Determining the Genetic Stability of Triploid Bermudagrass

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Off-types in bermudagrass (Cynodon dactylon x 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: 1) maize has a well characterized genetic map with many markers to choose from, so we know the chromosomal location of the clones we selected, 2) 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 which 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 may also be induced with application of mitotic inhibitor 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 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 are 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 ¼" 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% 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 units 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) than 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 polymorphisms 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 off-types, appearance of new RFLP band polymorphisms under treatment will guide us in determining application rates which will enhance the odds in our 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 which show an adequate signal in Southern blots of bermudagrass. Roughly 75% of those tested are usable, and there are three subclasses within this category- those showing strong signals on bermuda, those with moderate signals, and 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.