1992 ANNUAL PROGRESS REPORT
EXECUTIVE SUMMARY

REFINEMENT OF THE HOST-PATHOGEN INTERACTION SYSTEM

MARIA TOMASO-PETERSON AND DR. JEFFREY KRANS
MISSISSIPPI STATE UNIVERSITY

Research efforts in 1992 were focused on using the Host-Pathogen Interaction System (HPIS) to obtain creeping bentgrass germplasm with enhanced resistance to *Rhizoctonia solani* (Kühn), as well as developing an *in vitro* screening technique to verify enhanced resistance at the plantlet level.

Two co-culture procedures, simultaneous and delayed, were evaluated for obtaining bentgrass callus with resistance to *R. solani.* The simultaneous co-culture procedure was designed to allow the callus a gradual exposure to the toxic substances of *R. solani* over a period of 10 days whereas the delayed co-culture procedure exposed the callus to various concentrations of the toxic substances for only 24 hours. The results from both procedures indicate *R. solani* must be actively growing in the HPIS for at least 7d before the level of toxic substances is such that only 25% of the viable callus population can be recovered. From that 25% viable callus population, an average of two plantlets are regenerated. Some of these plantlets display enhanced resistance to *R. solani.*

The Host-Pathogen Interaction System Chamber was developed for screening the germplasm obtained from the HPIS refinement experiments. This system is similar to the HPIS in principle but is adapted for the unrestricted growth by the plantlets. The bottom compartment of the chamber consists of the actively growing *R. solani.* The top compartment has been modified by the addition of a 9.5 cm (high) by 9.0 (dia) glass cylinder. This expanded space in the upper compartment permits the use of additional growth medium required by larger plantlets, and provides adequate head space which plantlets require for optimum development. The plantlets were screened in the HPIS Chamber for two weeks. Thirty-three percent of the plantlets exposed to *R. solani* died. Those surviving plantlets were extremely stressed, displaying purple leaves and stunted growth. They were then transferred to tissue culture boxes where vigorous shoot and root development occurred. The plantlets have subsequently been transferred to soil and will be screened for resistance to *R. solani* at the whole plant level. This will provide critical and much needed evidence on the efficacy of the HPIS approach, as well as providing plants with enhanced resistance to *R. solani.*

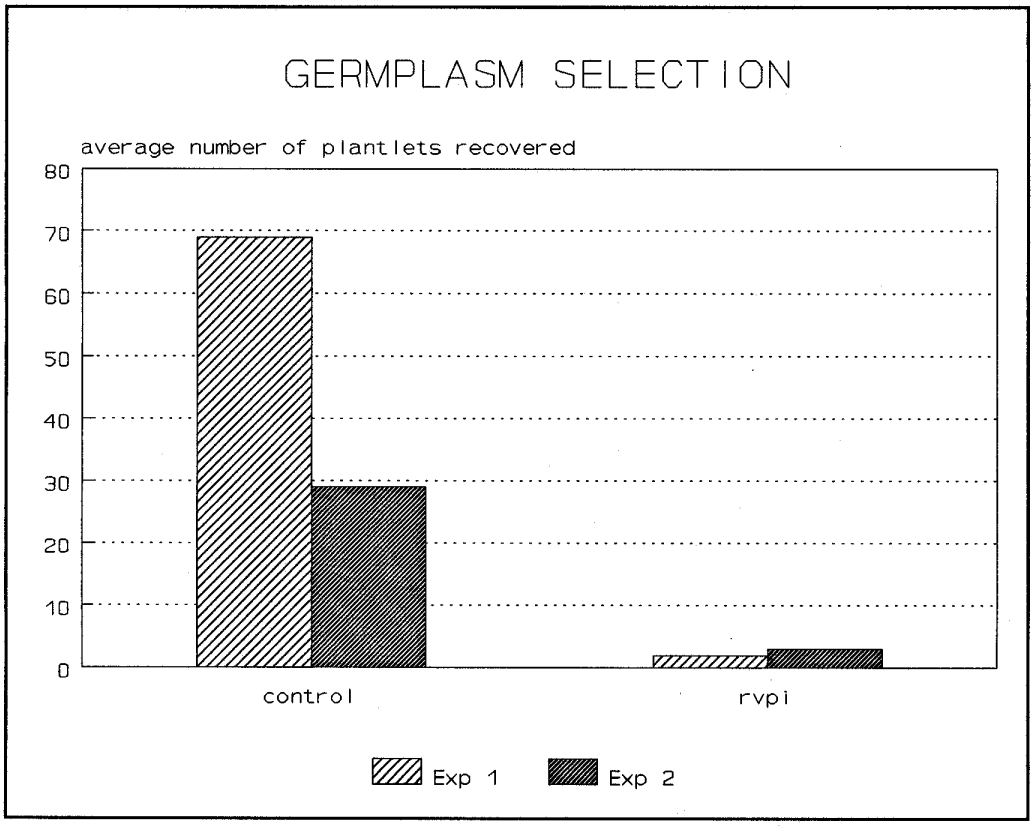
1992 USGA ANNUAL PROGRESS REPORT
2 NOVEMBER 1992

REFINEMENT OF THE HOST-PATHOGEN INTERACTION SYSTEM

MARIA TOMASO-PETERSON AND DR. JEFFREY KRANS
MISSISSIPPI STATE UNIVERSITY

I. GERMPLASM SELECTION AND PLANTLET DISEASE SCREENING:

Two germplasm selection experiments were conducted to obtain plantlets from callus that was exposed to *Rhizoctonia solani* isolate RVPI. The RVPI incubated 7d prior to the 24h co-culturing with the callus. Following the first co-culture, the HPIS plates were recycled in a second co-culture experiment. Only 21% and 18% of the callus population from experiments 1 and 2, respectively, survived the co-cultures. Therefore, the number of regenerated plantlets recovered from these calli was significantly less than the controls. About two plantlets/plate were recovered from callus exposed to RVPI.



These recovered plantlets were used to evaluate the HPIS plantlet screening technique. The HPIS was assembled, 0-MS was used as the nutrient medium for the plantlets and RVPI was actively growing in the bottom compartment. Following a week in the HPIS, both the RVPI-selected plantlets and the control plantlets displayed signs of stress and an overall reduction in plant vigor. At the end of two weeks, the 0-MS was dried out and almost all the plantlets had died. A major technical problem that arose with this technique was a lack of head space and depletion of nutrient medium within the HPIS compartment.

II. HPIS CHAMBER PRELIMINARY STUDY:

Modifications to the HPIS were made due to the results of the HPIS plantlet screening experiment. The primary components of the HPIS Chamber are:

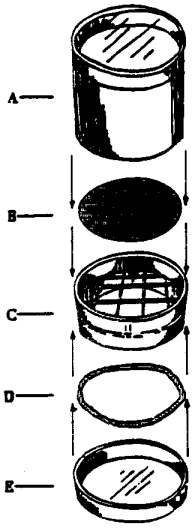
A. Cylinder for plantlet growth in HPIS Chamber.

B. Nuclepore membrane for restricting fungal growth.

C. Main body of HPIS chamber.

D. Caulking cord used to secure cylinder to HPIS plate.

E. Lid of fungal compartment, also sealed with caulking cord (D).



A cylinder, 9.5 cm (high) by 9.0 cm (dia), extends the plantlet compartment sufficiently so that adequate head space is available and an additional quantity of nutrient medium (50 ml) can be used to insure unrestricted growth by the plantlets. These modifications provide optimum conditions for plantlet growth. Young plantlets (10 mm tall) were transferred into the HPIS Chamber.

Initially all the plantlets grew vigorously and reached heights of near 60 mm. By the second week the plantlets from the RVPI treatments were extremely stressed and growth ceased, while plantlets in the control HPIS Chamber were lush and vigorous (figures 1 and 2). In general, the plantlets had long internodes, the purple pigmentation in the leaves turned brown and the new growth was grayish-green while the leaves were needle-like, appearing desiccated. Upon examination of the root systems, the root tips were

Figure 1. Creeping bentgrass variant (left) that co-cultured with *R. solani* for two weeks in the HPIS Chamber and (right) uninoculated control creeping bentgrass variant.

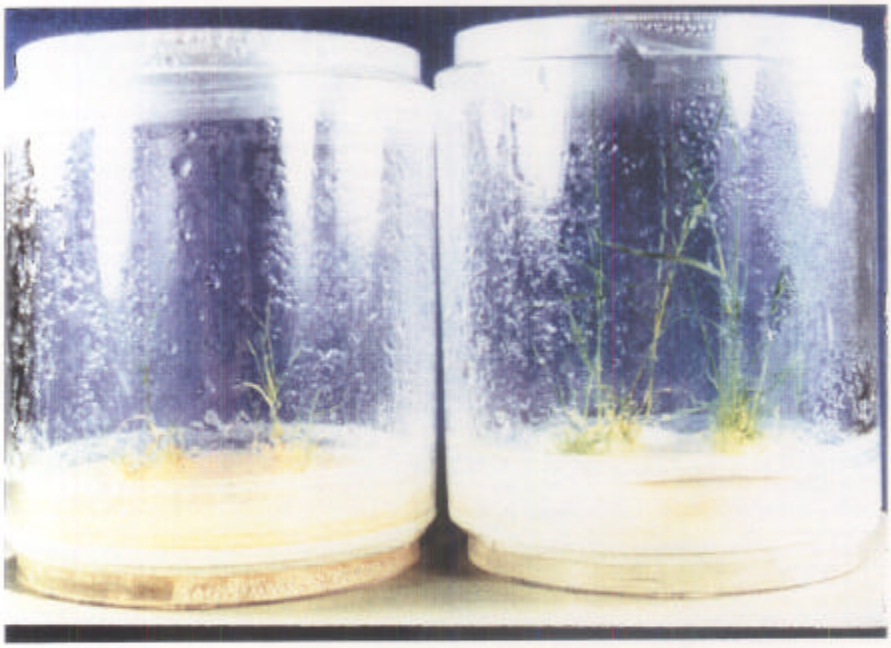


Figure 2. Creeping bentgrass variants co-culturing with *R. solani* in the HPIS Chamber (left) and creeping bentgrass variants in an uninoculated control HPIS Chamber (right).

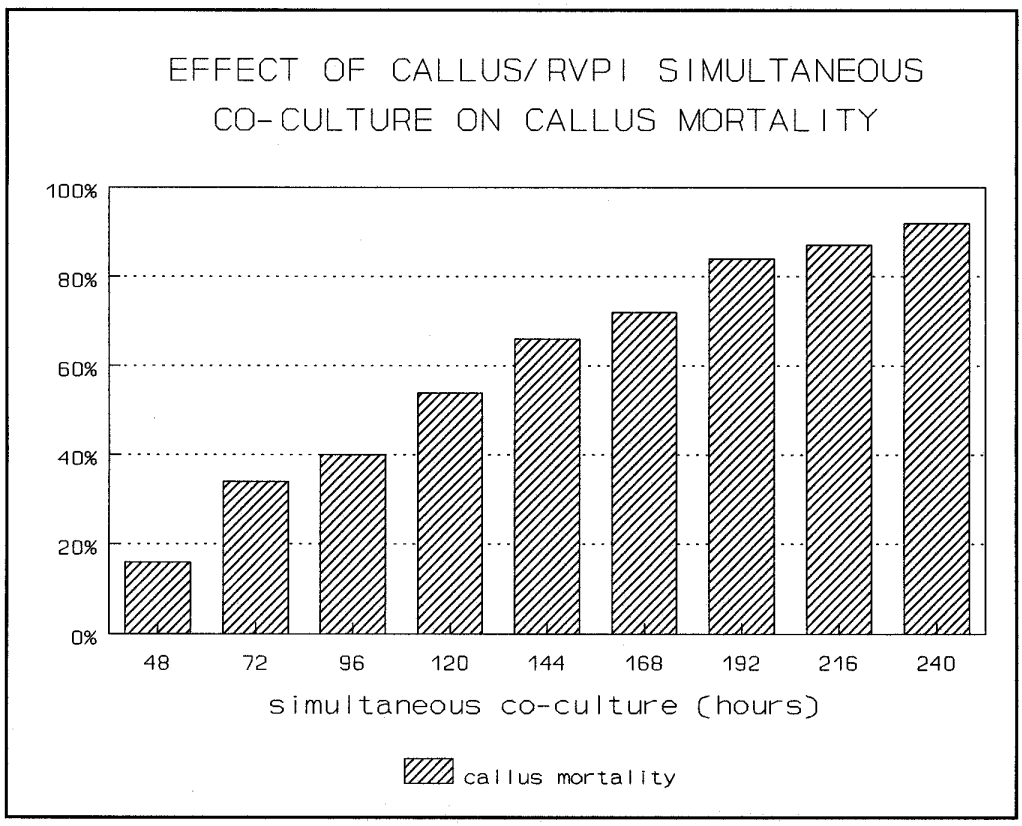
necrotic. It appeared the roots had absorbed toxic substance(s) produced by RVPI. Translocation of nutrients from the roots to the shoots may have been slowed down and even inhibited in some cases, thus causing death to some of the plantlets. Those plantlets that remained viable were carried forward as germplasm exhibiting enhanced resistance. Because some of the plantlets remain viable, the HPIS Chamber seems to be an effective tool for screening plantlets for enhanced resistance to *Rhizoctonia solani*. The HPIS Chamber will be used again in future plantlet screenings.

III. APPLICATION OF THE HPIS - CALLUS INDUCTION:

This experiment was conducted to determine if callus could be induced in the presence of RVPI, and to determine if that callus would exhibit resistance to RVPI. Two *R. solani* isolates, RVPI (pathogenic) and R12 (nonpathogenic) were cultured in separate HPIS plates. Uninoculated controls were also included. Penncross seedlings were transferred into the HPIS and maintained under conditions of low light. After 4 weeks, a very small amount of callus ($\leq$ 1mm dia) was present on the seedlings that co-cultured with RVPI. The R12-seedlings initiated shoot growth, while the control seedlings averaged 1.7mg callus per seedling (figure 3). Based on the results of this experiment, it was concluded that callus could not be induced satisfactorily in the presence of RVPI, probably because of the prolonged exposure to toxic substances. This method of callus selection, therefore, does not appear promising as a means for recovering resistant callus.

IV. APPLICATION OF THE HPIS - SIMULTANEOUS CO-CULTURE:

The simultaneous co-culture procedure consisted of introducing *Rhizoctonia solani* isolate RVPI and callus into the HPIS at the same time. This was designed to determine what affect a gradual exposure of callus to RVPI's toxic substances had on callus mortality. The durations of RVPI and callus co-culturing significantly affected callus mortality. Callus mortality increased as the duration of co-culturing increased.



A regression analysis of callus mortality versus duration of co-culturing revealed a linear function with an R^2 value of 0.97.

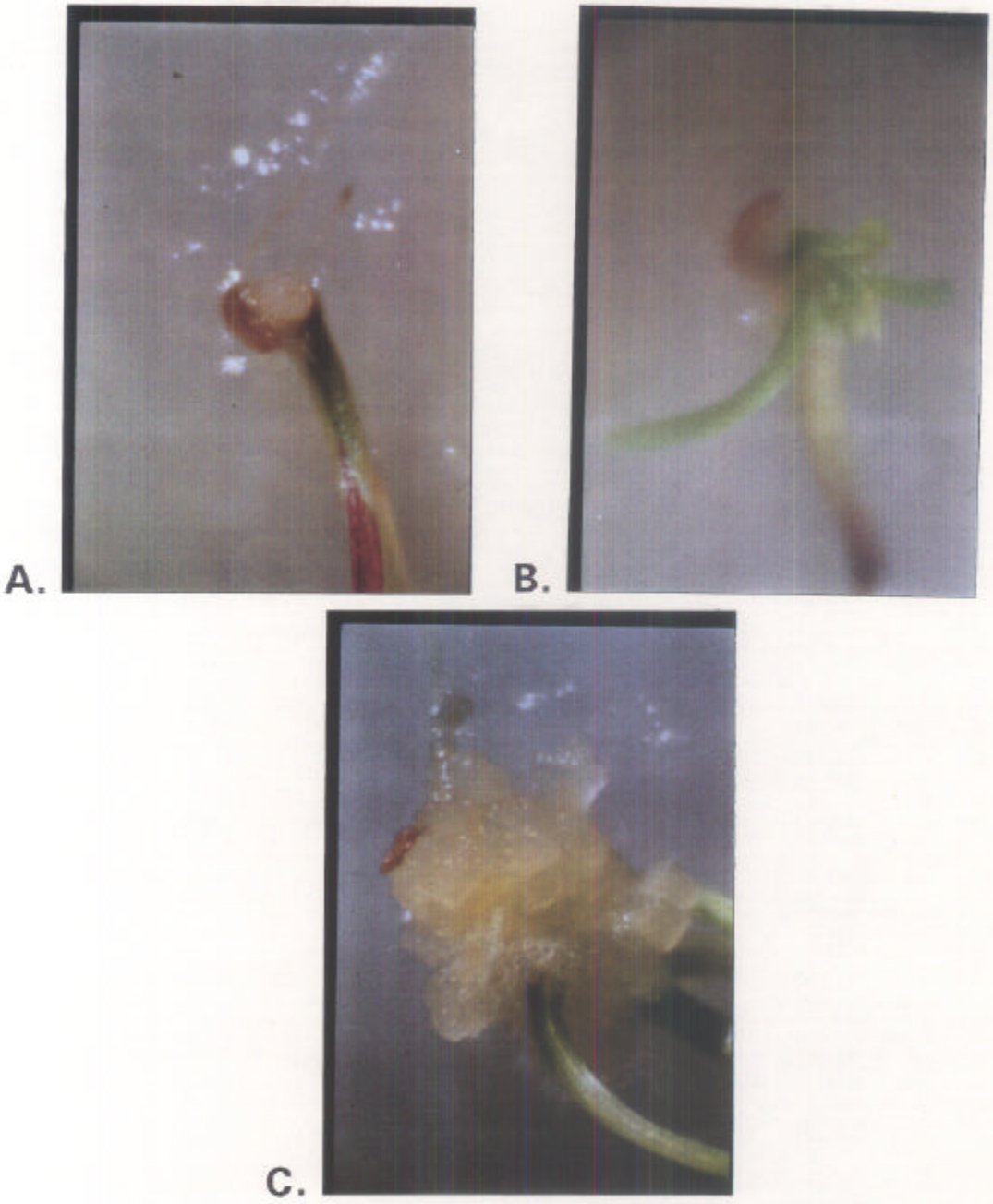


Figure 3. Bentgrass callus induction in the HPIS. Bentgrass seedlings co-cultured with RVPI (A), R12 (B), and uninoculated control (C) (6.3 magnification).

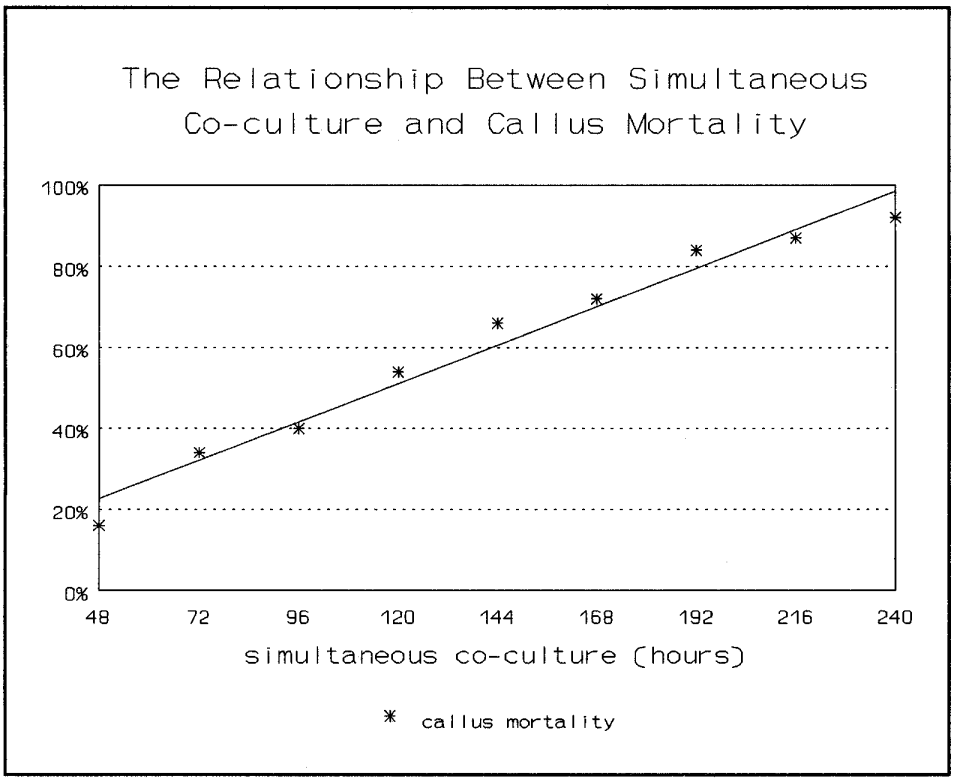
This linear regression equation can be summarized as follows:

$$M = 3.653 + 0.395T$$

WHERE:

M = % callus mortality

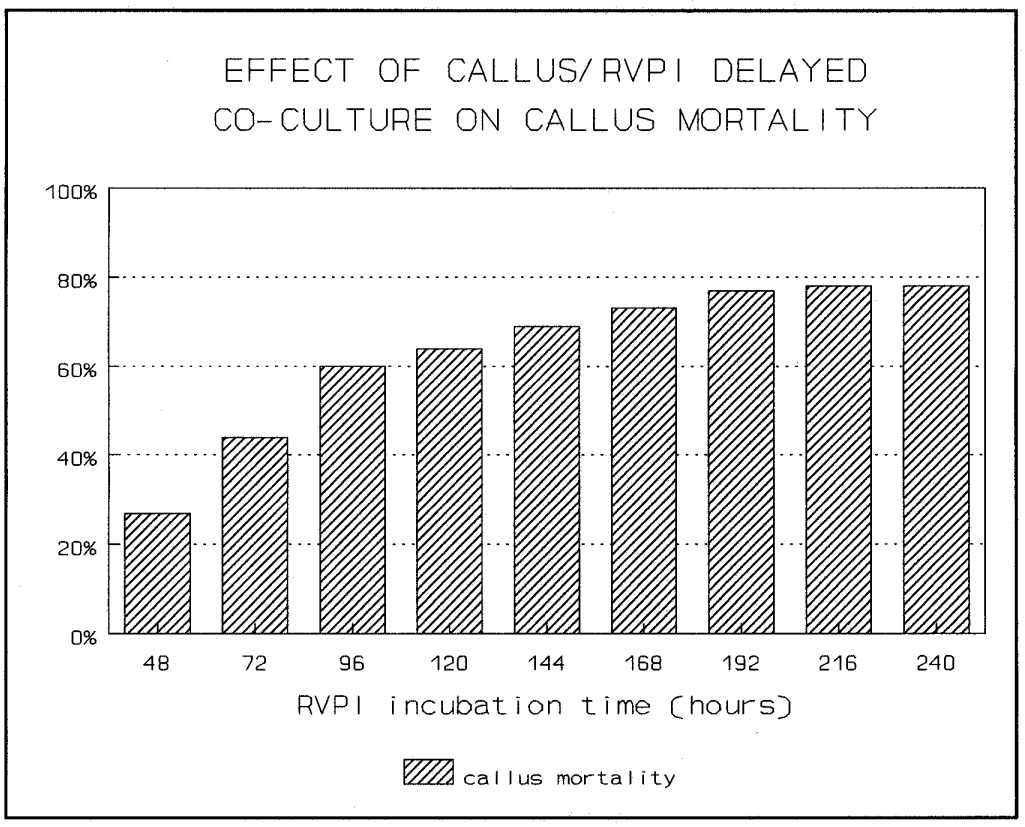
T = incubation/co-culture duration (h)



The regression function for simultaneous co-culture showed callus mortality increased 0.395% / hour of co-culturing. The high correlation coefficient (R^2 = 0.97) indicates good agreement between the regression equation and the observed data. The linear trend of this equation suggests that the host-pathogen interaction was cumulative given the parameters used in this experiment. This equation provides a basis to estimate the periods of co-culturing necessary to obtain desired levels of callus survival. This should be advantageous in maximizing the recovery of resistant bentgrass callus.

V. APPLICATION OF THE HPIS - DELAYED CO-CULTURE PROCEDURE:

The delayed co-culture procedure consisted of allowing *R.solani* to grow in the HPIS for predetermined periods of time prior to the introduction of the bentgrass callus. This allowed different quantities of toxic substances to accumulate in the medium but restricted the period of callus exposure and therefore any cultural acclimation by the callus to the toxins. As RVPI's incubation times increased, the level of callus mortality also increased.



A regression analysis of callus mortality at corresponding incubation times revealed a quadratic function with an R^2 value of 0.97.

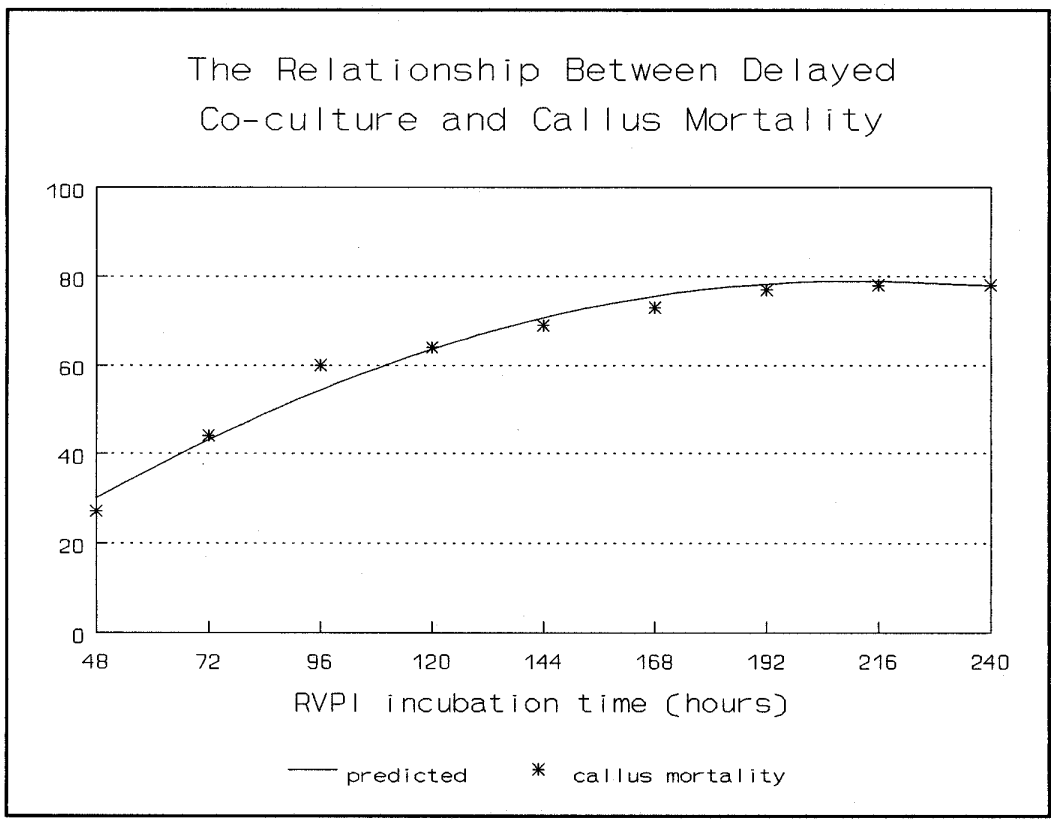
This quadratic regression equation describes the relationship between callus mortality and the duration of growth by RVPI in advance of callus introduction.

$$M = -3.210 + 0.782T - 0.00185T^2$$

WHERE:

M = % callus mortality

T = incubation time (h)



The regression equation for delayed co-culture displayed callus mortality was linear-like up to 144h, then starts to plateau, approaching 80% callus mortality as a limit. The high correlation coefficient ($R^2 = 0.97$) indicates a close agreement between the equation and the experimental results. The quadratic function of this equation illustrated the maximization of RVPI's toxic substances was at 168h. At incubation times above 168h, the 24h

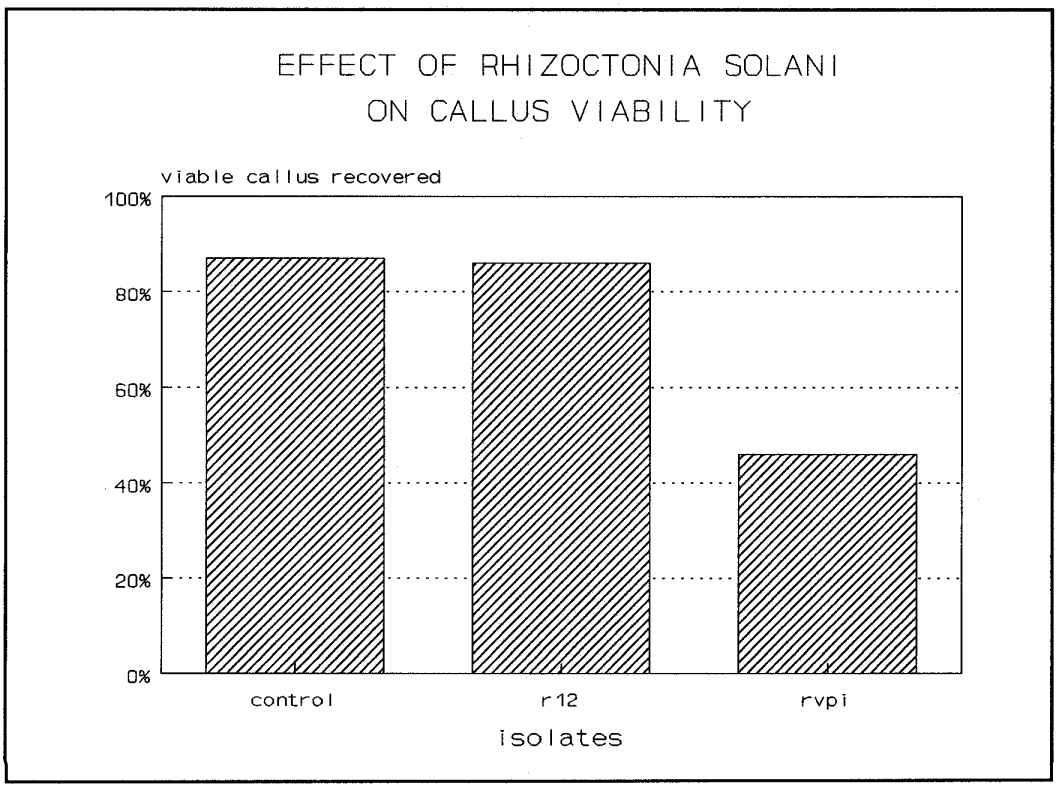
callus/RVPI co-culture duration was the limiting factor in obtaining higher levels of callus mortality.

Plantlets were recovered from both simultaneous and delayed co-culture procedures. These plantlets are currently being increased by vegetative means for evaluating *R. solani* resistance using *in vitro* leaf bioassay methods. Following the plantlet screening, the recovered variants will be evaluated at the whole plant level in greenhouse and field studies.

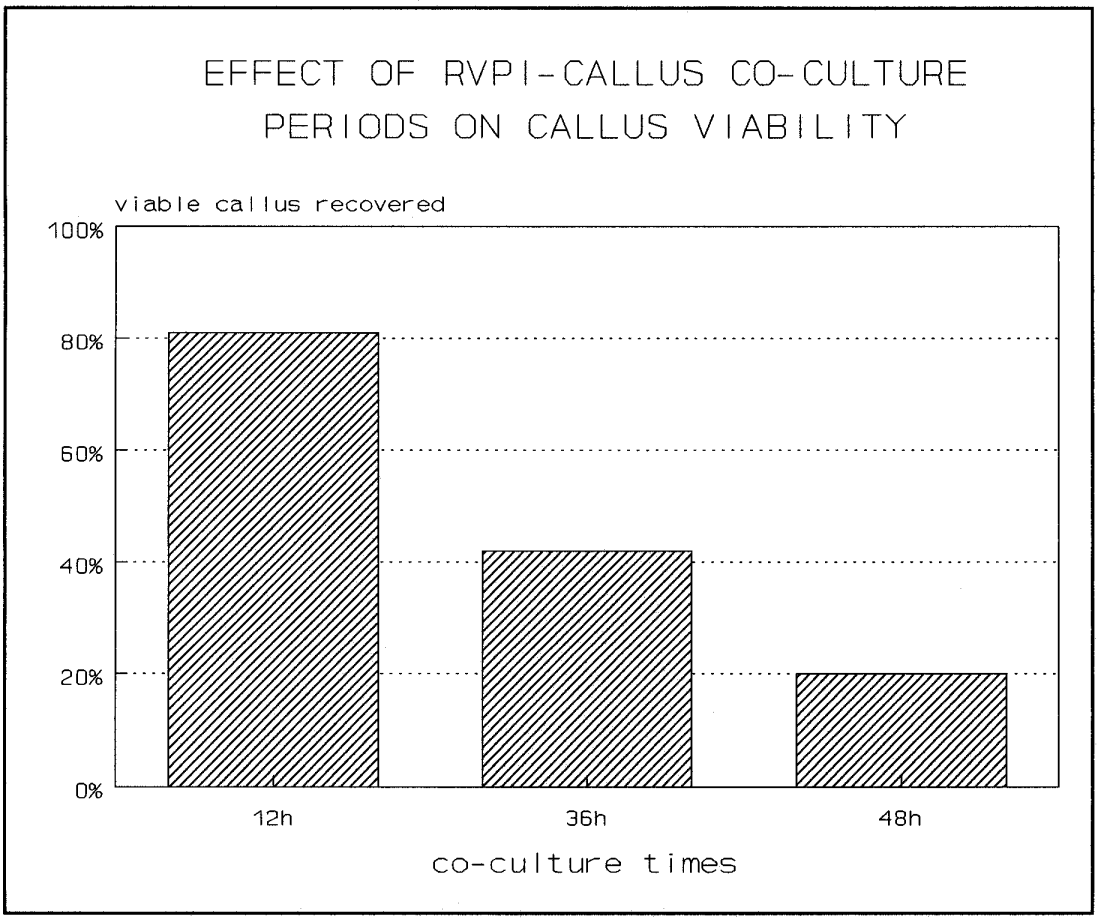
VI. APPLICATION OF THE HPIS - CALLUS CO-CULTURE AND PLANTLET REGENERATION:

A study evaluating co-culture time periods was carried out to define the optimum amount of time callus can be exposed to RVPI and not loose total plantlet regeneration capabilities. *Rhizoctonia solani* isolates RVPI and R12 were cultured in the HPIS along with an uninoculated control group. RVPI and R12 incubated 7d prior to co-culture with the bentgrass callus. The co-culture times included 12, 36, and 48 hours. Triphenyl tetrazolium chloride stain was employed to measure callus viability and plantlet regeneration was determined two weeks after exposure to the desired period of co-culturing.

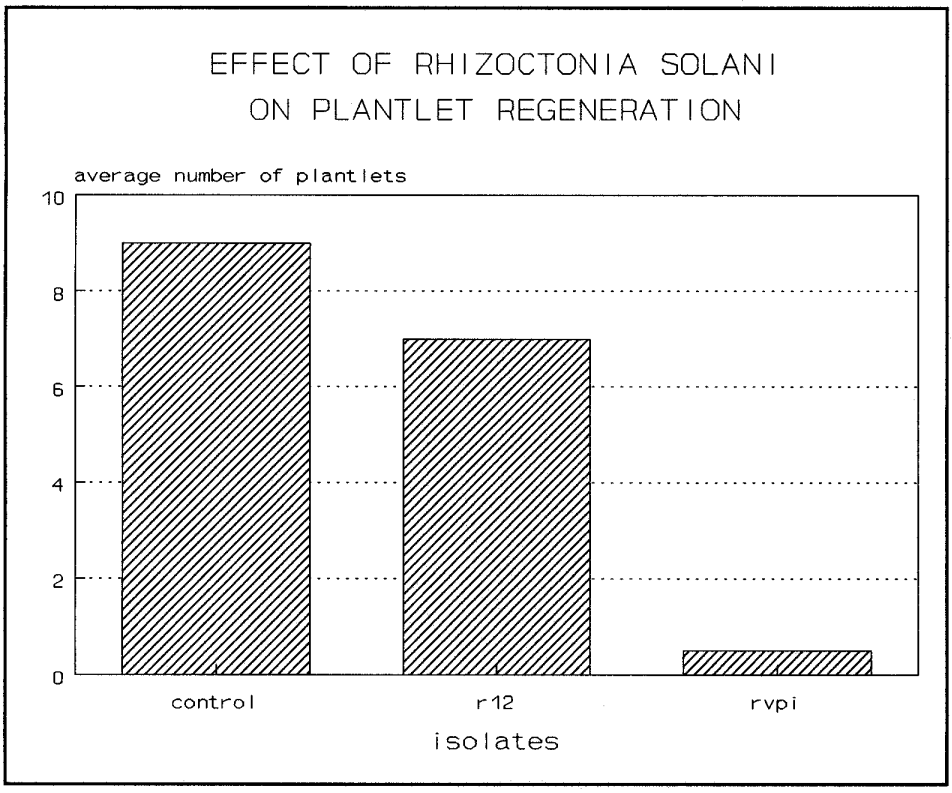
Callus viability was similar among control and R12 treatments (86%) over all co-culture times, while callus viability among RVPI treatments was significantly less (46%).



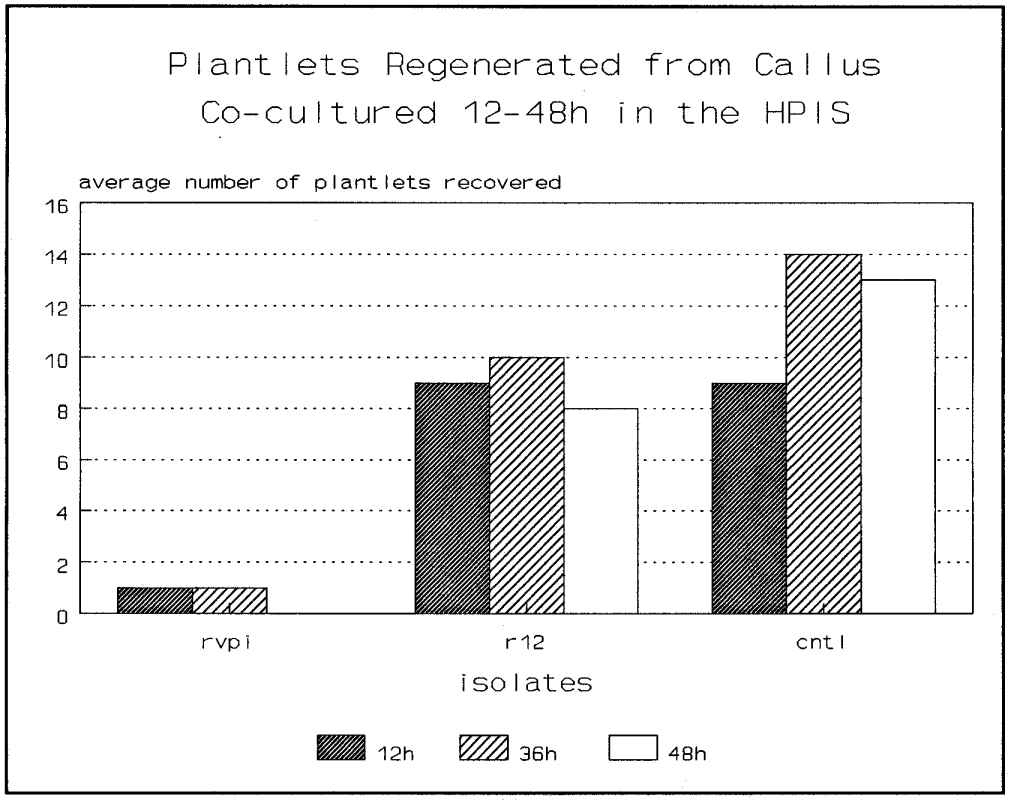
No interaction among the co-culture times and R12 or the control occurred. However, within the RVPI treatments, callus viability decreased significantly as co-culture time increased. Callus viability is inversely related to the length of co-culture time, ie. the longer the callus is exposed to RVPI, the less viable callus is recovered.



The callus that survived the HPIS co-cultures was transferred to 0-MS regeneration medium and maintained under continuous low light to induce plantlet regeneration. The number of regenerated plantlets was determined at end of two weeks. The number of plantlets regenerated/plate from callus exposed to RVPI (0.5) was significantly less than that of the R12 and control treatments (7.0 and 9.0, respectively).



Based on the results of the callus/RVPI co-cultures, bentgrass callus is capable of regenerating plantlets following exposure to RVPI for no longer than 36h. Callus exposed to RVPI exceeding 36h does not retain the ability to regenerate plantlets.



Therefore, when mass selecting for germplasm with enhanced resistance to *R. solani*, the callus/RVPI co-culture should not exceed 36h. This will insure some germplasm will be recovered for use in studies at the plantlet level using either the HPIS Chamber or a leaf bioassay method.

An *in vitro* plantlet screening with RVPI was conducted in the HPIS Chamber using the recovered plantlets from RVPI, R12 and control callus co-cultures. These plantlets were 4 weeks old when co-cultured with RVPI in the HPIS Chamber for two weeks. The plantlets exposed to RVPI appeared to stop growing after the second or third day in the HPIS Chamber. Thirty-three percent of the plantlets exposed to RVPI died. Those surviving plantlets were extremely stressed, displaying purple leaves and stunted growth. They were transferred to tissue culture boxes where vigorous shoot and root

development were initiated. The plantlets were transferred to soil when an adequate root and shoot system was developed and are currently being maintained in a growth chamber awaiting transfer to the greenhouse (figure 4). These variants should have enhanced resistance to *R. solani* due to the exposure at the cellular and plantlet levels.

VII. VARIANTS OBTAINED FROM CALLUS/RVPI CO-CULTURE EXPERIMENTS:

A large number of variants recovered from HPIS refinements experiments conducted in 1991 are established in a clonal repository at Pure Seed Testing, Inc. in Oregon and are also being maintained in the Agronomy greenhouse at Mississippi State University (figure 5). These variants were recovered from callus that survived co-cultures with RVPI during initial HPIS refinement experiments in the summer of 1991. These variants were not exposed to RVPI at the plantlet level. The plantlets were removed from tissue culture in the Fall of 1991 and allowed to "harden off" in the growth chamber. Each variant was subdivided, with one set sent to Oregon to be maintained in the clonal repository and the other set retained in the greenhouse at Mississippi State University.

In July, 1992, a crude whole plant inoculation was carried out on the variants as a preliminary mass screening. A mist chamber was set up in the greenhouse and the inoculated variants remained in 100% humidity for 48h. Within a week lesions were visibly evident. The variants were compared to Penncross on the basis of physical appearance. An average of 23% of the variants appeared healthier or less infected than Penncross, 16% were worse and 61% appeared about the same (figure 6). Some of the lesions resembled *R. solani* infection, but there were also various other lesions present, suggesting infection by other fungi. Lesions were collected and placed in pure culture to reisolate the pathogen *R. solani*. During this process other fungi in addition to *R. solani* were observed on the plates. Because several other pathogens infected the variants, it is hard to determine the resistance these variants have against *R. solani*. We think that the inoculation procedure stimulated other fungi in the soil to become active and infect the variants. In the future, a more controlled inoculation procedure will be employed for preliminary variant screening of disease resistance at the whole plant level.

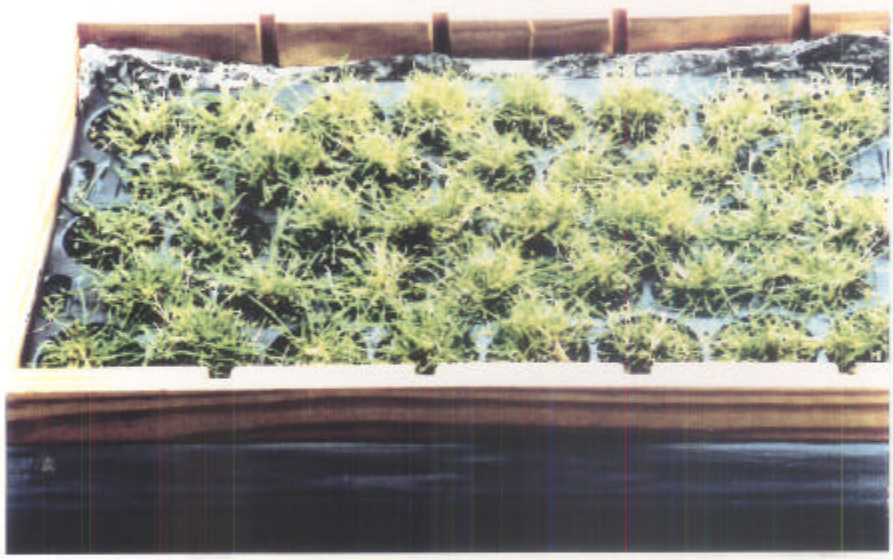


Figure 4. Bentgrass variants that have undergone selection for resistance to *Rhizoctonia solani* at the cellular and plantlet levels in the HPIS.

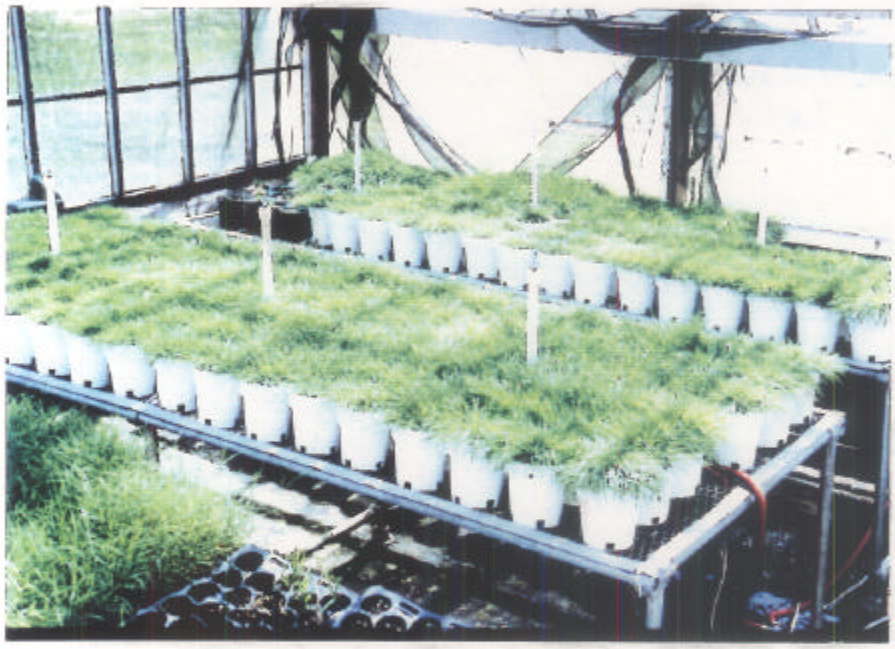


Figure 5. Bentgrass variants derived from callus/*R. solani* co-cultures in the HPIS.

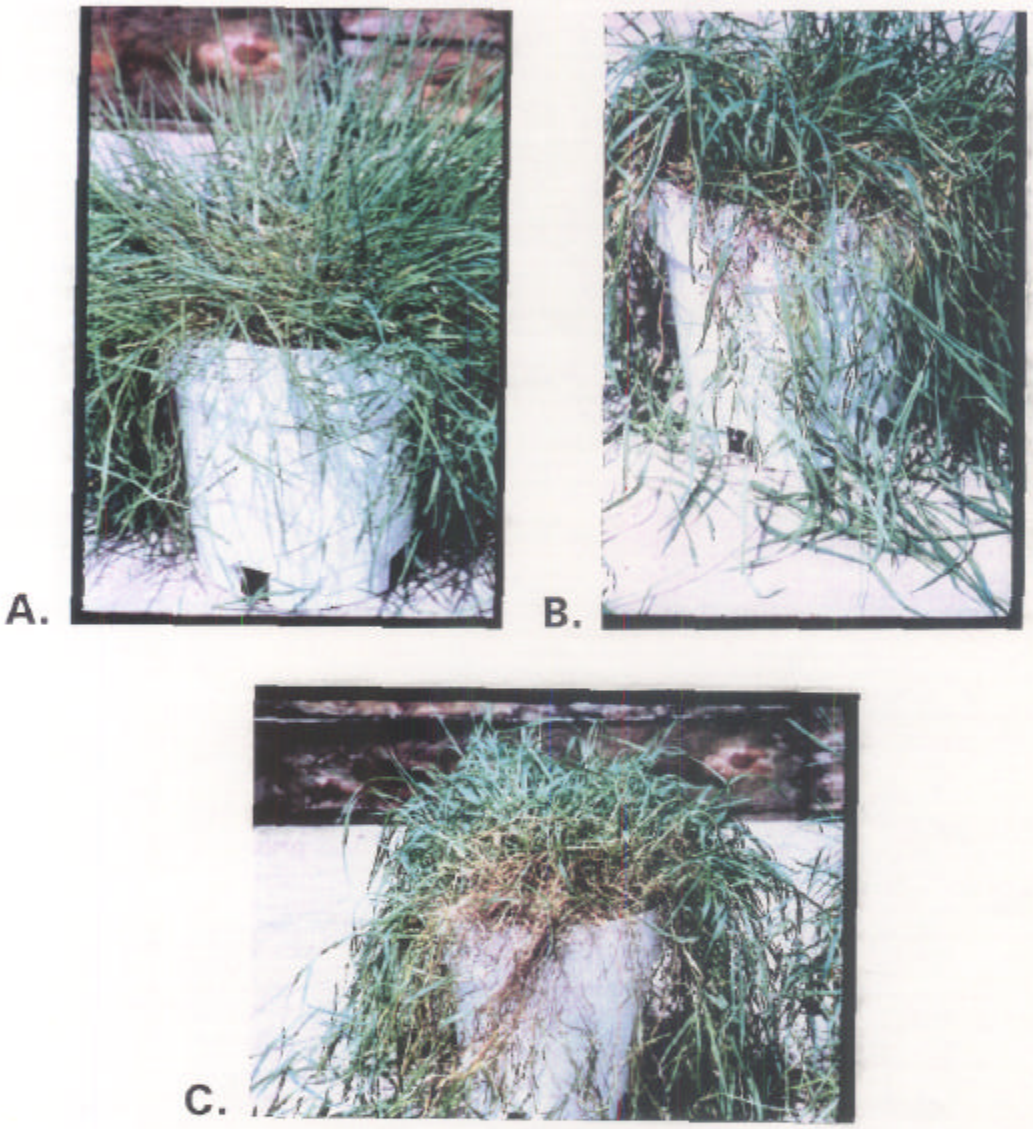


Figure 6. Bentgrass variants inoculated with RVPI. MT-Variant, slight infection (A), Penncross, moderate infection (B), and MT-Variant, extreme infection (C)