1993 USGA ANNUAL 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

Considerable progress has been made toward recovering *Rhizoctonia solani* resistant creeping bentgrass germplasm. In the laboratory, 203 plant variants were recovered from bentgrass callus/*R. solani* co-cultures via the Host-Pathogen Interaction System. Due to the success in recovering germplasm in the HPIS, we are able to select large numbers of variants, while maintaining a predictable production schedule of germplasm that will be subjected to additional screening in the laboratory, greenhouse and eventually in the field.

Laboratory studies addressing *in vitro* plantlet screening against *R. solani* is progressing favorably. The leaves of plantlets recovered from HPIS screening were inoculated with 1 mm plugs of *R. solani* mycelium and incubated. Preliminary results indicate segregation of resistance among the variants. A comprehensive study has been initiated based on these findings. If this procedure proves reliable, another level of screening will be incorporated into an already intense screening procedure. Bentgrass germplasm will undergo cellular, plantlet, and whole plant screening prior to field evaluation. Our objective is take superior lines to the field and evaluate them for enhanced resistance to *R. solani*. Ultimately we hope to isolate and provide *R. solani* resistant genetic material to the available germplasm collection for the purpose of developing superior creeping bentgrass varieties.

The variants we are working with at the whole plant level are being maintained in our newly acquired environmental control greenhouse (ECG). The ECG is equipped with a 3 ton heatpump air conditioner that allows us to provide optimum, stress-free environmental conditions for growing creeping bentgrass on a year round basis. Thus we can provide adequate light, optimum temperatures, humidity, and so forth while isolating the variants from various greenhouse contaminants. A fritted clay growth medium is used in propagating the variants and preparing them for the next level of *R. solani* screening experiments. These screenings are scheduled to begin in November 1993. The variants will be rated on their response to *R. solani* and the rate at which they recover from infection. Those displaying enhanced resistance will be maintained in a clonal repository for preservation, field evaluation, and seed production.

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Significant progress has been made in turf quality with the chemical control of weeds, insects, and diseases. However, chemical control measures provide only temporary relief from these plant pests and the negative impact some of these chemicals have on the environment are currently being realized. Permanent control of turfgrass pests depends on alternative approaches. The best alternative to chemical control is host plant resistance. The USGA grant entitled "Recovery of *Rhizoctonia solani* Resistant Creeping Bentgrass Germplasm using the Host-Pathogen Interaction System" is addressing the development of host plant resistance. The objectives being addressed during the first grant year, 1993, are: 1. Recovering *R. solani* selected bentgrass variants using the Host-Pathogen Interaction System (HPIS), 2. Screening the selected variants for enhanced resistance to *R. solani* at the plantlet (*in vitro*) level, 3. Verifying whole plant resistance to *R. solani* using greenhouse studies, and 4. Maintaining selected variants in a clonal repository to address future objectives.

ENVIRONMENTAL CONTROL GREENHOUSE

Growing healthy creeping bentgrass in a greenhouse in the deep South, particularly during the summer months, has been a great challenge for all those attempting it. Here at Mississippi State University, we have been involved in large-scale greenhouse production of creeping bentgrass since 1991. During the "heat-stressed" periods, May - October, shade cloth, frequent mistings, and other cooling techniques have been employed to maintain the bentgrass.

When it came time to address the most vital objective in this research grant, verifying whole plant resistance of bentgrass variants using greenhouse studies for determining *R. solani* enhanced resistance, we realized our greenhouse facilities did not permit the strict environmental control required to optimize the performance of the bentgrass variants or *Rhizoctonia solani*. Optimum temperatures for creeping bentgrass range from 16 - 24°C. While the optimum temperatures for *R. solani* range from 28 - 33°C. Through the support of the Mississippi Agricultural and Forestry Experiment Station, we were able to build an Environmental Control Greenhouse (ECG). A 27′ x 16′ x 8′ wooden structure was erected within an existing greenhouse bay. The structure is enclosed with a double layer of polyethylene greenhouse plastic and the layer is inflated using a blower fan.

Cooling is provided by a 3 ton heatpump air conditioner (figures 1 - 3). The ECG enables us to provide optimum temperatures for maintaining the bentgrass variants. Nighttime temperatures are maintained around 18 ± 2°C, while the daytime high often reaches 25 - 28°C in mid-afternoon. Prior to using the ECG, the bentgrass variants were exposed to temperatures frequently in the range of 38 - 42°C. At the point when screening experiments are initiated, the temperatures will be increased to promote the growth and vigor of *R. solani* to insure infection. The ECG has provided us with the flexibility to manipulate the environmental conditions required to insure our success in screening bentgrass variants for enhanced resistance to *R. solani*.

VARIANT SELECTION VIA HOST-PATHOGEN INTERACTION SYSTEM

In the laboratory, bentgrass germplasm was selected following callus/*R. solani* co-cultures in the HPIS. Two hundred three variants were recovered following 18, 24, and 36 h co-cultures. The plantlets were removed from the petri dishes in which plantlet regeneration was initiated and were transferred to culture boxes. A preliminary *in vitro* plantlet screening was conducted on 25 of the recovered variants. The experimental material was vegetatively propagated into culture boxes. Following two weeks of culture, each plantlet was inoculated with three, 1 mm plugs of *R. solani* and were incubated at 30°C in the growth chamber. After 48 h, some variants remained healthy, others developed lesions on the leaves, while the leaves on some variants became completely necrotic. This screening procedure is currently being repeated on the same 25 variants. If the results are consistent with the first experiment, further investigation into this *in vitro* screening technique will be conducted.

WHOLE PLANT VERIFICATION

The germplasm currently being evaluated was obtained from experiments carried out under the USGA 1991 project "Refinement of the Host-Pathogen Interaction System". To date this includes 104 variants that are divided into three groups. The first group are the "MT" variants that originated from the first plantlet recovery experiments via the HPIS. These variants underwent a greenhouse disease screening in 1992 but results were inconclusive. However, some variants remained significantly healthier than Penncross and were maintained for further evaluation.

To run the variants through another series of greenhouse inoculation studies with *R. solani*, we had to insure that they were free of all pathogens. This required the variants to be micropropagated via *in vitro* techniques. These techniques involved collecting nodal tissue from the variants, surface sterilizing and transferring tissue aseptically to MS tissue culture medium in large culture tubes. The nodes were cultured under continuous low light at least four weeks or until an adequate plantlet developed. At this point the plantlets were transferred to culture boxes, again containing MS medium and maintained under continuous low light. When

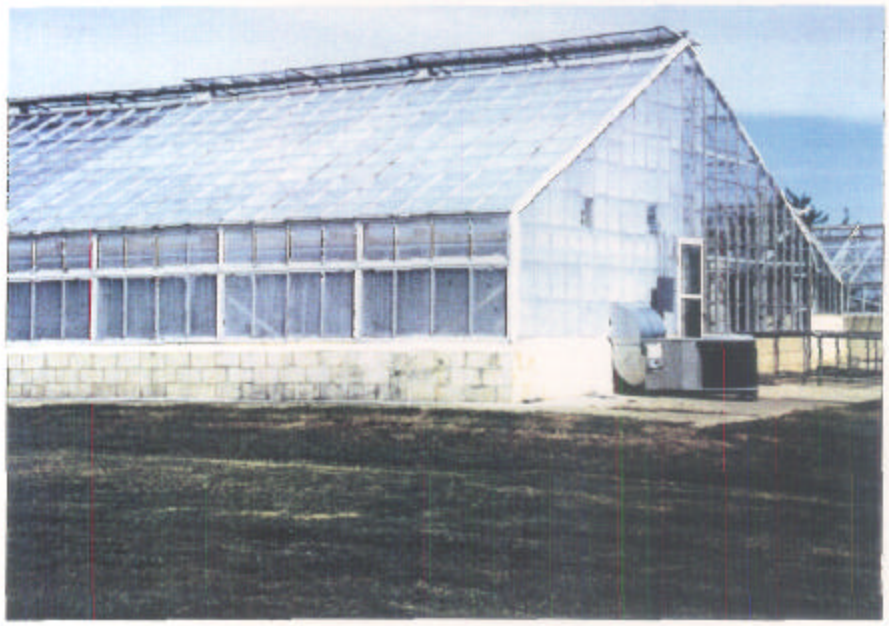

Figure 1. Renovated greenhouse at Mississippi State University. The environmental control greenhouse employs a 3 ton heatpump for cooling. White-washed glass and an 80% shade cloth covering the inside west window minimizes buildup of radiant heat.

Figure 2. Inside the greenhouse bay a 27' x 16' x 8' wooden frame was built. Two layers of greenhouse polyethylene covers the structure. Air is forced between the double layer of plastic to create a layer of insulation.

Figure 3. Inside the environmental control greenhouse bentgrass variants are maintained at 17°C. The plastic convection tubing suspended from the rafters serves as the duct work, providing optimum air circulation.

the plantlets developed adequate shoot and root systems, they were transferred to five cm pots containing sterile sand:soil mix and maintained in a growth chamber at 22°C with 16 h days.

The variants were transferred to the ECG once it became operational. In mid-August they were repotted in small cups containing fritted clay. The roots were trimmed off each plant just below the crown region and the sod plugs were planted into moistened fritted clay. The use of fritted clay seems to aid in moisture control, decreases algal growth, and reduces the threat of soil-borne pathogens invading the variants. The plants were initially watered three times per day and Hoagland's nutrient solution was applied twice a week. This culture method has proven to accelerate the growth of the variants thus, reducing the length of time between whole plant initiation and disease screening (figure 4).

These variants will undergo *R. solani* screening in November. The inoculum of *R. solani* is currently being developed using rye grain as the host. A small amount of ground rye inoculum will be placed in the center of each replicated variant. Variants will be maintained under 100% humidity for 48 h. Daytime temperatures will range between 27 - 33°C and nighttime between 26 - 29°C. The inoculation and screening procedure will be repeated three times on different replications with one control group included. Susceptibility and/or resistance will be determined by the number of lesions present on the leaves and the portion of total turf area (8 cm) damaged by the fungus.

The second group of variants is referred to as "LH" variants. These were recovered from HPIS refinement studies carried out by a former graduate research assistant. In December 1992, the LH variants were vegetatively propagated to increase the number of replications per variant. A greenhouse *R. solani* inoculation study was scheduled for Spring 1993, but in the interim the variants became infected with *Pythium*. It was observed that some of the variants were displaying extreme symptoms while some were only slightly infected, and others appeared very healthy. The fungicide, Subdue®, was applied to the variants and the infection seemed to be arrested. Following the recovery of the variants, they were inoculated with plugs of *R. solani* and maintained in a mist chamber. Due to operational difficulties with the fogger, the variants remained in the mist chamber four days (instead of two) and then were transferred back to greenhouse benches. Fifteen days following inoculation, some of the variants began to display symptoms of disease. The variants were visually rated according to the percent disease or damaged leaf tissue present. Lesions were collected from the infected leaves, surface sterilized, and maintained on Potato Dextrose Agar in a 26°C incubator to identify the organism. The organism present in the leaf tissue was identified as a *Helminthosporium spp*, another turf pathogen. Unfortunately, growth conditions were highly favorable for *Helminthosporium* development resulting in almost total suppression of pathogenicity by *R. solani*. New replicates were propagated in sterile, sandy soil. The LH variants are currently being maintained, (ie. trimmed

Figure 4. MT variants growing in fritted clay. The plants are clipped to simulate a putting green surface.

and fertilized), and will be transferred to fritted clay and screened for enhanced resistance to *R. solani* in the future.

The third group are termed the "MT-II" variants. These were recovered from HPIS studies conducted in the Summer of 1992. As plantlets, these variants were screened in the Host-Pathogen Interaction System Chamber. Upon completion of whole plant screening, the selected variants will have undergone three levels of exposure to *R. solani*: cell, plantlet, and whole plant. The variants are currently being vegetatively propagated in the fritted clay medium (figures 5 and 6). The number of replicates per variant is being increased to 24. This will allow three inoculation screenings with seven replications per screening and three replications per variant serving as controls. *R. solani* screening studies will be initiated as soon as the turf covers the 8 cm diameter of the cup.

SUMMARY

In review of the first years activities addressing the recovery of *Rhizoctonia solani* resistant creeping bentgrass germplasm much progress has been made. In the laboratory we have 203 variants derived from callus/*R. solani* co-cultures via the HPIS. Due to the success of recovering germplasm using the HPIS, we are able to generate large numbers of variants, while maintaining a predictable production schedule of germplasm that will be subjected to additional screening in the greenhouse and eventually in the field. Laboratory studies concentrating on *in vitro* plantlet screening against *R. solani* is progressing favorably. This objective is very important because success at this screening level will lend support to passing material forward for resistance screening at the whole plant level. The *in vitro* screening also helps reduce the numbers of variants to a somewhat more manageable level.

The variants at the whole plant level are being maintained in the environmental control greenhouse under optimum conditions for creeping bentgrass. Our goal in the ECG is to provide an environment that is essentially stress-free. This includes optimum temperatures for growth, low humidity, and most importantly, control of undesirable soil-borne pathogens. The fritted clay growth medium seems beneficial for promoting vigorous growth, managing moisture, and retarding disease. Thus the variants at the time of *R. solani* inoculation screenings should be healthy, vigorous plants. Following inoculation and infection, the variants will be rated for their response to *R. solani* and the rate at which they recover from infection. Those variants displaying enhanced resistance will be maintained in a clonal repository for preservation, field evaluations, and ultimately seed production.

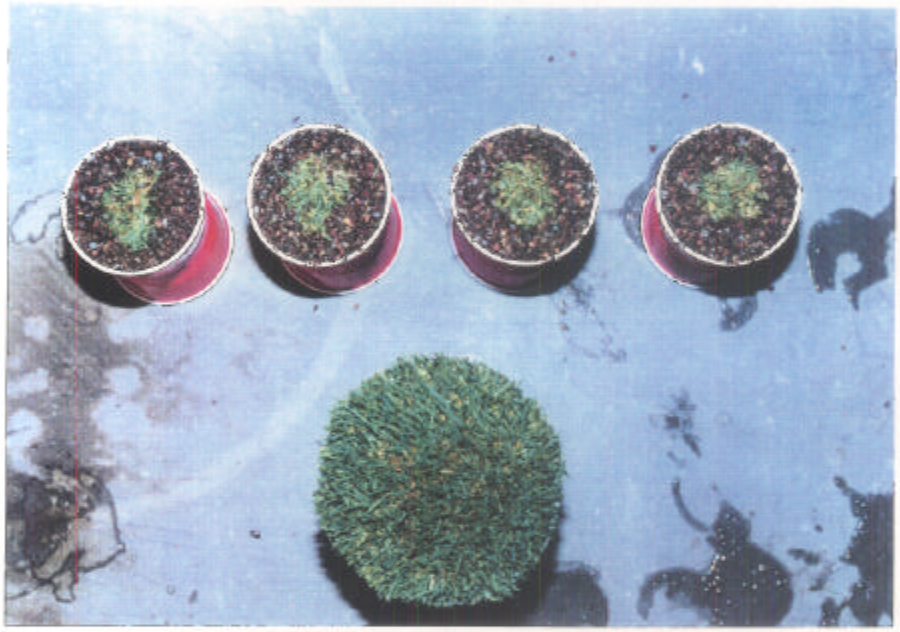

Figure 5. MT-II variants undergoing vegetative propagation. The 10 cm pot is quartered and the plugs are planted in fritted clay.

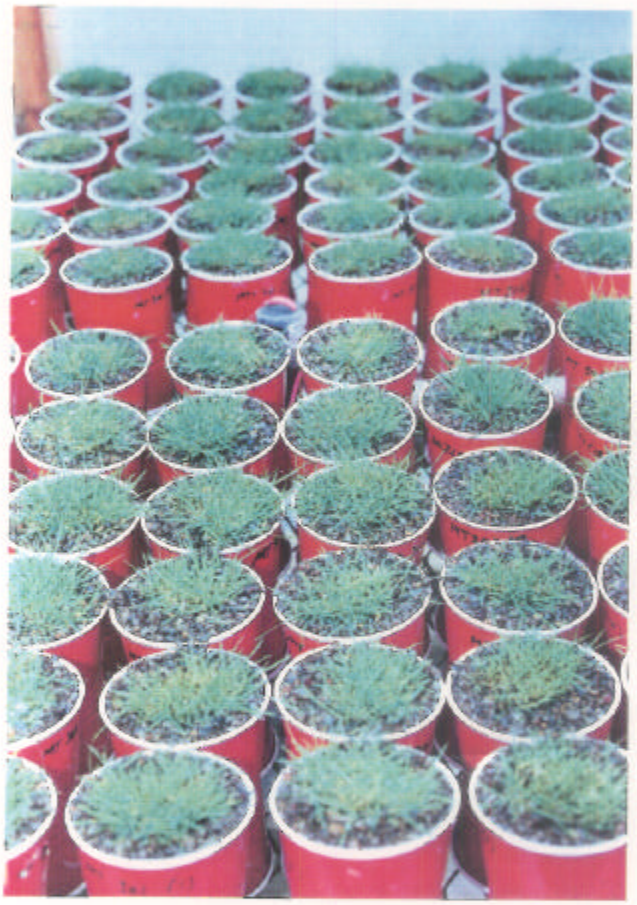

Figure 6. MT-II variants are healthy and vigorously growing after one week in fritted clay.