

USGA 2000 Executive Summary
Mississippi State University

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Application of dinitroaniline class pre-emergent herbicides is an integral part of triploid bermudagrass sod production. The active ingredients in their formulation are known to cause chromosome lagging at mitosis, which can give rise to aneuploids. Aneuploids are plants with higher or lower chromosome numbers than expected. Aneuploidy can cause changes in leaf blade width and length; similar changes are often observed in naturally-occurring off-types. Another alternative which might explain off-types is that long-term, chronic dinitroaniline usage is mutagenic to DNA of perennially-grown bermudagrass; therefore, dinitroaniline herbicides may be a causal agent in off-type formation. A third possibility is that herbicide usage may favor spontaneous (naturally-occurring) mutations which cause off-types.

Mutation detection tests performed on mammals and bacteria have found that highly-purified oryzalin and pendimethalin are not mutagenic. While these tests supply useful evidence about the safety of the active ingredient, they do not give us a "yes or no answer" about the potential mutagenicity (to plant tissues) of long-term chronic applications of the formulations as they are applied in the field.

Our goal is to learn more about off-type formation using a 2 phased experiment. The first phase has been an "off-type induction" experiment. Six selected triploid bermudagrass varieties ('Champion', 'Floradwarf', 'MS-Supreme', 'Tifdwarf', 'Tifeagle', and 'Tifgreen') are subjected to acute (2X) doses of oryzalin and pendimethalin, to determine if off-type plants can be produced. This has been performed in greenhouses using methyl bromide-sterilized growth medium, to eliminate contamination as a factor contributing to off-type recovery. A total of 6 cycles of induction will be completed at project's conclusion.

The second phase of the experiment is an "off-type detection" experiment. We are using 3 separate routes to classify any off-types which might be induced by the off-type induction experiment. These are: morphological trait classification, AFLP profiling (a method of DNA fingerprinting), and chromosome number counting.

Morphological traits (leaf blade length and width, internode length and diameter) showed no significant variation among cycles or among herbicide treatments, indicating that no morphological off-types were found by our random sampling. The only significant differences simply traced to pre-existing intervarietal variation.

Our molecular results to date show that the majority of missing bands occurred in the pendimethalin treatment. However, since missing AFLP bands occurred in the untreated control, we cannot make a firm conclusion regarding what caused their disappearance; analysis of further cycles will help form a clearer picture. All varieties have shown at least one band loss.

Table 1. Triploid bermudagrass variety band loss over 3 cycles of dinitroaniline herbicide treatment.

Cycle Number	Untreated Control	Oryzalin (2X)	Pendimethalin (2X)
0	No missing bands	No missing bands	No missing bands
1	No missing bands	No missing bands	No missing bands
2	MS-Supreme; 113 bp band missing	Floradwarf; 360 bp band missing	Champion; 105 bp band missing MS-Supreme; 342 bp band missing Tifeagle; 157 bp band missing
3	No missing bands	No missing bands	Tifdwarf; 128 and 142 bp bands missing Tifgreen; 81, 110, 115, and 128 bp bands missing

Our cytology data has only been completed through Cycle 2. To date, no aneuploids were detected, with all samples showing the expected 27 chromosomes. Our chromosome counting technique requires fresh tissue (so we cannot use fixed specimens), which are unobtainable during dormancy, so this off-type detection method has lagged behind the other 2 methods.

Additionally, we have conducted a small experiment to determine if bermudagrass contains active transposable elements (also known as "jumping genes") similar to those characterized in other grass species, such as rice and maize. These naturally-occurring elements are responsible for many of the spontaneous morphological mutations found in those species. To date, we have performed PCR-based experiments to determine if these elements are present in bermudagrass. We have found 4 elements from maize which are also in triploid bermudagrass DNA. These are named "Grande", "Huck-2", "Prem-1", and "Prem-2". Since there is some evidence that off-types may be a spontaneous occurrence, we hope to document the presence and activity of these elements as a possible explanation for bermudagrass off-types.

Expected Research Results for 2001:

Currently, we are performing AFLP profiling of Cycle 4 grass and statistical analysis of Cycle 4 morphological traits. These will be completed in December 2000.

The chromosome counts for cycles 3-6 will be completed during April-July of 2001.

Currently, we are growing Cycle 5 grass for dna treatment in spring 2001, and we will finish Cycle 6 in the summer of 2001.

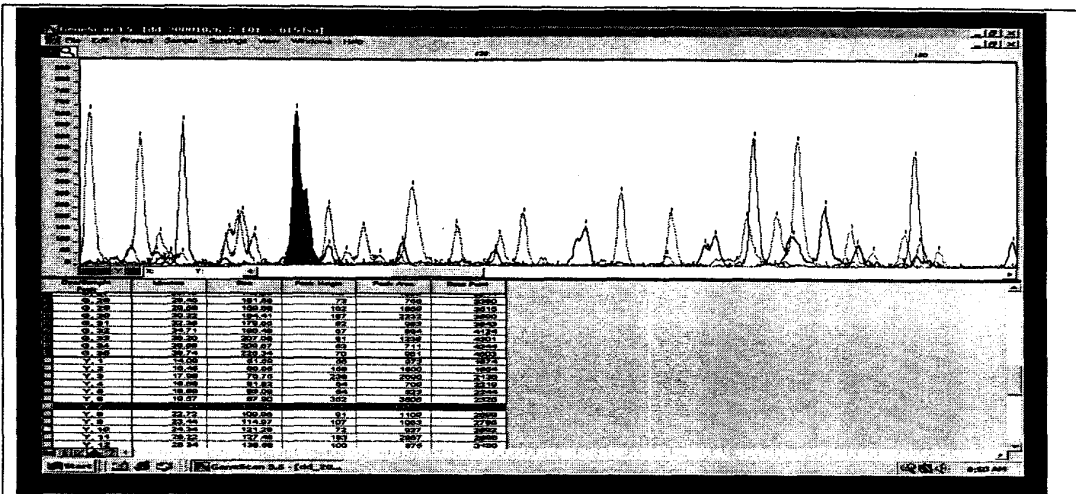
In the event that we are unable to induce off-types, we have assembled a small collection of off-types from golf courses and sod farms in Mississippi and Louisiana. These will be analyzed for differences in AFLP profile and chromosome number from their progenitor varieties.

Turf research improvements made with this grant:

This grant has facilitated the introduction of 2 new methods to turfgrass genetic analysis:

First is the use of capillary electrophoresis for rapid production of AFLP profiles (see Figure 1). This technique has not been previously used on warm-season turfs. This facilitates genetic analysis in three ways- first, it is not gel-based, so it is simpler to perform. Second, it produces more accurate estimates of DNA band size than previously possible (resolution is accurate to within 1 base pair). Finally, it makes data output simpler by automatically exporting DNA band data into spreadsheet form.

Figure 1. Capillary electrophoresis image of a triploid bermudagrass AFLP DNA fingerprint (note the spreadsheet data underneath the colored peaks). The different colored peaks correspond to different AFLP primer pairs which are electrophoresed simultaneously).



Second is the use of nitrous oxide for reproducible production of accurate bermudagrass chromosome counts. Prior to introducing this technique, accurate chromosome counts in bermudagrass were fairly difficult to obtain; counts from the older methods often resulted only in ploidy determination rather than in detection of the exact chromosome number of individuals.

The research results contained in this summary were previously presented in a talk by DW Davis at the American Society of Agronomy Meetings in Minneapolis, MN on 11/9/2000; "Determining the Genetic Causes of Off-type Formation in Triploid Bermudagrasses."