ANNUAL REPORT TO

THE UNITED STATES GOLF ASSOCIATION

GREEN SECTION

Project: MONOCLONAL ANTIBODIES FOR RAPID DIAGNOSIS OF PATCH AND NECROTIC RING SPOT DISEASES OF TURFGRASS

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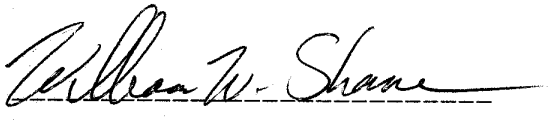

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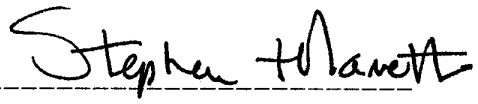

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Executive Summary

The diagnosis of summer patch and necrotic ring spot diseases of Kentucky bluegrass, caused by Magnaporthe poae and Leptosphaeria korrae, respectively, can take months with current techniques. Monoclonal and polyclonal antibodies which are specific for these two pathogens are being developed to speed the diagnostic procedure.

Enzyme-linked immunosorbent assay techniques were developed with a rabbit-derived polyclonal antibody against LK. Seventeen antibody-producing monoclones were developed that 'recognize' L. korrae strain 56289. Additional work is continuing to develop assay tests with the desired sensitivities for detection of L. korrae and M. poae in clinic samples.

Rationale

The diagnosis of summer patch and necrotic ring spot diseases of Kentucky bluegrass, caused by Magnaporthe poae and Leptosphaeria korrae, respectively, is very difficult with current techniques due to the non-distinctive disease symptoms and culture characteristics. New technologies are available that allow the rapid identification of micro-organisms through the use of antibodies derived from animal serum. The impact of this new approach is already being felt in the area of turfgrass management. Antibody-based kits are in use or being developed by private companies for detection of pythium blight, brown patch and dollar spot of turfgrass.

Development of antibody-based detection kits for necrotic ring spot and summer patch by private companies in the near future will likely be less likely because 1) these two newly discovered disease complexes are not well understood, and 2) the two pathogens attack crowns and roots rather than foliage, making sampling and assaying more difficult.

The immediate benefit of antibody-based assay systems for summer patch and necrotic ring spot will be for investigating the characteristics of the two pathogens. A second benefit will likely be a straightforward diagnostic test suitable for use by clinics and turf managers.

Progress Report

Funds from the USGA for this project became available on approximately February 1, 1987. Approximately 8 months have elapsed in the 12 month grant period. Our work has focused primarily on the development of an antibody detection system for Leptosphaeria korrae--similar work now beginning with Magnaporthe poae will benefit from the experience we have gained with the previous system.

Both polyclonal and monoclonal antibodies are being developed against LK and MP. Polyclonal antibodies can be obtained more rapidly than monoclonal antibodies. Polyclonal antibodies have certain advantages over monoclonal antibodies and visa-versa. In some instances combinations of both poly- and monoclonal antibodies are used in plant disease diagnosis. Monoclonal antibody production in most cases is preferable because the antibody source is consistent and inexhaustible. We are increasing the likelihood of developing a usable antibody system through the use of both monoclonal and polyclonal antibodies.

The following is a summary of progress on the project to date.

Preparation of antigen

American Type Culture Collection strains of Leptosphaeria korrae (LK), and Magnaporthe poae (MP) (formerly known as Phialophora graminicola) were grown in shake cultures of potato-dextrose broth for 2-3 weeks. The resultant fungal mycelia were washed with distilled water, lyophilized, ground to a fine powder in liquid nitrogen, and stored at 4 C until needed for antibody production.

Polyclonal antibody production

A female New Zealand white rabbit was injected in the left inner thigh with a 50 mg dry weigh of LK strain 56289 suspended in 1.5 ml of 0.01 M phosphate buffered saline pH 7.1. This was followed 7 days later by a second injection. The rabbit was bled through a marginal ear vein 6 weeks following the second injection. A second bleeding was performed 2 months later.

Antibody sensitivity to LK mycelia was tested with an Enzyme linked immunosorbent assay (ELISA). Using 0.215 ng LK/ml as an antigen concentration ELISA results revealed the antisera titer for bleed #1 was 1:1024 and for bleed #2 was 1:4096. These titers are very promising, however, the selectivity of the antisera has yet to be determined.

Cultures of Magnaporthe poae are being prepared in the same manner as LK for injection into a second rabbit for production of polyclonal antibodies to MP.

Monoclonal antibody production

Production of monoclonal antibodies against LK is being done with the technical assistance of the monoclonal facility under the direction of Dr. Bruce Zwilling, Dept. of Microbiology, Ohio State University.

Ten mice were immunized with LK followed by a booster inoculation. The mice were sacrificed and the spleen cells were harvested. The spleen cells were fused with myeloma cells to obtain 18 perpetually growing, antibody producing hybridoma cells (Fig. 1).

Culture filtrates from these 18 hybridoma cell lines were screened against LK at 0.215 ng/ml with ELISA to determine the reactivity of the antibodies. Out of the 18 culture filtrates tested five reacted strongly, five reacted moderately, seven reacted weakly, and one did not react at all (Fig. 2). The sensitivity of serum from the 10 best clones appears to be very adequate for our purposes. The selectivity of the clones has yet to be determined.

Future Steps

Continued screening of poly- and monoclonal antibodies to LK will be

done to determine their specificity and sensitivity to additional strains of LK (Table 1). Other fungi that may be found associated with patch or ring symptoms will also be screened to verify that the chosen antibodies have the desired selectivity (Table 1). Antibody sensitivity and selectivity will be examined by testing grass tissue infected with LK. The screening process is a time-consuming and very critical phase of the project. The polyclonal and monoclonal antibodies must be well-characterized for a wide range of situations to obtain a high degree of confidence for their use in diagnosis.

Cultures of MP have been prepared in the same manner as LK for submission to the monoclonal antibody facility. We are well satisfied with the work done by this facility for us.

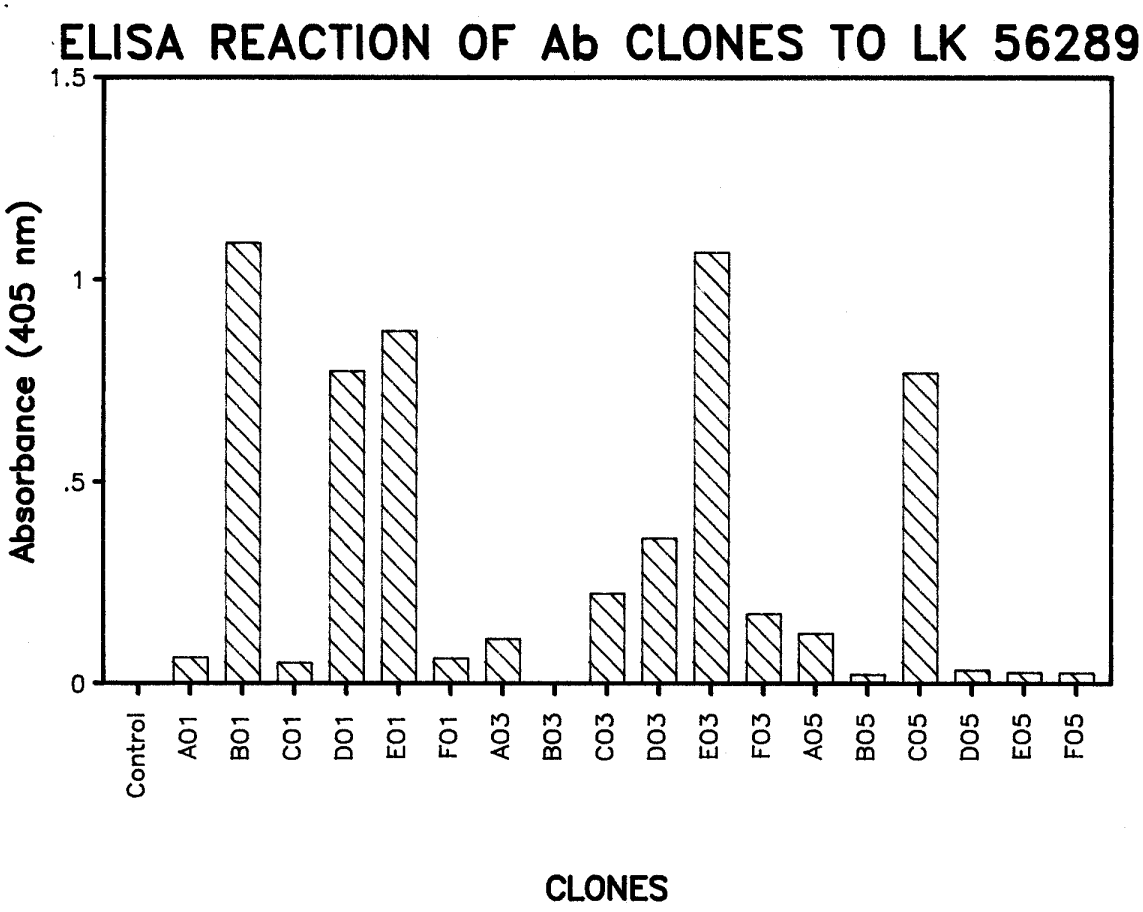
ELISA REACTION OF Ab CLONES TO LK 56289
Absorbance (405 nm)
1.5
1
.5
0
Control
A01
B01
C01
D01
E01
F01
A03
B03
C03
D03
E03
F03
A05
B05
C05
D05
E05
F05
CLONES

Figure 2.

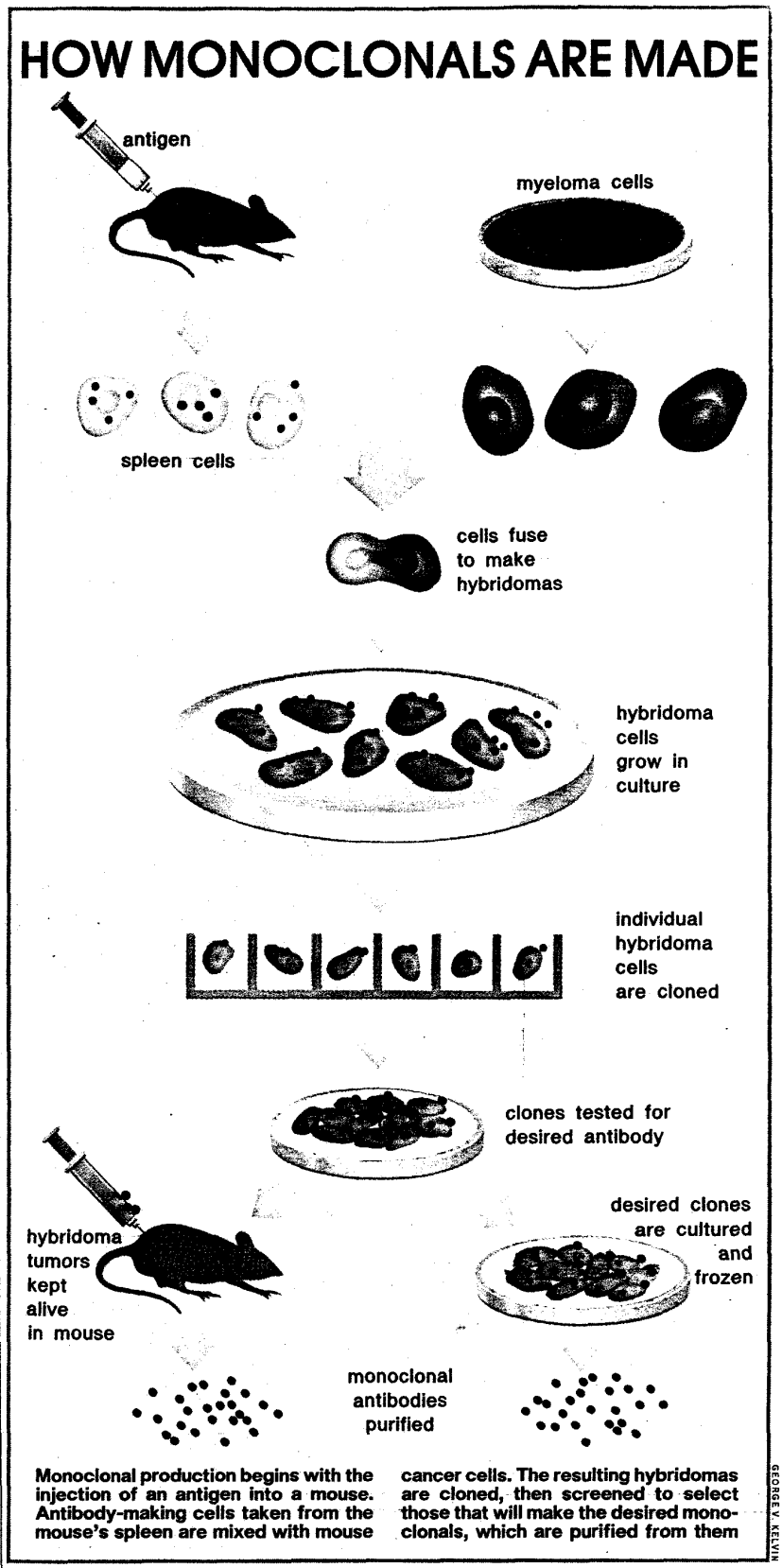
HOW MONOCLONALS ARE MADE
antigen
myeloma cells
spleen cells
cells fuse to make hybridomas
hybridoma cells grow in culture
individual hybridoma cells are cloned
clones tested for desired antibody
hybridoma tumors kept alive in mouse
desired clones are cultured and frozen
monoclonal antibodies purified
Monoclonal production begins with the injection of an antigen into a mouse. Antibody-making cells taken from the mouse's spleen are mixed with mouse cancer cells. The resulting hybridomas are cloned, then screened to select those that will make the desired monoclonals, which are purified from them
GEORGE V. KELVIN

Figure 1

Table 1. Fungal strains to be utilized in screening antibodies for specificity to necrotic ring spot disease.

Strain	Taxonomic Identity	Source
13-1J	*Leptosphaeria korrae*	Michigan
56289	*L. korrae*	New York
13-1C	*L. korrae*	Minnesota
13-1D	*L. korrae*	Minnesota
13-1E	*L. korrae*	Minnesota
13-1F	*L. korrae*	Minnesota
13-1G	*L. korrae*	Wisconsin
13-1K	*L. korrae*	Ohio
13-1L	*L. korrae*	Ohio
30-1A	*Drechslera poae*	Ohio
5787B	*Curvularia spp.*	Ohio
5787C	*Curvularia spp.*	Ohio
str#1	*Rhizoctonia cerealis*	Ohio
str#2	*R. cerealis*	Ohio
5787D	*Fusarium* spp.	Ohio
7-1A	*Sclerotinia homeocarpa*	Ohio
7-1F	*Sclerotinia homeocarpa*	Ohio
7-1G	*Sclerotinia homeocarpa*	Ohio
#5	*Leptosphaerulina* spp.	Ohio
GG6286	*Gaummanomyces tritici*	Ohio
GG6186	*Gaummanomyces tritici*	Ohio
56773	*Magnaporthe poae*	New York
60239	*Magnaporthe poae*	New York
PAWS1	*Pythium aphanidermatum*	Ohio
5787A	*Alternaria* spp.	Ohio
none	*Trichoderma* spp.	Ohio

Expenditures to date:

Production of monoclonal antibodies to LK strain 56289, monoclonal production service, Department of Microbiology	$ 3,000.00*
Material and supplies	917.07
University Indirect Costs (overhead)	1,379.00
	5,295.07

*The price for production of monoclonal antibodies by Microbiology increased from $2,500 to $3,000. An additional $3,000 of the original grant money will be used to pay for production of monoclonal antibodies against Magnaporthe poae.