EXECUTIVE SUMMARY

PROJECT:	Low temperature and drought regulated gene expression in bermudagrass
INVESTIGATOR(S):	Wm. Vance Baird (P.I.) and Landon C. Miller
INSTITUTION:	Clemson University (Horticulture Department)
SPONSOR:	United States Golf Association, Green Section

Nuclear DNA samples have been isolated from three cultivars of bermudagrass ('Midiron', Tifway' and 'Tifgreen'). 'U-3' will be included in these studies. The DNA preparations are of sufficient quality (high molecular weight, and free of contaminating proteins and polysaccharides) and quantity that restriction endonuclease digestion can readily be performed. The digested DNA will be used in differential hybridization analysis (Southern blots) of the bermudagrass nuclear genome. Heterologous gene probes for nuclear sequences correlated with induction at low-temperatures or by exposure to conditions of water deficit, originally isolated from dicotyledonous species, will be used to screen these membrane blots. Initial surveys by dot/slot-blot analysis, using undigested genomic DNA and entire plasmid clones as gene probes, indicate that many of these genes will likely detect related sequences in these cultivars, and these may prove to be evolutionarily conserved in bermudagrass. A postdoctoral research associate (Dr. Jiyu Yan) has recently joined the project, and is focusing here current efforts on this preliminary characterization of the bermudagrass nuclear genome. Institutional support for the purchase of additional growth chambers has been acquired. These chambers will be utilized in establishing standard conditions of temperature, light and humidity to reproduce the environmental parameters necessary for low-temperature acclimation. Characterizing the expression of sequences related to the heterologous gene probes under these standardized environmental conditions is the next objective of this project.

PROGRESS REPORT: May 1 to October 31, 1993

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INSTITUTION: Clemson University

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Following an eight month search for a graduate student who would focus their degree research upon this project, I was fortunate to hire Dr. Jiyu Yan - - a 1993 graduate of Virginia Polytechnic Institute and State University, in Blacksburg, VA. She completed her Ph.D. degree in the Department of Crop and Soil Environmental Science under the direction of Prof. R. E. Schmidt (and Prof. D. M. Orcutt of the Department of Plant Pathology, Physiology and Weed Science). The title of her dissertation is "The Influence of Plant Growth regulators on Turfgrass Membrane Lipids, Tolerance to Drought/Salinity Stress and Nutrient Efficiency". During here degree work she gained extensive experience in analyzing the growth regulator, lipid and sterol composition of different turfgrasses.

Dr. Yan has been here a little over a month and is working into the new laboratory and greenhouse environment very well. She is mastering the methods for nucleic acid isolation, both from bermudagrass and bacteria harboring plasmids containing heterologous gene clones. She has completed preparations of high quality DNA from bermudagrass cultivars such as 'Midiron', 'Tifgreen' and 'Tifway'. She has used both leaf and stem material with similar positive results. We recently received a shipment of 'U-3' bermudagrass (A-12182, courtesy of Prof. C. M. Taliaferro at Oklahoma State Univ.) and this material will be processed before the end of November, once the plugs "green-up" in our hot house.

DNA isolation has proven to be somewhat problematic. Although yields from young fresh (or frozen) leaf material are very high, the DNA may be degraded, as a result of its susceptibility to physical stresses (e.g., shearing) during the isolation process, unless overt care is taken. This problem is encountered regardless of cultivar used or which salts/chaotropic agents are employed in the isolation buffer. However, with care very pure (as evaluated by spectrophotometry), high molecular weight DNA (as evaluated by size fractionation in agarose gels) can be routinely isolated. Dr. Yan has confirmed that her DNA isolations are appropriate

substrates for restriction endonuclease digestion. She, with the aid of my technician (John Wells), is currently preparing genomic Southern blots for the untreated cultivars. Dr. Yan is modifying the nucleic acid isolation procedure to optimize it for the isolation of pure, intact RNA, from which she will prepare poly-A^+ (i.e., mRNA) enriched fractions. Our initial results have shown that only standard RNAse-free conditions need to be maintained in order to isolate intact, high quality RNA from bermudagrass.

My technician has prepared four of the twelve heterologous gene probes we have available for analysis of the bermudagrass nuclear genome. He is teaching the isolation and labeling procedures to Dr. Yan and she will complete the processing of the remaining gene clones, or others we may acquire. These probes include COR (cold regulated) gene sequences from *Arabidopsis*, the osmotin gene from tobacco and the betaine-aldehyde dehydrogenase gene from spinach. Initial dot-blot analyses using uncut genomic DNA and entire plasmid clones are encouraging in that it appears many of these sequences will prove to be conserved in bermudagrass.

Clemson University and the Horticulture Department have provided funds ($32,500) for the purchase of two additional environmental growth chambers (Conviron, model E-15). These chambers are capable of maintaining temperatures near freezing with or without the light banks activated, a necessity for us to mimic conditions conducive to low temperature acclimation. We finished the specification write-ups and purchase order in mid-September. I received word today that the contract has been sent to the manufacture, so we anticipate that the chambers will be in place by the new year.