RUTGERS UNIVERSITY

Endophytes of Turfgrasses: New Tools and Approaches

1991 Research Grant: $40,000
(Second year of support)

Dr. Peter Day
Dr. Reed Funk
Principal Investigators

This project was proposed and initiated by Dr. Peter Day, AgBIOTECH Center and Dr. Reed Funk, Department of Crop Science, Rutgers. Dr. T.M.A. Wilson, AgBIOTECH Center, is responsible for supervision of the laboratory work of the project. Program goals are to: 1) produce a germplasm collection of fungal endophyte-infected grasses concentrating on *Poa* and *Agrostis* species; 2) produce a collection of endophyte cultures for classical and molecular analysis; 3) produce endophyte-specific DNA probes; 4) use the probes to characterize endophyte variability and produce RFLP maps for taxonomy; 5) develop gene transfer methods for fungal endophytes; and 6) identify the genes responsible for insect repellent alkaloid biosynthesis and metabolism.

After extensive screening of turfgrass germplasm collections, particularly *Poa* and *Agrostis* species throughout the U.S.A. and Europe (in collaboration with Dr. Jim White, Auburn University, Alabama), we have obtained a limited number of fungal endophyte-infected grasses in these two genera. To date, however, the presence of fungal endophytes in Kentucky bluegrass and creeping bentgrass continues to elude us. Recently acquired endophytes in other *Poa* and *Agrostis* species are being cultured with a view to introducing them into germinating seedlings of Kentucky bluegrass and creeping bentgrass.

A collection of fungal endophyte cultures has been established on agar plates and contains representative isolates from a wide variety of turfgrass genera. Selected examples are being used for DNA cloning and genetic fingerprint analysis. Fungal endophyte-specific DNA probes have been produced by the polymerase chain reaction (PCR) and diagnostic fingerprints of DNA sequences generated by randomly amplified polymorphic DNA (RAPD)-PCR methods.

The RAPD-PCR technique has demonstrated an exquisite sensitivity for variation in total genomic DNA sequences, and in many cases, has shown as much inter-isolate variation as inter-specific and inter-generic variation at the DNA level. This result highlights the primitive nature of fungal endophyte taxonomy, as well as providing diagnostic banding patterns for a particular endophyte "species". We have therefore elected to provide more precise DNA sequence information to aid fungal endophyte taxonomy.

This work is in collaboration with Dr. Christopher Schardl (University of Kentucky, Plant Pathology Dept.) and requires that we obtain the DNA sequences of the poorly conserved spacer regions between the more highly conserved ribosomal RNA genes. Schardl has already developed an evolutionary tree of turfgrass fungal endophytes of the *Acremonium* genus (anamorph *Epichloe*) from a limited number of grasses and our data will provide additional resolution to this taxonomic device. Work on the development of

gene transfer methods for fungal endophytes has progressed in a parallel project through production of fungal protoplasts and attempts to introduce recombinant DNA plasmids containing convenient antibiotic resistance genes. In principle, the technique looks feasible; however, some additional selectable marker genes must be sought as *Acremonium* spp. have a high endogenous resistance to hygromycin.

Because of the absence of widely available, natural endophytes in *Poa* and *Agrostis* species of interest to the USGA, we have elected to take a different route toward production of insect-resistant or otherwise modified turfgrasses for golf courses. Since March 1991, the project has therefore focused on the development of techniques for *Poa* and *Agrostis* tissue culture and regeneration of mature culms from single cells or disorganized calli (grass tumors). This work has been extremely successful and highly regenerable embryogenic turfgrass tissue cultures have been developed.

We have also investigated the possibility of introducing foreign genes into turfgrass cells by DNA particle bombardment techniques. To date, the level of transient reporter gene expression has been encouraging and we are currently selecting for stable transformed turfgrass cell lines which express a gene conferring resistance to the herbicide bialaphos (Basta™). In parallel with bialaphos resistance, we are negotiating with several commercial organizations for genes which might confer insect resistance, virus resistance, growth retardation, resistance to fungal and bacterial pathogens and a variety of other single-gene traits. We consider this to be a major technological breakthrough for the production of transgenic turfgrass with improved agronomic performance through insertion of one, or a few desirable genes.