USGA 1999 Executive Summary
Mississippi State University

Dr. JM Goatley Jr., Dr. JV Krans, and DW Davis. Dept. of Plant & Soil Science, Mississippi State University, Mississippi State, MS 39762.

The goal of the turfgrass breeding and management group at Mississippi State is to determine the source of off-type sectors that occur in bermudagrass (*Cynodon dactylon x transvaalensis* Burtt-Davy) putting green varieties.

Off-types which have been studied in other grass species are frequently due to absence of one or more chromosomes, a condition known as aneuploidy. This chromosome loss may occur spontaneously, or be induced by application of mitotic inhibitor compounds, such as dinitroanilines. For example, oryzalin is frequently used in place of colchicine to induce chromosome doubling in lab experiments. When a seedling receives a dinitroaniline dose which is inadequate for suppression of cell division, aneuploidy may sometimes be the result. If aneuploidy occurs in bermudagrass greens varieties which have been chronically treated with dinitroanline, we wish to correlate it with the formation of off-types.

Our multiple-cycle experiment integrates results from laboratory, greenhouse, and field tests. Each cycle begins with a greenhouse phase. The varieties Tifgreen, Tifdwarf, Tifeagle, MS-Supreme, Champion, and Floradwarf are established from small stolon pieces (2 nodes in length) in horticultural flats containing a methyl bromide-fumigated 3:1 masonry sand:peat moss mix, and grass is maintained at about ¼" mowing height to encourage lateral growth in the flats. When the grass reaches 75% coverage of the flat, they are subjected to a single drench application equivalent to a 2X rate of oryzalin or pendimethalin (controls receive no herbicide). At the conclusion of the herbicide application, the flats are left unmowed so they can recover from the stringent treatment.

After the treated grasses recover, sod samples are collected and shredded into sprigs. The sprigs are used for two purposes- first of which is to establish new flats for the next cycle of treatment. The second is for our field experiment phase; the samples will be planted in a fumigated field plot which has been set aside for long-term "archiving" of all grass samples taken from the treated flats during the experiment. This plot was planted with tall fescue for 6 seasons prior to the fumigation and has no observable bermudagrass contamination. The purpose of archiving these samples is to give any potential variant sectors a chance to grow out and express their altered phenotypes. The plots are 4' x 4' and are replicated within each variety x treatment x cycle combination. The plots are allowed to reach full coverage without mowing, to lower the chance of inter-contaminating the hybrid bermudas in our test. As of November 1999 three cycles of herbicide treatment have been completed and a fourth is underway. We intend to complete a total of six to seven cycles.

The laboratory phase of our experiment involves screening of AFLP primer pairs to establish a control DNA fingerprint for each of the 6 varieties. The technique allows us to visualize radio-labeled DNA bands which have been

separated on a 5% polyacrylamide gel. To date 38 out of 64 commercially available AFLP primer pairs (from Keygene, Inc, distributed by Gibco/BRL) have been screened on the controls. All six of our triploid bermudagrass genotypes can now be clearly distinguished from one another. Furthermore, this technique produces so many potentially informative DNA fingerprint bands, that it should allow monitoring of changes in fingerprint within each variety, whether they occur in response to treatment or as a result of spontaneous mutation. As our treated material becomes available, it will be tested against these primer pairs to detect fluctuations from the control samples.

A new technique which greatly simplifies root-tip cytology in bermudagrass has been adapted from a technique used in maize cytology. Chromosome counts are made from bermudagrass root-tip cells which have been subjected to 150 psi nitrous oxide gas for 2 hours. These are fixed in ice-cold 90% acetic acid for 30 minutes, digested in 1% cellulase, 0.5% pectinase and then suspended in 1:1 methanol:acetic acid. The root-tips are then smeared on a regular microscope slide and air-dried for 10 minutes before applying 2% acetocarmine for visual examination of chromosomes. This technique routinely yields several dozen clearly countable mitotic figures per slide. Our cytological examinations of the six varieties to date have only revealed the expected triploid number of 27 chromosomes.

Dr. JM Goatley Jr., Dr. JV Krans, and DW Davis, Dept. of Plant & Soil Science, Mississippi State University, Mississippi State, MS 39762.

Research results anticipated:

To date, we have completed three cycles of treatment and a fourth is underway. Cycle 1 was established in the field in October, cycle 2 is ready to plant in the field in the spring, and cycle 3 treated material will be planted immediately after cycle 2. We anticipate doing two to three more cycles in 2000 for a total of six to seven treated cycles.

The most critical issue to resolve in the grant this fall was that of producing reliable DNA fingerprints for each of the 6 genotypes. As of November 1999, our AFLP technique has provided us with unique fingerprints for all of our genotypes. The technique is straightforward and reproducible, and is without a doubt the best method currently available for detecting DNA polymorphisms that may arise among our treated material.

Using the gel rigs available at Univ. of Missouri, 96 samples can be screened per day. As the quality of DNA is paramount to success using AFLPs, we are currently experimenting with a new DNA isolation protocol which we hope will speed up that part of the process. At present, DNA isolation must be done using cesium chloride gradients, which require much time and labor. If we succesfully gear up the DNA isolation procedure, all of the samples that have been archived at Mississippi State could be processed in about 1 month, and DNA analysis of the entire experiment (456 samples if we complete only 6 cycles, 532 if we complete 7) could be completed in 3-4 months time.

The new root-tip technique learned from Dr. Akio Kato at Univ. of Missouri will also aid in making a timely cytological analysis of the material. Using the large nitrous oxide chamber that Dr. Kato has built, 50 samples can be processed per day. Unlike using the traditional squash technique, the slides can be stored in a dessicator for quick counting after fact.

In our initial efforts in 1998, we attempted to visualize bermudagrass off-types in greenhouse flats after herbicide treatment, without providing the treated material any means of spreading out. With that approach, we could only visualize off-types for plant height, color, or the dimensions of unmowed leaves. We followed this course with the aim of absolutely controlling the factor of contamination. This was a worthy goal. However it was not feasible to exercise this level of control over contamination while concurrently trying to answer the question we were really interested in- which was how do off-types in bermudagrass really occur?

Further, we want to know how these off-types arise in mowed plots and how the chronic application of dinitroanlines might promote their appearance. The modifications made to the experiment by adding a field phase will greatly improve our chances of recognizing any variant plant sectors produced, and will be more relevant to turf in the way it is managed on real golf courses.