glufosinate ammonia was confirmed to have fungicidal as well as herbicidal properties. Therefore, we have been able to simultaneously control weeds and turfgrass pathogens (mainly *Sclerotinia ulnocarpal* and *Rhizoctonia solani*) by spraying this herbicide on transgenic creeping bentgrass expressing the gene under greenhouse conditions.

Studies have shown that the chitinase genes can make transgenic plants resistant to pathogenic fungi such as *R. solani*, etc. Research in Dr. Vargas' laboratory has shown that our transgenic creeping bentgrass clone 711, transcribing the elm chitinase gene controlled by the cauliflower mosaic virus 35S promoter, has improved resistance of plants to *R. solani* under controlled environmental conditions. Recently, Dr. Sticklen's laboratory has constructed a plasmid containing the elm chitinase gene controlled by rice actin promoter (shown to provide greater gene expression in grass family than the 35S promoter) and transformed creeping bentgrass with this construct. Theoretically, using this grass-specific promoter, we could improve the level of expression of the chitinase gene, and the degree of resistance to *R. solani* in transgenic creeping bentgrass plants.

The mannitol 1-phosphate dehydrogenase (mt1D, known for its drought tolerance) gene that we have used to transform creeping bentgrass is also confers salt tolerance, A preliminary experiment performed by Dr. Baird's laboratory has not shown any drought tolerance of transgenic plants. More studies are needed to confirm whether these plants have any tolerance to drought and/or salt.

Determining the Genetic Stability of Triploid Bermudagrasses

Mississippi State University

Michael Goatley, Jr.

Start Date: 1998
Number of Years: 3
Total Funding: $72,790

Objectives:

Determine the origin of the off-types that arise in hybrid (triploid) bermudagrass golf greens through a combination of cytological and molecular analyses.

Off-types in hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis* Burtt-Davy) putting green varieties are a persistent problem in southeastern golf courses. They disrupt green uniformity and interfere with ball roll; their effects sometimes necessitate green replacement. Our current goal at Mississippi State is to learn if their formation has a genetic and/or cytological basis. To minimize contamination to the smallest practical extent, we are conducting a greenhouse study using sterilized growth medium.

The genetic detection phase involves cross-species hybridization between bermudagrass DNA and RFLP clones from maize (*Zea mays* L.), and chromosome counts of bermudagrass root-tip cells for the cytology. We chose to use maize clones for two reasons. First, maize has a well characterized genetic map with many markers to choose from, so we know the chromosomal location of the clones we selected. Second, there is considerable evidence that gene order among the grasses is strongly conserved. Therefore, we can select clones from maize with reasonable assurance that we are monitoring a large portion of the bermudagrass genome, rather than using markers that are potentially biased towards small regions of the genome. Additionally, we are attempting to learn if there is a relationship between off-type formation in bermudagrass green varieties and chronic application of mitotic inhibitor herbicides such as pendimethalin and oryzalin.

Off-types in other grasses, most notably the cereal grain species, are frequently due to absence of one or more chromosomes, a condition known as aneuploidy. This chromosome loss may occur spontaneously, but it also may be induced with the application of mitotic inhibiting compounds. Oryzalin is now frequently used in place of colchicine to induce chromosome doubling for production of doubled haploids in lab experiments, mainly because it is much less toxic to humans than colchicine. When a plant's exposure to oryzalin is inadequate, chromosome doubling is incomplete, and aneuploidy sometimes results. If this occurs in bermudagrass putting green varieties, we hope to correlate it with the formation of off-types.

To meet this goal, six varieties are being subjected for one month to weekly drench applications of a 0.5X rate of oryzalin or pendimethalin in a replicated greenhouse experiment, designed as a randomized complete block. This is intended to expose the plants to a cumulative 2X rate application. The varieties are *TIFGREEN, TIFDWARF, TIFEAGLE, MS-SUPREME, CHAMPION,* and *FLORADWARF*. The grasses were established from small stolon pieces (2 nodes in length) in horticultural flats containing an approximate 75:25 masonry sand:peat moss mix, and are maintained at about 0.25-inch mowing height to encourage lateral growth in the flats. As a safeguard against latent contamination, the flats are irrigated for 10 days prior to stolon planting to encourage germination of seed or other dormant propagules so that they can be eliminated.

When the grass reaches 75 percent coverage of the flat, herbicide treatment is commenced. At the conclusion of the herbicide applications, the flats are left unmowed to detect any morphological off-types that might arise. Presence of differences will be determined by comparing leaf blade length and width, as well as internode length and width, between untreated checks and the treated clones using a two-tailed Dunnett's test (following a significant ANOVA). Stolons from these treated flats are then sampled to establish new flats. To date we have completed one cycle of the experiment and are initiating a new cycle with stolons from the previous round of treatment.

To test the efficacy of the herbicides, leachate from the final herbicide application at the end of the first treatment cycle

was used in a bioassay. Herbicide effectiveness was measured as suppression of annual ryegrass seed germination. Oryzalin suppressed germination about as effectively (54.9% of the untreated control) as pendimethalin (55.7% of the untreated control). This will allow us to adjust the treatments to achieve similar *efficacy rates* for both herbicides.

Concurrently with the greenhouse experiment, we are using selected RFLP markers taken from maize (*Zea mays* L.) to check for appearance of DNA polymorphism that might stem from chronic exposure of the grasses to the herbicide. It is unknown if these compounds are mutagenic; however, many organic compounds, including some herbicides, have mutagenic activity at high rates and/or chronic exposure levels). RFLPs will allow us to monitor phenotypically silent mutations. Although these do not result in observable off-types, appearance of new RFLP band polymorphism due to the treatments will guide us in determining application rates that will enhance the odds in favor of producing an off-type.

To date, 71 maize cDNA clones have been tested in cross-species hybridizations against bermudagrass DNA to identify those that show an adequate signal in Southern blots of bermudagrass. Roughly 75 percent of those tested are usable, and there are three subclasses within this category: a) those showing strong signals on bermuda, b) those with moderate signals, and c) those that show a weak signal (these three subclasses are present in fairly equal proportions). These selected probes will be used to probe Southern blots of bermudagrass genomic DNA samples from flats subjected to the herbicide treatments.

Cytological examinations of the six varieties to this point have revealed only the expected number of chromosomes for triploid bermudagrass ($2n = 3x = 27$). I

Genetic Enhancement of Paspalum for Recreational Turf

University of Georgia

Ron R. Duncan

Start Date: 1998
Number of Years: 5
Total Funding: $125,000

Objectives:

1. *Ecotype evaluations off-site and industry collaboration.*
2. *Creation of additional genetic diversity within the species.*
3. *Genetic profiling of ecotypes.*

AP-10 (greens) and Fwy-1 (PI 509018-1: fairway/tees) ecotypes are slated for submission to the University of Georgia germplasm release committee during early 1999. Sufficient vegetative material will be available if the releases are successful. These seashore paspalums have exhibited excellent aggressiveness and performance on golf courses and under sod production. The darker green genetic color and turf quality traits are parallel to or better than most dwarf bermudagrasses. Genetic analysis research involving simple sequence repeats (SSRs or microsatellites) has progressed to the point of effectively profiling individual ecotypes for plant variety protection using trinucleotide repeats.

Wear tolerance mechanisms differ between paspalum and bermudagrass. Recoverability rates were identical between the two species. Wear tolerance in paspalum was attributed to leaf total cell wall contents (50% of the variability) while tolerance in bermuda was due to stem moisture (41%) and stem cellulose (32%).

Fertility studies have revealed that paspalum is more highly responsive to $CaNO_3$ than NH_4NO_3, NH_4SO_4, or urea. These highly soluble fertilizers appear to be critical for rapid establishment and recoverability, and may be important during long-term management in stressed environments. I

Long-Term Preservation of Clonally Propagated Turfgrass Species

Colorado State University

Harrison G. Hughes

Start Date: 1998
Number of Years: 2
Total Funding: $49,701

Objectives:

1. *Develop suitable micropropagation procedures for selected genotypes of bermudagrass, zoysiagrass, saltgrass and buffalograss.*
2. *Develop suitable shoot tip culture media (STCM) for the four species.*
3. *Examine cryopreservation of the four species using vitrification methodologies.*

Clones of saltgrass (6), buffalograss (3), bermudagrass (1), and zoysiagrass (1), were established in the greenhouse and grown for a source of materials to put into tissue culture. It is important to establish *in vitro* protocol for each clone because cryopreservation requires very small growing points which will need to be established *in vitro* after freezing. If the tissue contains bacteria or fungal contaminates, they will likely overgrow any plant tissue thus obscuring positive results.

Various techniques involving different bleach treatment times and *PPM* (a commercially patented compound with antibiotic activity) concentrations, were used to disinfect tissue samples of buffalograss, bermudagrass, and saltgrass. Basal medium used was half strength MS and Nitsch and Nitsch vitamins plus 5 mg L^{-1} thiamine, 2 mg L^{-1} glycine and 30 g L^{-1} sucrose. Best results were obtained when small sections (1 to 2 cm) were used. In addition, either a bleach soak for 20 minutes for buffalograss, or 10-minute soak in bleach containing 5 mg