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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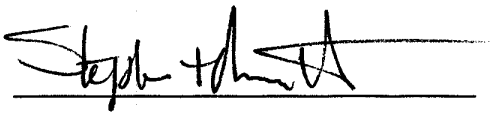

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EXECUTIVE SUMMARY

Necrotic ring spot and summer patch diseases of Kentucky blue grass, annual blue grass, and other turf grasses are extremely difficult to diagnose with traditional techniques. Research at Ohio State University is focused on the use of immunological techniques for rapid diagnosis of these two diseases.

A monoclonal antibody-producing clone, LKc50 was developed that was selective for the causal agent of necrotic ring spot (Leptosphaeria korrae (LK)). The antibody, used in an indirect ELISA test, reacted strongly against all verified strains of LK (>40) tested in laboratory studies, including strains from bermuda grass displaying symptoms of spring dead spot. The antibody did not react significantly with 42 non-LK antigens including Magnaporthe poae (summer patch pathogen), Gaeummanomyces (take-all), plant tissue, and common plant-inhabiting fungi.

Approximately 50 % of cultures displaying LK characteristics received or isolated at Ohio State University have not produced the sexual stage (ascospores). The ELISA test for LK has allowed us to identify these non-sporulating cultures as LK with relatively high certainty.

The ELISA test for LK also allows us to detect the pathogen directly on infected plant tissue. Samples of Kentucky bluegrass naturally-infected with LK were collected from Ohio, Washington, and Colorado during the summer and fall of 1989. Antibody of LKc50 reacted significantly with all plant samples from LK-infected turf but not with healthy turfgrass.

The ELISA test for LK in conjunction with standard culturing procedures can verify the presence of this pathogen in turfgrass with low infestations within 9 days. Infected plant tissue is plated on standard isolation media. Next, the ELISA test for LK is done when the fungus has grown out sufficiently to allow sampling with a 7 mm diameter cork borer (approximately 7 days). This technique is appropriate when amounts of the pathogen on the turf sample are too low to detect directly with the ELISA test.

Monoclonal antibodies for the fungus causing summer patch, Magnaporthe poae (MP), are still under development. The first set of monoclonal antibodies for MP proved to be unsatisfactory. A second set of immunizations has been done with a new set of mice to obtain a set of clones with better selectivity. We expect to have clones with the desired selectivity by summer of 1990.

RATIONALE

The diagnosis of summer patch (Magnaporthe poae) and necrotic ring spot (Leptosphaeria korrae) diseases of Kentucky bluegrass, annual bluegrass, bermuda grass, and other grasses is very difficult with current techniques. Disease symptoms resemble those for other patch diseases and for some abiotic disorders. Isolation of these fungi from infected plant tissue is difficult as is their identification even when growing in a petri plate. 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.

It is not expected that private companies will produce antibody-based detection kits for necrotic ring spot and summer patch in the near future because 1) these disease complexes are not well understood, 2) the pathogens attack crowns and roots, making assaying more difficult, and 3) the diseases are not as prevalent as dollar spot, brown patch, and Pythium blight.

The primary goal is to provide a clinic tool for diagnosing necrotic ring spot and summer patch diseases. As a research tool the antibodies will allow us to survey the abundance of the pathogens, examine the diversity within the two pathogen populations and to study population dynamics over time in turf grass stands. A future possibility is the development of a rapid assay suitable for use in the field by turf grass managers.

PROGRESS REPORT

SUMMARY OF 1987 ACTIVITIES (condensed from 1987 report)

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. Eighteen original clones were subcloned, purified, and screened against LK and non-LK fungi, soil, plant tissue, and other common components. The result was 10 clones with good selectivity for LK.

SUMMARY OF 1988 ACTIVITIES (condensed from 1988 report)

NECROTIC RING SPOT WORK

Putative LK strains from 9 states were gathered for antibody clone evaluation. Various methods were used in order to induce

the sexual stage which must be seen to verify the identity of putative LK strains. Twenty-four out of 58 strains produced ascospores characteristic of LK--many of the remaining strains resemble LK.

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 three from bermuda grass) and against all but one of the 34 putative LK strains.

LKc5 antibody was tested against a wide array of non-LK antigens. The LKc50 antibody did not react with any Magnaporthe poae strains and did not react with 42 of 44 antigens. A weak reaction was noted with one strain of Fusarium and one of Ascochyta sp. The current 3 step assay method requires 24 hr.

A paper on the necrotic ring spot work was presented at the national Phytopathological Society meeting in San Diego and at a discussion session at the 1989 American Phytopathological Society meeting.

SUMMER PATCH WORK

Monoclonal antibodies for summer patch (Magnaporthe poae) were developed. 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.

Nine putative strains of Magnaporthe were collected from 4 states and grown in shake culture for mycelia production. The identities of the cultures are being verified by induction of the perfect stage. The perfect stage is produced by pairing M. poae strains of the opposite mating types 'A' and 'a'. Additional M. poae strains are being collected for a more complete screening procedure.

WORK ACCOMPLISHED DURING 1989

NECROTIC RING SPOT WORK - 1989

Selectivity of LKc50

Strains of LK were collected from other sources to test the selectivity of antibody from LKc50. Antibody from LKc50 reacts well with all 40 verified strains of LK tested to date, including strains from Bermuda grass. An additional 40 fungal strains have been collected that resemble LK in colony morphology--however,

these fungi have not produced the sexual stage, which is needed for positive identification. The ELISA test for LK reacts positively against these strains, indicating that they are indeed LK.

Assay of turf naturally-infected with necrotic ring spot

The LKc50 antibody was tested for the ability to detect Leptosphaeria korrae in necrotic ring spot-infected turfgrass. Symptomatic Kentucky bluegrass turf samples were obtained from sites in Ohio, Washington and Idaho. Significant absorbance readings were obtained with the ELISA assay on with samples (Table 1).

Turf samples were also obtained from Wisconsin (via Dr. G. Worf) and Pennsylvania (Dr. P. Landschoot)--however LK infestation levels in the samples were too low to allow direct detection of the pathogen.

Of some concern is the weak but appreciable reaction of the LKc50 antibody to non-LK fungal strains 3375 (Ascochyta sp.) and one Fusarium strain. Other strains from these fungal groups were tested and did not cause a positive reaction.

A paper on the necrotic ring spot work was presented at a discussion session at the 1989 American Phytopathological Society meeting in Richmond, Virginia.

SUMMER PATCH WORK - 1989

Additional cultures of M. poae were collected, grown in culture, and characterized. A total of 14 verified strains of M. poae have been collected (Table 2). An additional 30 fungal isolates resembling M. poae were collected in 1989 from sites in Ohio and Kentucky. The identity of these isolates are currently being determined thorough induction of the sexual stage by pairing with known mating types.

Monoclonal antibodies for the fungus causing summer patch, Magnaporthe poae (MP), are still under development. The first set of monoclonal antibodies for MP proved to be unsatisfactory although the preliminary tests were promising.

A second set of immunizations has been done with a new set of mice to obtain a set of clones with better selectivity. We expect to have clones with the desired selectivity by summer of 1990. In this second round, the screening procedures in the early stages of clone selection will be expanded to increase the likelihood of obtaining a good clone.

WORK IN PROGRESS

Necrotic ring spot

We are working to improve the performance of the LK ELISA test by varying assay conditions and reagents.

We will continue to test the LK antibody with samples from the field. We are arranging to have additional samples of LK-infected turf sent to us during 1990. The focus will continue to be on necrotic ring spot disease of Kentucky bluegrass and fine fescue. Samples of spring dead spot-infected bermuda grass will also be tested since limited tests indicate the ELISA assay can detect LK on this host.

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. John Stier, a graduate student at Ohio State University, has undertaken a study of LK distribution on turfgrass plants in the field.

The time may come when it is appropriate to see if antibody of LKc50 clone is suitable for use in the public sector outside of Ohio State University. The LK antibody has utility in at least three arenas: as a tool in research, as a clinic tool for diagnosis, and as a diagnostic tool on-site. Use in research and in clinics would require relatively little antibody, whereas, production for use by the turfgrass manager is a large undertaking.

A candidate company for wide-scale production is Agri-Diagnostics, Assoc. Cinnaminson, NJ, because of their prominence in the turf area. The 10 minute rapid assay format of their new Reveal kit line would be convenient for on-site testing. Other companies have expressed interest (e.g. Neogen, Ciba Geigy), but not to the extent of Agri-Diagnostics. Only antibody and not the living cells that produce the antibody will be provided to interested companies for their preliminary evaluations. Further negotiations will involve the USGA and the Ohio State University.

Summer patch work

Additional putative Magnaporthe poae strains will be collected and identified by pairing with known mating partners. Several test sites have been identified for tests with naturally-infected turfgrass.

The progress in the summer patch project has been slowed by the low selectivity of the antibody of the first set of clones. The next round of clone selection resulting from the second set of

immunizations should go rapidly because all the tester strains are already collected and prepared for utilization. The screening procedures in the early stages of clone selection will be expanded to increase the likelihood of obtaining a good clone. We expect to have antibody to M. poae by summer of 1990.

CREDITS

Technical assistance during 1987 and 1988 was provided by Vicki Wills (microbiology student). Jennifer Jacubowski (microbiology student) has worked on this project since the winter of 1989. John Stier, a M.S. student has begun work on the necrotic ring spot/summer patch antibodies for his thesis project. John will focus on the use of the antibodies for monitoring pathogen populations in the field.

With both the necrotic ring spot and summer patch work the following people provided cultures, comments and/or suggestions:

Dr. Gary Chastagner, Dr. Joe Vargas, Dr. Gail Worf, Dr. Peter Landschoot, Dr. Noel Jackson, Dr. David Grothaus, Dr. Peter Dernoeden, Dr. Dave Thompson, Dr. Ned Tisserat, Dr. Phil Larsen, and Dr. Richard Smiley.

TABLE 1. Reaction of Leptosphaeria korrae-specific MAB Lkc50 in indirect ELISA (A405 nm) to field samples of necrotic ring spot infected and healthy Kentucky bluegrass.

Sample	Necrotic ring spot symptoms/signs on Turf stand	Basal stem	L. korrae isolated	Absorbance
Ohio samples (Trial 1)				
diseased				
1	patch	moderate[1]	yes	0.250[2]
2	patch	slight	yes	0.210
3	patch	moderate	yes	0.173
healthy[3]				
4	none	none	no	0.055
5	none	none	no	0.095
6	none	none	no	0.063
-	buffer	-	-	0.000
Washington samples (Trial 2)				
1A	patch	intense	yes	0.370
1B	patch	moderate	yes	0.409
2A	patch	low	yes	0.272
2B	patch	intense	yes	0.465
3A	patch	intense	yes	0.269
3B	patch	intense	yes	0.519
Idaho samples (Trial 2)				
4A	patch	low	yes	0.254
4B	patch	moderate	yes	0.218
GH1 [4]	none	none	no	0.096
-	buffer	-	-	0.000

[1] Rating system for ectotrophic colonization (possibly Leptosphaeria korrae) of basal stem, crown and roots is as follows--intense: colonization with hyphae aggregations on basal stem is evident with unaided eye, moderate: with aid of dissecting microscope runner hyphae can be easily seen on all basal stem pieces, low: runner hyphae found on some plant specimens.

[2] Average value of four subsamples. For each sample 20 washed basal stem + roots (1.5 cm length) were ground in 5 ml buffer, filtered through 1 layer of tissue and used to sensitize plates. Plates were coated with 360 µl of sample, blocked with 1% BSA, and subjected to 1/16 dilution of MAB from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an anti-mouse IgG.

[3] Samples from apparently healthy Kentucky bluegrass turf at same location.

[4] Non-infected 1 month-old Kentucky bluegrass plants grown in greenhouse

[2] Homogenized, lyophilized mycelia of Leptosphaeria korrae strain ATCC 56289 at a 77.4 µg/well.

TABLE 2. Verified strains of Magnaporthe poae collected for use in development of monoclonal antibodies [1]

Strain designations and alternate names	Mating type	Original host	Geographical origin	Source	Date of Isolation
12W-ri1 = RI1	A	turf grass	Rhode Island		
12W-ri2 = RI2	-	turf grass	Rhode Island		
ATCC 64131 = WR-1-85 = 12RI-WR-1-85	A	Poa annua	Rhode Island		
ATCC 64412 = 12RI-73-1	a	Poa sp.	Rhode Island		
ATCC 60239 = 12W-PG57 = 57-84 = Pg57	A	Poa pratensis	New York		
ATCC 56773 = 60	A	Poa pratensis	New York		
12W-pg197	A	turf grass	New York		
12RI-ARL-1	a	Poa pratensis	Arlington, VA		
22588	A	Poa annua	Columbus, OH	J. Stier	1988
23688	A	Poa annua	Cleveland, OH	J. Stier	1988
ATCC 64411 = 73-15	-	Poa pratensis	New York	P. Landschoot	8/85
NAV-A-1	-	Poa annua	New Jersey	P. Landschoot	8/88
PIT-A-1	-	Poa annua	New Jersey	P. Landschoot	8/88
MICH-1	-	Poa annua	Michigan	R. Detweiller	8/84

[1] The identities of these strains was verified by induction of the sexual stage by mating with known 'A' and 'a' mating types of M. poae.

Descriptions of slides
Nameth and Shane
Annual Report 1989
1 November 1989

1. Title slide of research project of Shane and Nameth

2. Isolation from Kentucky bluegrass basal stem tissue. Fusarium colonies (light colored colonies) are taking over the plate, obscuring the dark colored Leptosphaeria korrae (necrotic ring spot) colonies. With the LK monoclonal antibody test we can sample the dark colored colonies with a cork borer and determine whether or not they are Leptosphaeria korrae.

3. Reaction of monoclonal antibody against necrotic ring spot infected or healthy turfgrass from the field. This slide shows that we can clearly pick out the Leptosphaeria korrae-infected tissue (high readings = tall bars -- i.e., dark reaction in an ELISA test). The samples were taken directly from the field from naturally infected turfgrass sites.

4. Progress in development of monoclonal antibodies. This slide shows the steps that are involved in the formation of the monoclonal antibody. At the present time with the Leptosphaeria korrae antibody we are at the field test/optimize test step. With the Magnaporthe poae antibody (summer patch) we will shortly harvest the spleens of mice showing good reaction against Leptosphaeria korrae. We were at the "screen with target and non-target antibody" step, however, the clones were unsatisfactory and so we began again.