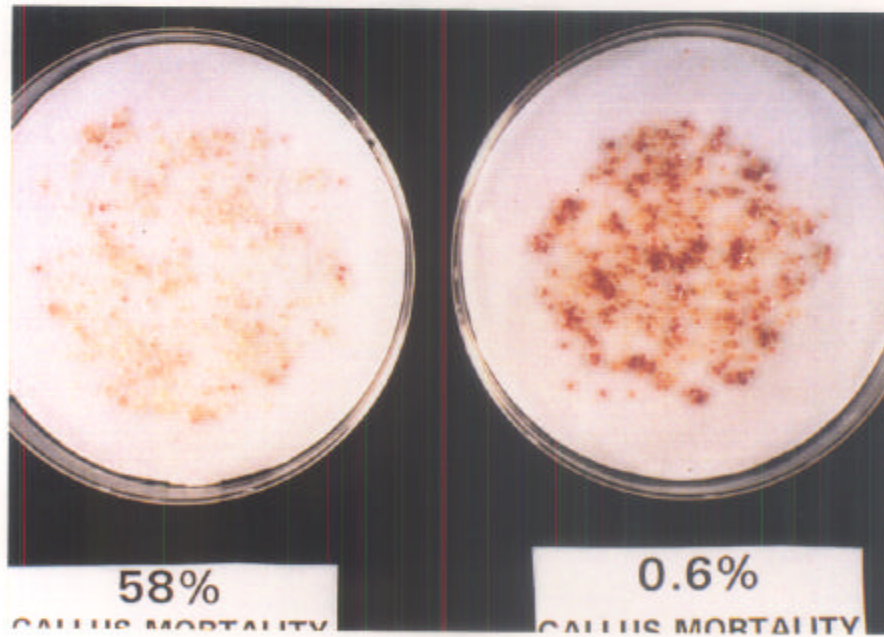
Callus mortality is determined using a 1% triphenyltetrazolium chloride stain. Presence of red stain indicates callus viability. Evaluation of callus mortality verifies HPIS as an in vitro cell selection system.
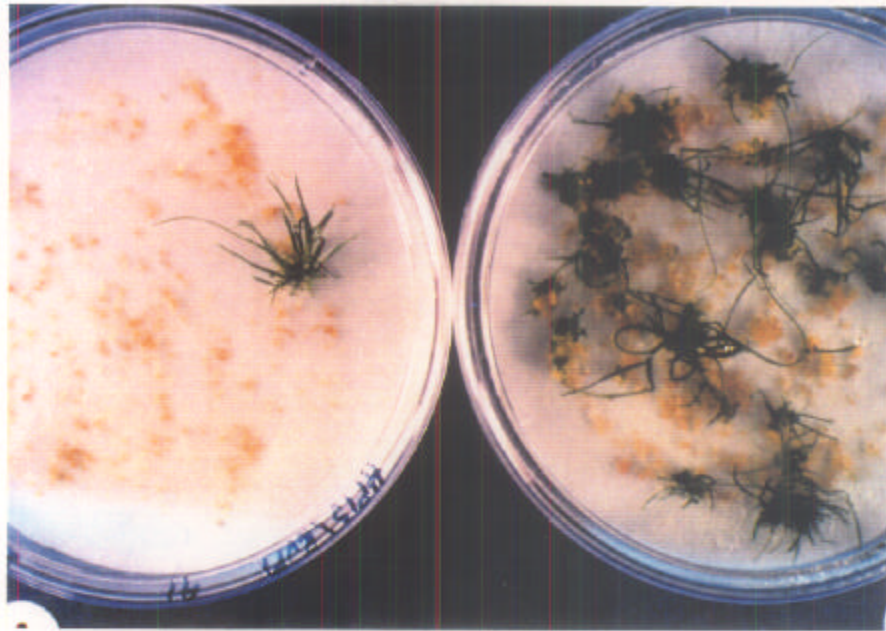
Callus is cultured on regeneration medium under continuous low light to induce plantlet regeneration. Bentgrass germplasm is recovered from callus (A) that survived co-culture with R. solani in HPIS. Over 60 plantlets regenerated from control callus (B).
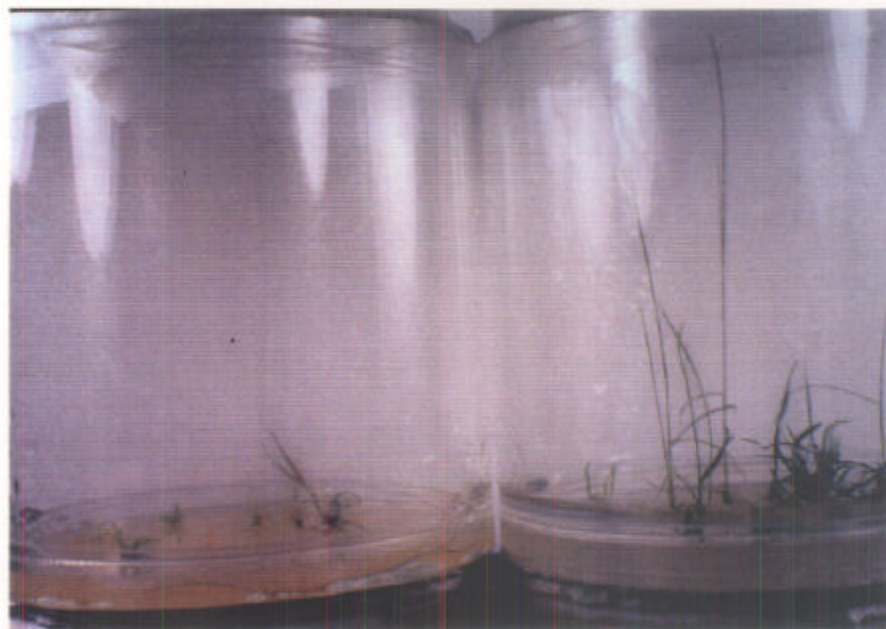

Bentgrass germplasm recovered from resistant callus co-cultured with R. solani in the HPIS chamber (left). Over 66% of the screened germplasm experienced a decline in plant vigor, but survived. Control plantlets thrived (right).
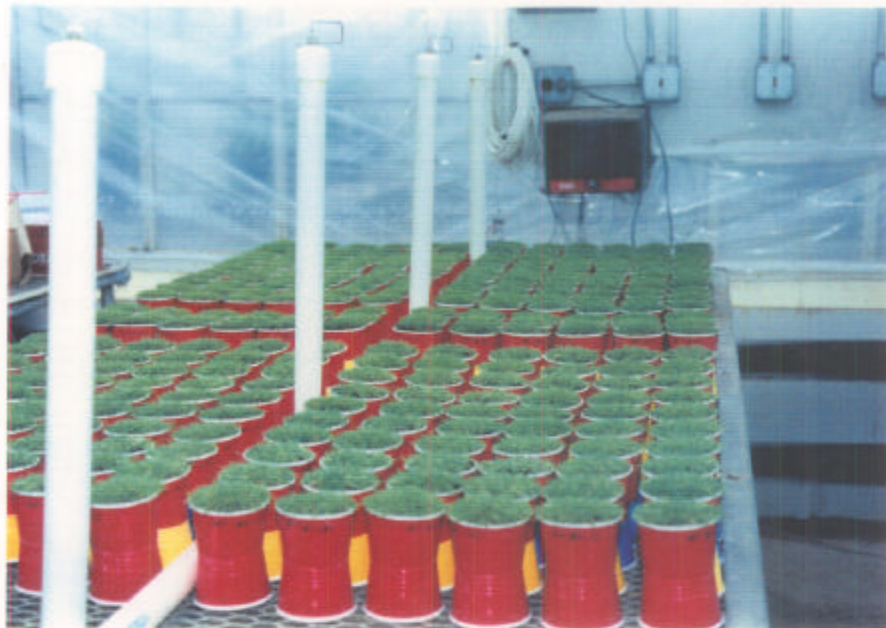
Bentgrass germplasm (variants) recovered from HPIS chamber selections are transferred to cups containing fritted clay. Variants are maintained in an environment controlled greenhouse (ECG) where temperatures average 22 C.
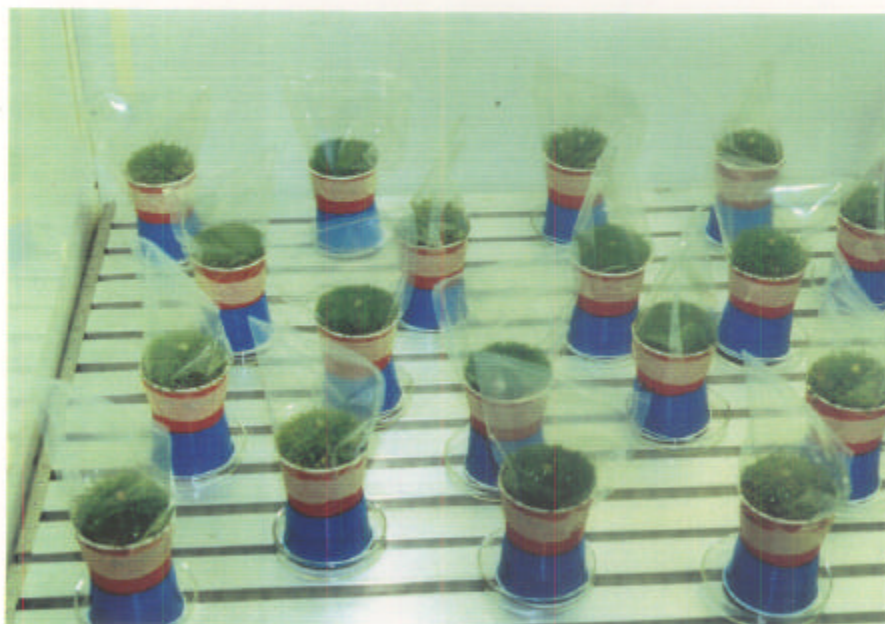
Bentgrass variants are inoculated with R. solani and incubated in a growth chamber 41 h. Variants are rated on disease response by measuring the diameter of diseased turf.
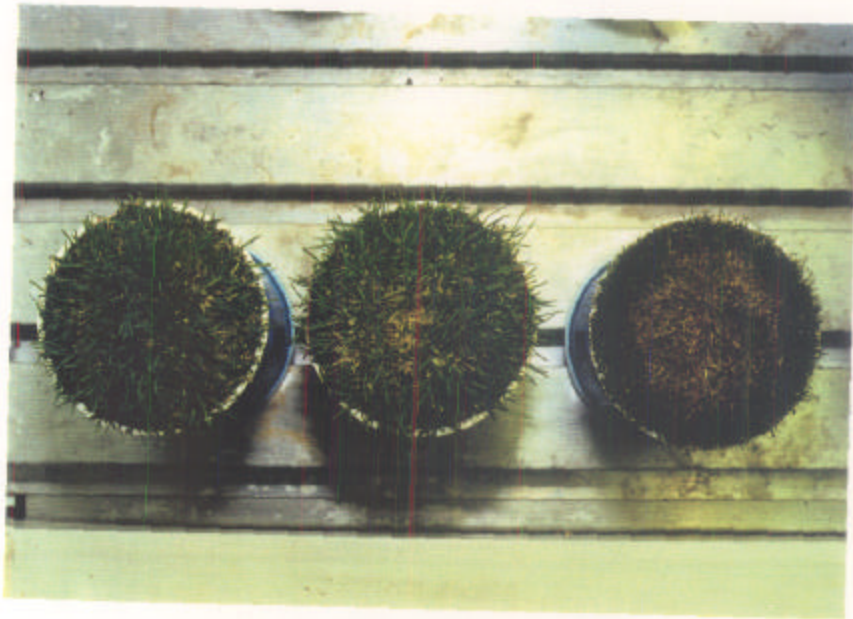
Inoculated bentgrass variant MT 323 (center) exhibited 21.6 mm diseased turf vs Penncross (right) with 50.0 mm. Uninoculated control MT 323 (left).
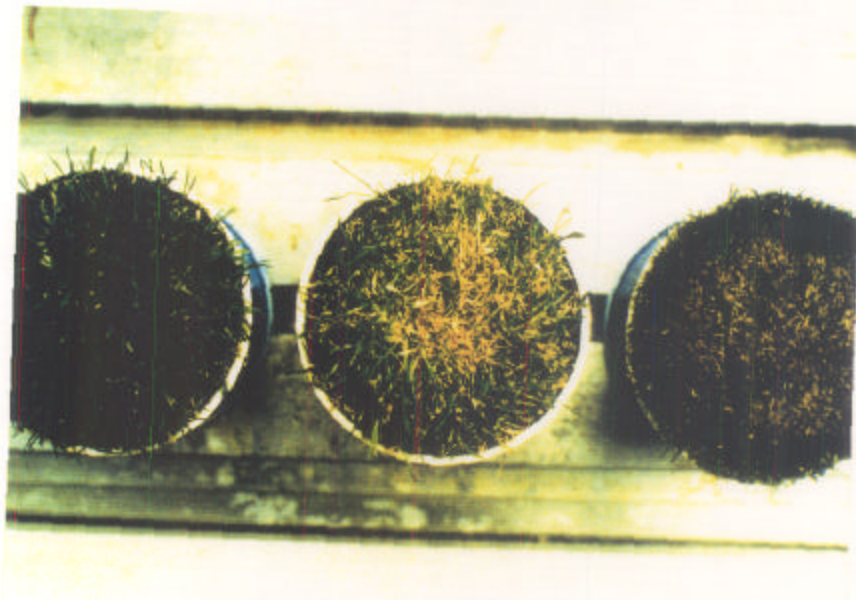
Inoculated bentgrass variant MT 325 (center) exhibited 35.0 mm diseased turf, similar to Penncross (right). Uninoculated control MT 325 (left).

Rhizoctonia solani Disease
Screening on Bentgrass Variants

bentgrass variants

■ less disease vs Penncross ■ similar disease as Penncross
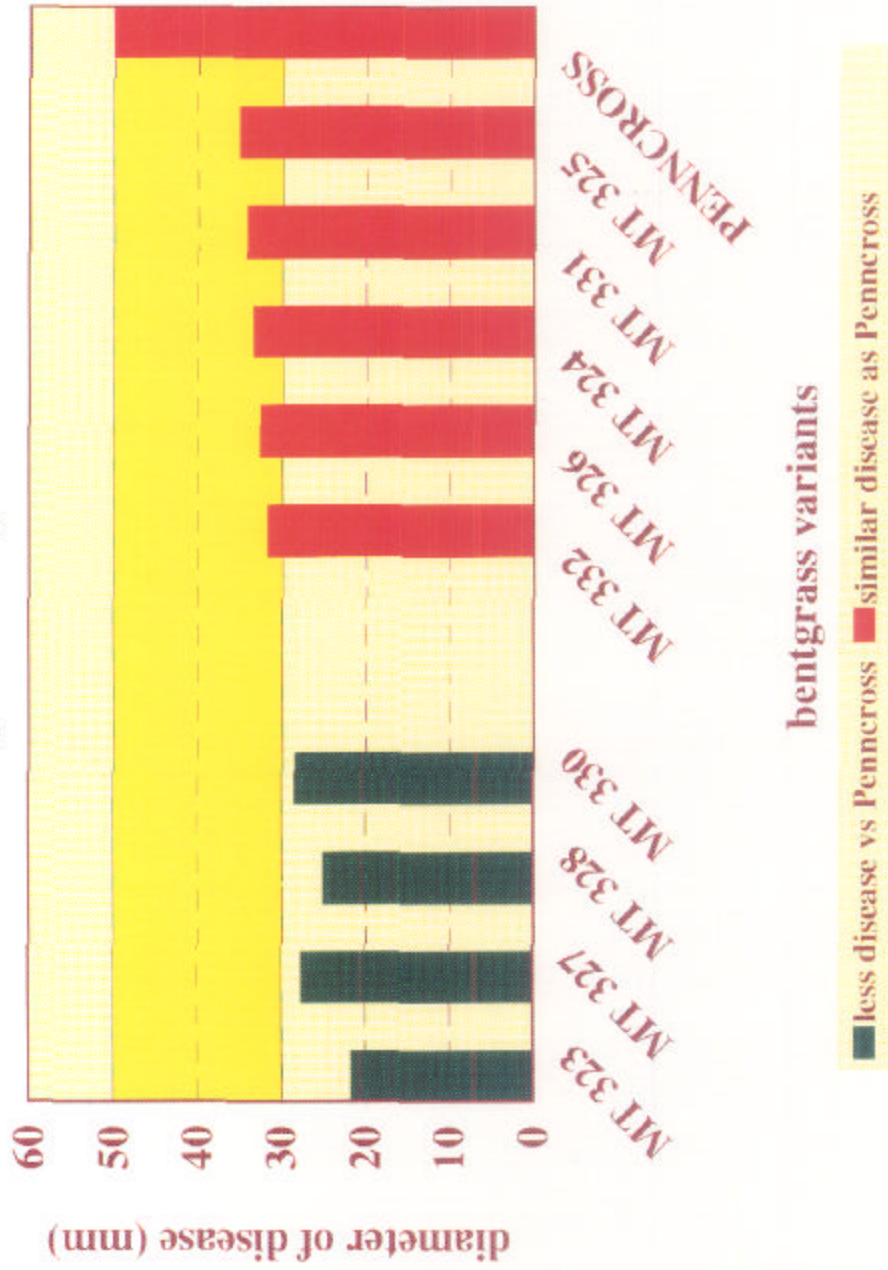
significant at P = 0.05 (LSD)
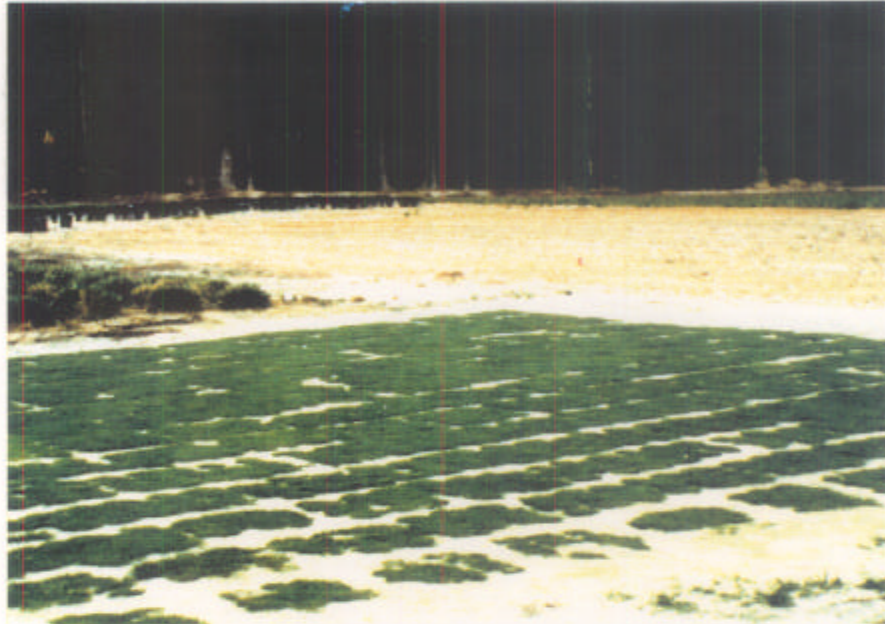
Bentgrass germplasm evaluations are established at Pure Seed Testing, Inc., Rolesville, NC (above) and Old Waverly Golf Course, West Point, MS (below).
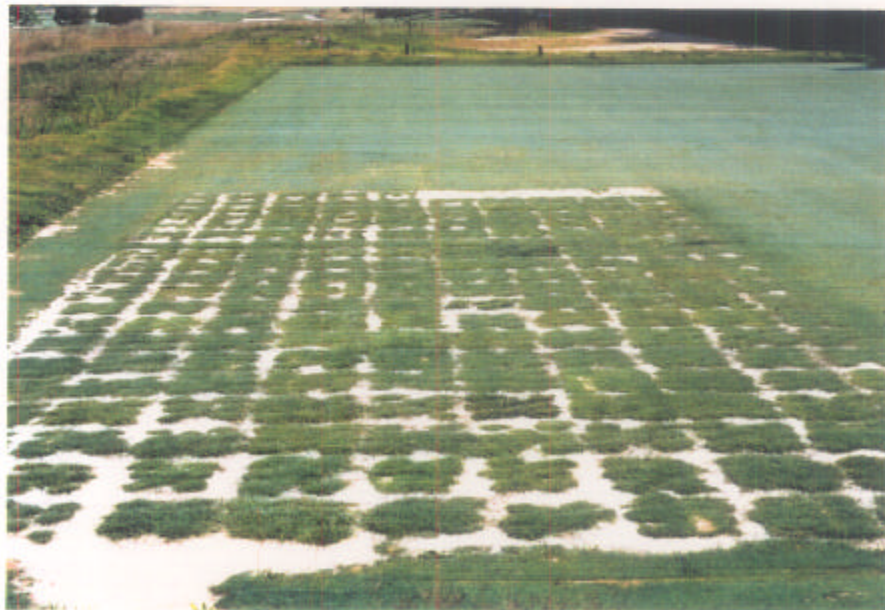
Preliminary Observations of *R. solani* (brown patch) Presence Among Bentgrass Variants
- Pure Seed Testing Location -

Variant (blocks 1,2,3)	Brown Patch	No Brown Patch
MT 323 (1&2)		
MT 327 (2)		
MT 328 (1-3)		
MT 330 (2)		
MT 332 (3)		
MT 326 (1)		
MT 324 (1&3)		
MT 325 (1)		
Penncross (3)		

SUMMARY

Tobacco (*Nicotianea tabacum* L.), alfalfa (*Medicago sativa* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), corn (*Zea mays* L.), and several other food and fiber crops have been improved for disease resistance through *in vitro* cell selection (Helgeson et al. 1972; Miller, 1983; Kroon, 1991; Behnke, 1979; Gengenbach and Green, 1975).

Bentgrass variants have been exposed to *R. solani* at three levels of development; cellular, plantlet, and whole plant. This cycle of selection included *in vitro* HPIS selection techniques and traditional inoculation procedures. The final phase of HPIS germplasm selection will be implemented in 1996. Bentgrass variants will be exposed to *R. solani* under natural, putting green conditions. Ultimately, selected bentgrass variants with enhanced resistance to *R. solani* will be evaluated for use as parental lines in creeping bentgrass nurseries.

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12.   Tomaso-Peterson, M. and J.V. Krans. 1990. Evaluation of a new *in vitro* cell selection technique. Crop Sci. 30:226-229.

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PRESENTATIONS:

"Recovery of *Rhizoctonia solani* resistant creeping bentgrass germplasm using the host-pathogen interaction system" 1995. American Society of Agronomy - St. Louis, MO.

"Meeting the challenges of growing creeping bentgrass in a greenhouse in the deep south" 1994. American Society of Agronomy - Seattle, WA.

"Verifying pathogenicity of *Rhizoctonia solani* using the host-pathogen interaction system" 1993. American Society of Agronomy - Cincinnati, OH.

"Overview of creeping bentgrass research at Mississippi State University" 1993. Mississippi Turfgrass Conference - Mississippi State University.

"Selecting brown patch resistant creeping bentgrass callus using the host-pathogen interaction system" 1992. American Society of Agronomy - Minneapolis, MN.

"Evaluation of a new *in vitro* cell selection technique" 1990. American Society of Agronomy - San Antonio, TX.

PUBLICATIONS:

"Creeping Bentgrass Research at Mississippi State University - Disease Resistance" 1995. Turfgrass Field Day - Mississippi State University.

"Creeping Bentgrass Research at Mississippi State University" 1994. Turfgrass Field Day - Mississippi State University.

"Selection for brown patch resistance in creeping bentgrass" 1994. M. Tomaso-Peterson. Mississippi Turfgrass Research Progress Report. Information Bulletin 274. pp 6-7.

"Test tube turfgrass" May 1994. M. Tomaso-Peterson and J.V. Krans. Golf Course Management.

"Selecting brown patch resistant creeping bentgrass" 1993. Turfgrass Field Day - Mississippi State University.

"Selecting brown patch resistant creeping bentgrass" 1992. Turfgrass Field Day - Mississippi State University.

"Refinement and evaluation of the Host-Pathogen Interaction System (HPIS) using *Rhizoctonia solani* and 'Penncross' creeping bentgrass" 1992. Lihong Zhao. Master of Science Thesis.

"Disease resistant screening with Penncross creeping bentgrass" 1991. Turfgrass Field Day - Mississippi State University.

"Evaluation of a new *in vitro* cell selection technique" 1990. M. Tomaso-Peterson and J.V. Krans. Crop Science. 30:226-229.

The following publications have cited the Host-Pathogen Interaction System as an intregal part of their research: (reprints attached)

1. Souissi, T. and R. J. Kremer. 1994. Leafy Spurge (*Euphorbia esula*) Cell Cultures for Screening Deleterious Rhizobacteria. Weed Science. 42:310-315.

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STUDIES CONDUCTED WITHIN USGA FUNDED RESEARCH PROJECTS
1990-1995

I.   Refinement of the Host-Pathogen Interaction System for obtaining disease resistant creeping bentgrass 1990-1992 (see appendix A)

   USGA isolate
   Media
   Leaf blade
   Callus rinse
   HPIS recycling
   Immediate co-culture
   RVPI inoculation
   Germplasm selection and plantlet disease screening
   HPIS chamber
   Callus induction in HPIS
   Simultaneous co-culture
   Delayed co-culture
   Callus co-culture/plantlet regeneration

II. Recovery of *Rhizoctonia solani* resistant creeping bentgrass germplasm using the Host-Pathogen Interaction System 1993-1995 (see Appendix B)

   Environment Controlled Greenhouse
   Variant selection (germplasm recovery)
   Whole plant verification
   Rye grain inoculum
   Hyphal plug-RVPI
   Hyphal plug-R31
   R. solani isolate/pathogenicity
   HPIS variant selection
   Field establishment (2 locations)
   Turf quality
   Brown patch observation