

MISSISSIPPI STATE UNIVERSITY

Refinement of the Host-Pathogen Interaction System

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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 HPIS is a unique cell selection technique which permits the simultaneous transfer of various substances from a disease organism to a callus culture during concurrent growth, yet which avoids direct physical contact between the organisms. The assembly and application of the HPIS evolved through a series of experiments dating back to 1988. With an understanding of its application for *in vitro* cell selection, we have focused our efforts in 1991 on refining HPIS protocol.

Isolates from the USGA culture collection of *Rhizoctonia* spp., (courtesy of Dr. Phil Colbaugh, Texas A&M University), were co-cultured (concurrently grown) with creeping bentgrass callus in the HPIS. The pathogenic isolates inhibited callus growth and development, whereas the non-pathogenic isolates had no effect on callus viability. Studies were conducted, to determine effects of various tissue culture media on vigor and pathogenicity of *R. solani*, primarily hormones and energy source concentrations. Various HPIS cultural studies were conducted, focusing on the length of incubation, duration of concurrent growth-interactions, establishing cultural practices for calli following co-culturing in the HPIS, and examining the persistence of toxicity within the HPIS plates.

Some important questions pertaining to HPIS protocol have been answered by these refinement studies. We know that: 1) pathogenicity at the whole plant level is similar to pathogenicity at the cellular level; 2) media components, especially growth hormones and energy sources, play an important role in the pathogenic expression of *R. solani* in the HPIS; 3) we can maximize the use of HPIS plates with successive co-cultures; and 4) *R. solani* must incubate at least seven days in the HPIS and there must be a 24-hour duration of concurrent growth-interactions between *R. solani* and creeping bentgrass callus before resistant callus can be recovered.

We are rapidly achieving an understanding of how to optimize the HPIS toward our overall and final goal of developing disease resistant variants. Remaining questions will be addressed in current and future HPIS studies.