ANNUAL PROJECT SUMMARY
MONOCLONAL ANTIBODIES FOR RAPID DIAGNOSIS OF NECROTIC RING SPOT AND SUMMER PATCH

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EXECUTIVE SUMMARY

MONOCLONAL ANTIBODIES FOR RAPID DIAGNOSIS OF SUMMER PATCH AND NECROTIC RING SPOT DISEASES OF TURFGRASSES

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Columbus, Ohio

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Slow-growing patch diseases are among the most difficult problems to diagnose on turfgrasses. Research at the Ohio State University, Department of Plant Pathology has focused on the development and use of immunological techniques for rapid diagnosis. We previously reported our success in developing a monoclonal antibody-producing clone that was selective for Leptosphaeria korrae, the causal agent of necrotic ring spot. The antibody, a small protein that can bind to the fungus, can now be grown in great quantity in a laboratory flask. The antibody allows us to test for the presence of the pathogen in a plant sample. Our antibody was highly reactive against all fungal strains of Leptosphaeria korrae tested.

The usefulness of the antibody for L. korrae has been tested thoroughly against diseased turfgrass submitted to the Ohio State University Plant and Pest Diagnostic Clinic and additional Kentucky bluegrass samples collected by our laboratory. L. korrae was successfully isolated from all Kentucky bluegrass samples exhibiting a significant reaction with the LK antibody. The LK antibody was used to study the distribution of L. korrae in the various regions of "frogeye" patches and on turfgrass plant parts. Sampling techniques for detection of L. korrae were optimized.

The LK antibody successfully detected Leptosphaeria korrae in certain bermuda grass sites with spring dead spot symptoms. The antibody will be useful in determining the causal agent of spring dead spot. Currently, at least three fungi (L. korrae, Ophiosphaerella herpotricha, and Gaeumannomyces graminis) have been shown to be causes of this disease.

Development of monoclonal antibody against the causal fungus of summer patch (Magnaporthe poae) is in progress. A third set of mice have been immunized using an improved protocol. Reactivity of mouse serum will be tested in November 1990, followed by production of monoclonal antibody clones. Screening of clones will begin in mid December, followed by field testing in the summer of 1991. The result will be a fast, reliable method to diagnose and monitor this disease.

RATIONALE FOR WORK

Numerous pathogenic fungi cause patch symptoms on turfgrass. Several of the patch diseases, such as necrotic ring spot, summer patch, and spring dead spot, are difficult to diagnose with traditional techniques.

New technologies are available that allow the rapid diagnosis of fungal diseases through the use of polyclonal or monoclonal antibodies. The impact of this new approach is already being felt in the area of turf grass management. Antibody kits are in use for detection of Pythium blight, brown patch, and dollar spot.

Antibody-based detection kits for necrotic ring spot and summer patch because 1) these disease complexes are not well understood, 2) the pathogens attack crowns and roots, making assaying more difficult, 3) the prevalence of these diseases has not been fully appreciated.

OBJECTIVES

The primary goal is to develop antibody for diagnosing necrotic ring spot and summer patch diseases. Procedures for assaying turfgrass for the pathogens will be determined. We will determine the suitability of the antibody for the measurement of pathogen populations.

Little is known about the population dynamics of the necrotic ring spot pathogen throughout the year although miscellaneous observations suggest that greatest populations will be detected in the spring and fall. Assay procedures need to be determined through detailed sampling of various plant parts over time.

A final goal is to promote the development of a rapid antibody assay for these diseases in a form suitable for use by turf grass managers.

PROGRESS TO DATE

NECROTIC RING SPOT

Monoclonal antibodies against Leptosphaeria korrae (LK) strain ATCC 56289 were produced with the technical assistance of a monoclonal facility in the Department of Microbiology, Ohio State University. A single clone, 412E4IA9G5 (LKc50 clone), showing the best selectivity was increased for bulk antibody production. The antibody from this clone was extensively evaluated for its selectivity against many LK and non-LK antigens. The clone Lkc50 reacted strongly against lyophilized mycelia of all 24 verified strains of LK (including one from bermuda grass) and against all verified LK strains.

Lkc50 reacted negatively to 39 of 42 isolates of non-LK fungi and negative to 2 grass samples. Reaction of MAb LKc50 was moderately positive to three isolates--33375, an Ascochyta sp., CURV1, a Curvularia sp., and 40-1A, an Exserohilum holmii strain.

Assay of Kentucky bluegrass samples

The LKc50 antibody was tested for the ability to detect Leptosphaeria korrae in Kentucky bluegrass naturally-infected with necrotic ring spot in Ohio, Washington, and Ohio. Significant absorbance readings were obtained in both 1989 and 1990 from all samples from which L. korrae was isolated (Table 1).

We are investigating the usefulness of the LK antibody for microscopic studies of the distribution of L. korrae on turfgrass roots. We are using a transmission electron microscope to examine thin sections of necrotic ring spot infected roots that have been stained with gold-labelled LK antibody. Through this work we wish to learn the selectivity of the LK antibody, i.e., what types of L. korrae hyphae (young, old) are detected.

Assay of bermuda grass with spring dead spot symptoms

Samples of bermuda grass with spring dead spot symptoms were collected by researchers in North Carolina, Maryland, Kentucky, and Kansas during the early summer of 1990. Significant antibody reaction were obtained with all samples from which L. korrae-like fungi were isolated (Table 2). Positive identification by of the L. korrae-like fungi from the Maryland and North Carolina samples has not been completed by the time of this report. Dr. Peter Dernoeden had isolated L. korrae from the Maryland sample site on a previous occasion.

Although the samples from Kansas had a strong reaction with the antibody test, the only significant fungus found was a Ophiosphaerella-like organism. Dr. Ned Tisserat of Kansas State University has shown that Ophiosphaerella herpotricha can cause a spring dead spot-like symptom on bermuda grass. However, the Ophiosphaerella-like fungi we isolated did not react with the antibody

in subsequent tests, indicating that we did not successfully isolate the entity that reacts with the antibody. Further testing with samples from the Kansas site is needed to see if L. korrae is indeed present.

Conclusion of Project on Necrotic Ring Spot

The USGA/Ohio State University research project on necrotic ring spot will reach a successful conclusion at the end of 1990 with the prospects for a contractual arrangement with a commercial company for production of antibody kits for detection of Leptosphaeria korrae. The antibody can be used to detect this fungus on Kentucky bluegrass, fine fescue, and bermuda grass.

John Stier, a graduate student supported by a Ohio State University assistantship, is concluding a two year study in which he has used the LKc50 antibody to study the distribution of the necrotic ring spot fungus on turfgrass plants. He will submit and defend his masters thesis at the end of 1990.

SUMMER PATCH WORK

Isolates of M. poae were collected from 4 states, grown in culture, and characterized. A total of 14 verified strains of M. poae have been collected and stored. Additional fungal isolates resembling M. poae were collected in 1989 and 1990 from sites in Ohio and Kentucky. The identity of these isolates are currently being determined thorough induction of the sexual stage by pairing by pairing M. poae strains of the opposite mating types 'A' and 'a'.

We are determining the pathogenicity of the M. poae isolates in greenhouse and field studies. Preliminary information from Ohio and other states suggests that fungi known as M. poae may be a fairly diverse group.

We are in the process of developing monoclonal antibody for summer patch (Magnaporthe poae). Immortal cell lines were produced by fusing antibody-producing cells from spleens of six immunized mice with myeloma cells. Cell lines were subcloned and tested for antibody production and immortal growth characteristics.

The first set of monoclonal antibodies for MP proved to be unsatisfactory although the preliminary tests were promising. A second set of immunizations was done using a different strain of M. poae and a new set of mice in late 1989. The second set of mice appear to have antibody with low reactivity, even with several booster immunizations.

A third set of mice was immunized with M. poae in Oct 1990 using a revised protocol--reactivity of the mouse serum will be tested during the second week of November. We will proceed with the production of immortal cell lines producing antibody in late November provided that

the mouse blood serum shows good reactivity. Selection of the desirable monoclonal antibody clones will begin in 1991.

We hope to continue our work to develop an antibody against Magnaporthe poae (summer patch) under a new project submitted to the USGA for 1991. A rapid diagnostic test for this pathogen is needed by researchers, turf managers, clinics, and chemical sales representatives.

PUBLICATIONS SINCE LAST ANNUAL REPORT

Nameth, S. T., W. W. Shane, and J. C. Stier. 1990. Development of Monoclonal Antibody for Detection of Leptosphaeria korrae, the Causal Agent of Necrotic Ringspot Disease of Turfgrass. Phytopathology 80:000-000 (in press).

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Table 1. Reaction of Leptosphaeria korrae-specific monoclonal antibody Lkc50 in indirect ELISA to field samples of necrotic ring spot infected and healthy Kentucky bluegrass

1989	Absorbance (A_{405})[1]
Ohio necrotic ring spot samples	0.250, 0.210, 0.173
Washington necrotic ring spot samples	0.370, 0.409, 0.272 0.465, 0.269, 0.519
Idaho necrotic ring spot samples	0.254, 0.218
apparently healthy plants	0.063, 0.550, 0.095
1990	
Ohio necrotic ring spot samples	0.270, 0.388, 0.230 0.165, 0.200, 0.214
apparently healthy plants	0.009, 0.082, 0.012

[1] Each value is the mean for a sample from a different location

Table 2. Reaction of Leptosphaeria korrae-specific monoclonal antibody Lkc50 in indirect ELISA (A_{405}) to field samples of spring dead spot infected and healthy bermuda grass, 1990

	ELISA Absorbance	L. korrae-like fungus isolated	Gaeumannomyces-like fungus isolated	Ophiosphaerella-like fungus isolated
North Carolina samples - (L. Lucas)				
1	0.429	yes[1]	yes	no
2	0.170	no	yes	yes
3	0.854	yes	no	no
4	0.134	yes	yes	no
Maryland samples (P. Dernoeden)				
1	0.452	yes	no	no
2	0.423	yes	no	no
3	0.080	yes	no	no
Kansas samples (N. Tisserat)				
1	1.114	no	no	yes[2]
2	1.073	no	no	yes
3	0.882	no	no	yes
Kentucky samples (P. Vincelli)				
1.	0.001	no	no	yes
2.	0.012	no	no	yes
3.	0.018	no	no	yes
healthy plants	0.000	-	-	-

[1] Leptosphaeria korrae-like fungi isolated from North Carolina samples 1, 3 and 4 were very positive in subsequent ELISA tests for L. korrae using fungal hyphae from purified culture.

[2] Ophiosphaerella-like cultures isolated from Kansas samples 1, 2, and 3 were negative in subsequent ELISA tests for L. korrae using fungal hyphae from purified culture.