# *Transformation of Bermudagrass for Improved Fungal Resistance*

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**Objectives:**

1. Isolate, identify, and characterize chitinases and glucanases and their genes that have high activity against Spring Dead Spot (SDS).
2. Develop an efficient protocol to transform (genetically engineer) bermudagrass.
3. Transform and characterize bermudagrass with the antifungal chitinase and/or glucanase genes directed against the SDS casual organism.

**Start Date:** 1998
**Project Duration:** 5 years
**Total Funding**: $125,000

Spring dead spot (SDS) is a major bermudagrass disease in the southern United States. The causal agents are *Ophiosphaerella herpotricha* and *O. korrea.* Improvement in resistance in sexually isolated lines requires the development of genetic transformation protocols.

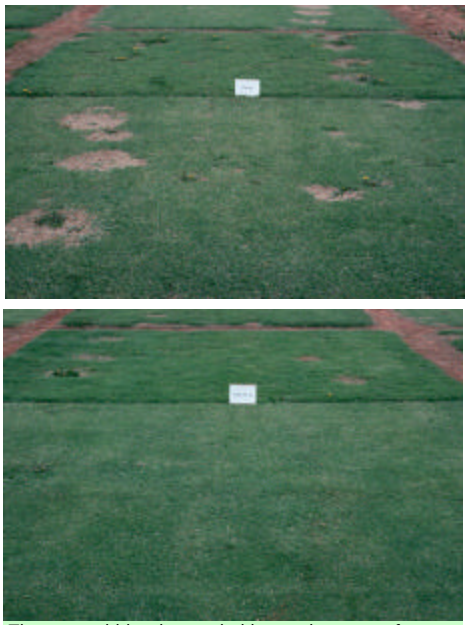
The objectives of this work are to (1) develop an efficient bermudagrass transformation system, (2) isolate genes and/or factors with specific activity against the causal agents of spring dead spot and other fungal diseases, and (3) utilize these agents or genes to increase resistance to fungal diseases.

Transformation of the bermudagrass variety 'Brazos' was performed using the biolistic bombardment with DNA containing an expression cassette that included the bar gene coupled to a constitutive ubiquitin promoter. The bar gene codes for an enzyme that metabolizes the herbicide Liberty (bialaphos).

Evaluation of all 671 putative transformants indicated that none of these plants were transgenic. New transformation experiments utilizing higher concentrations of bialaphos have been initiated.

A gene construct with a hygromycin resistance gene is also being assembled to be evaluated for bermudagrass transformation. Screening of elite lines in the bermudagrass breeding program has identified a turf-type genotype (PCR-58) with tissue culture and plant regeneration capabilities similar to 'Brazos'. PCR-58 will be utilized in future transformation experiments.

A microorganism with potent activity against *O. herpotricha* was recently isolated and identified. Activity of the antifungal factor was stable *in vitro* over a six-month period. The microorganism was identified to the genus level using



*The new cold-hardy, seeded bermudagrasses from Oklahoma State University (below) have superior spring dead spot resistance than older seeded cultivars (above).*

GC-FAME, Biolog, and 16S ribosomal sequence analysis. Activity was due to the secretion of several potent antifungal factors that were stable under the harshest conditions.

Isolation of the factor using preparative SDS-PAGE and reverse phase chromatography was successful. In addition, a new purification procedure was developed to permit the purification of large quantities of the bioactive substance. The active compounds were identified. We are interested in developing either the microorganism or the antifungal factor as a biocontrol agent.

We also isolated eight additional endogenous microorganisms with antifungal activity from bermudagrass crown tissues. The microorganisms were identified using their 16S ribosomal sequence. Characterization of the active components is in progress. We developed a sensitive assay that is capable of quantitatively determining the relative infection state of bermudagrass crown tissues.

A functional genomic analysis was initiated during the past year to study gene expression profiles between tolerant (OKS 91-11) and susceptible (Jackpot) varieties when infected with *O. herpotricha.*

Suppression-substractive hybridization (SSH) libraries were created to identify genes that are up or down-regulated in response to fungal infection. These clones are currently being sequenced and they will be utilized to study bermudagrass defense responses to *O. herpotricha.*

## Summary Points

- Transformation techniques for bermudagrass using biolistic gun are being developed.
- A turf-type genotype, PRC-58, was identified with tissue culture and plant regeneration capabilities.
- A sensitive assay was developed to determine the relative infection state of bermudagrass crown tissue.
- Genomic analysis was initiated during the past year to study gene expression profiles between tolerant (OKS 91-11) and susceptible (Jackpot) varieties when infected with *O. herpotricha.*
- A microorganism with potent activity against *O. herpotricha* was isolated and the anti-fungal factor it produces was stable *in vitro* for six months.