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<sub>3</sub> than NH<sub>4</sub>NO<sub>3</sub>, NH<sub>4</sub>SO<sub>4</sub>, 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<sup>-1</sup> thiamine, 2 mg L<sup>-1</sup> glycine and 30 g L<sup>-1</sup> 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