Development of a Monoclonal Antibody for Detection of *Leptosphaeria korrae*, the Causal Agent of Necrotic Ringspot Disease of Turfgrass

S. T. Nameth, W. W. Shane, and J. C. Stier

Assistant professors and graduate research associate, Department of Plant Pathology, The Ohio Agricultural Research and Development Center, The Ohio State University, Columbus 43210-1087.

We thank Vicki Wills and Jennifer Jakubowski for valuable technical assistance.

Salaries and research support were provided by state and federal funds. Additional support was provided by the Ohio Turfgrass Foundation and the United States Golf Association. Article 102-90.

Accepted for publication 3 July 1990 (submitted for electronic processing).

ABSTRACT

Nameth, S. T., Shane, W. W., and Stier, J. C. 1990. Development of monoclonal antibody for detection of *Leptosphaeria korrae*, the causal agent of necrotic ringspot disease of turfgrass. Phytopathology 80:1208-1211.

Monoclonal antibody (MAb) LKc50 was developed against *Leptosphaeria korrae* strain ATCC 56289. The antibody was capable of detecting *L. korrae* from cultures and in naturally infected Kentucky bluegrass samples from three states. In cross-reactivity tests using indirect ELISA, MAb LKc50 reacted positively to all 24 isolates of *L. korrae* screened, including strains from six states, from both Kentucky bluegrass and bermudagrass. MAb LKc50 reacted negatively to 38 of 42 isolates of related and nonrelated fungi and negatively to apparently healthy grass. The limit of detection was less than 2 μg/ml of lyophilized mycelial homogenate. MAb LKc50 provides a means for rapid detection of *L. korrae*, an ectotrophic root-invading fungus that is difficult to identify using conventional methods.

Leptosphaeria korrae J. C. Walker & A. M. Smith is the causal agent of necrotic ring spot (13,10), a fungal disease of the basal stem, crown, and roots of Kentucky bluegrass (*Poa pratensis* L.) and fine fescue (*Festuca rubra* L.). *L. korrae* has also been identified as one of the causal agents of spring dead spot on bermudagrass, *Cynodon dactylon* (L.) Pers. (2,3,13). With both diseases, infected tissues are often covered with dark ectotrophic "runner" hyphae of *L. korrae* (10). Necrotic ring spot is difficult to distinguish from several diseases associated with Kentucky bluegrass, including summer patch (caused by *Magnaporthe poae* Landschoot & Jackson), yellow patch (caused by *Rhizoctonia cerealis* Van der Hoeven), and Fusarium blight (caused by *Fusarium culmorum* (Wm. G. Sm.) Sacc. and *F. poae* (Peck) Wollenweb.). Grass plants, especially if under stress, are commonly infested with weak pathogens and/or saprophytes, such as *Fusarium, Curvularia, Cladosporium*, and *Alternaria* spp., that make the diagnosis of specific diseases difficult (9).

Identification of *L. korrae* based on cultural characteristics in vitro is only tentative (10,13). *L. korrae* has no known anamorph (13). Definitive diagnosis of necrotic ringspot requires the presence of the teleomorph, which is rarely detected on field samples (14). Production of the teleomorph in culture can take months using current techniques (14).

The use of pathogen-specific antibody could provide a rapid, sensitive technique for diagnosis of necrotic ring spot on turfgrass. This paper describes the production of monoclonal antibody against *L. korrae* and its use for the detection of *L. korrae* in infected turfgrass. A preliminary report has been published (8).

MATERIALS AND METHODS

Fungal strains. Fungal isolates (Tables 1 and 2) were collected and maintained on potato-dextrose agar (PDA) slants at 4 C or on hydrated, sterilized pearl millet seed at either 4 C or room temperature. Fungal biomass for antibody production and testing was grown in shake cultures (80 rpm at room temperature) containing either 100 or 250 ml of potato-dextrose broth (PDB) (Difco Laboratories, Detroit, MI) for approximately 1 mo or until sufficient biomass could be obtained. Each fungal culture was harvested and washed with 80 to 160 ml of distilled water, depending on the size of the mycelial mat, using Whatman No. 1 filter paper in a Buchner funnel apparatus. Washed fungal biomass was lyophilized, frozen in liquid nitrogen, ground to a fine powder with a mortar and pestle, and stored at 0 C.

Putative *L. korrae* strains were grown on PDA at laboratory light and temperature. Two plugs (7 mm in diameter) from the margins of actively growing colonies were transferred to petri dishes (6 cm in diameter) containing 10 ml of 15 g/l Bacto agar (Difco Laboratories, Detroit, MI) and approximately 50 twice-autoclaved hard fescue seeds (*Festuca longifolia* 'Scaldis') scattered on the agar surface. Plates were sealed with Parafilm (American National Can Company, Greenwich, CT) and incubated at 22 C and a 12 hr photoperiod under fluorescent lights at 450 lux. Plates were examined weekly for pseudothecia and ascospores of *L. korrae* (13).

Production of monoclonal antibody (MAb). MAb was developed against strain ATCC 56289 of *L. korrae* (American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD). Antibody clones were developed by the Ohio State University Monoclonal Antibody Facility, Department of Microbiology. One hundred μg of homogenized mycelia of *L. korrae* in Freund's complete adjuvant was used to immunize BALB/c mice. Mice were given a booster injection of 100 μg of mycelia of *L. korrae* in sterile 0.090 M $NaCl_2$ 3 wk later and rested for 3 wk. Spleen lymphocytes were harvested for fusion 3 days after a final immunization with 100 μg of mycelia of *L. korrae*. Mouse spleen cells at a concentration of 1.5×10^8/ml were fused with P3X63Ag.853 myeloma cells at equal concentrations in 50% polyethylene glycol (Fisher Scientific, Cincinnati, OH) as previously described (6). On day 1 following fusion, cells were exposed to 0.1 ml of HAT medium (100 μM hypoxanthine, 16 μM aminopterin and 0.4 μM thymidine) in 96-well culture plates. On days 2, 3, 5, 8, and 11, half of the HAT medium was removed from each well and 0.1 ml of fresh HAT medium was added. After day 11, medium was exchanged every 3–4 days. Culture supernatant fluids were sampled after 21 days and screened for the presence of antibody. Cultures producing antibody that reacted with *L. korrae* were selected and cloned twice by limiting dilution. Antibody stocks were stored at −60 C.

Hybridoma supernatants were screened using an indirect enzyme-linked immunosorbent assay (I-ELISA). Hybridoma supernatants were screened during the first two selection cycles against

ATCC 56289 and against four isolates of *L. korrae* and four non-*L. korrae* isolates in the third and fourth cycles. Polystyrene ELISA plates (Nunc-InterMed, Thousand Oaks, CA) were sensitized with homogenized mycelia at a concentration of 215 μg dry wt/ml in sodium carbonate (Na_2CO_3)/sodium bicarbonate ($NaHCO_3$) buffer, pH 9.6, and blocked with 1% bovine serum albumin. Sensitized plates were stored in a desiccated state at 4 C until needed. Binding of mouse antibodies was determined with a peroxidase-labeled, affinity-purified antimouse antibody with 2, 2′-azino-di-[3-ethyl-benzthiazoline sulfonate] as the substrate (Beckman Inc., Palo Alto, CA). Absorbance values were determined at 405 nm with a Model 700 Microplate reader

TABLE 1. Reaction of monoclonal antibody LKc50 against lyophilized mycelia extracts of *Leptosphaeria korrae* and nontarget fungi using indirect enzyme-linked immunosorbent assay[a]

Strain[b]	Geographic origin	Source	Absorbance (405)[c]
L. korrae isolates			
ATCC 56289	New York	R. M. Smiley	1.022
13-1J	Michigan[d]	J. M. Vargas, Jr.	1.464
13-300	Novi, MI	J. C. Stier	1.306
13-500, 13-501, 13-502, 13-503	Columbus, OH	J. C. Stier	1.304, 1.468, 1.416, 1.507
SHU/MOS, DDEAN, DSODV, WARD	Idaho[d]	D. Thompson	1.030, 1.356, 1.315, 1.334
W43	Renton, WA	G. A. Chastagner	1.383
W64	Mill Creek, WA	G. A. Chastagner	1.284
W129	Spokane, WA	G. A. Chastagner	1.386
W87, W96, W99, W102, W103	Coulee Dam, WA	G. A. Chastagner	1.400, 1.385, 1.509, 1.300, 1.662
W107, W109, W110	Pullman, WA	G. A. Chastagner	1.360, 1.160, 1.210
W144	Puyallup, WA	G. A. Chastagner	1.140
CA2	California	R. M. Endo	1.334
Non-target fungi			
ATCC 64414 *Phialophora graminicola*	Narragansett, RI	P. Landschoot	0.044
1-13A *Fusarium* spp.	Ohio	L. H. Rhodes	0.030
30-1A *Drechslera poae*	Ohio	P. O. Larson	0.057
ATCC 56773 *Magnaporthe poae*	New York	R. M. Smiley	0.061

[a] Identities of *L. korrae* strains were verified by induction of ascospores in vitro.
[b] Strain CA2 was isolated from bermudagrass—all other *L. korrae* isolates are from Kentucky bluegrass.
[c] Mean values of 2 experiments, each with 6 replicate wells per antigen. Plates were coated with 77.4 μg antigen in 350 μl per well, blocked with 1% bovine serum albumin, washed, and treated with an 1:16 dilution of MAb from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an antimouse IgG.
[d] Strain designations by the original collector are not available for these cultures.

TABLE 2. Reaction of monoclonal antibody LKc50 against lyophilized mycelia extracts of non-target fungi, grass, potato dextrose broth, and *Leptosphaeria korrae* using indirect enzyme-linked immunosorbent assay

Antigen designation	Antigen description	Host	Source	Absorbance (405 nm)[a]
ALT1, ALT2	*Alternaria* spp.	Turf grass	W. W. Shane	0.059, 0.018
3375	*Ascochyta* spp.	Kentucky bluegrass	W. W. Shane	0.821
CURV1	*Curvularia* spp.	Kentucky bluegrass	W. W. Shane	0.593
14-1A	*Diaporthe phaseolorum*	Soybean	K. Kmetz	0.006
25-1A	*Drechslera erythrospila*	Turf grass	P. O. Larsen	0.210
30-2A	*Drechslera catenaria*	Turf grass	P. O. Larson	0.014
40-1A	*Exserohilum holmii*	Turf grass	P. O. Larsen	1.212
FUS1, FUS2	*Fusarium* spp.	Turf grass	W. W. Shane	0.021, 0.016
1-3A	*Fusarium* spp.	Turf grass	L. H. Rhodes	0.017
1-4A	*Fusarium tricinctum*	Turf roots	Dept. Pl. Pathol., Ohio State Univ.	0.016
1-11E	*Fusarium roseum*	Turf grass	P. O. Larsen	0.024
ATCC 64420	*Gaeumannomyces cylindrosporus*	Kentucky bluegrass	N. T. Jackson	0.025
ATCC 64419	*Gaeumannomyces graminis* var. *graminis*	Zoysia grass	P. J. Landschoot	0.030
ATCC 64418	*Gaeumannomyces* spp. type 'A'	Kentucky bluegrass	P. J. Landschoot	0.029
Gg6186, Gg6286	*Gaeumannomyces graminis*	Wheat	P. E. Lipps	0.007, 0.012
7-1A, 7-1F, 7-1G, 7-1X	*Lanzia/Moellerodiscus* spp.	Turf grass	Dept. Pl. Pathol., Ohio State Univ.	0.004, 0.008, 0.010, 0.007
LEPTO-5	*Leptosphaerulina* spp.	Alfalfa	L. H. Rhodes	0.005
ATCC 60239	*Magnaporthe poae*	Kentucky bluegrass	R. M. Smiley	0.007
NIGR	*Nigrospora* spp.	Turf grass	W. W. Shane	0.035
PA1	*Pythium aphanidermatum*	Unknown	Agri-Diagnostics	0.028
PA243	*Pythium aphaenidermatum*	Turf grass	A. F. Schmitthenner	0.003
PG223	*Pythium graminicola*	Kentucky bluegrass	A. F. Schmitthenner	0.005
PG365	*Pythium graminicola*	Unknown	A. F. Schmitthenner	0.018
PT68, PT244	*Pythium torulosum*	Turf grass	A. F. Schmitthenner	0.024, 0.010
PU211	*Pythium ultimum*	Poinsettia	A. F. Schmitthenner	0.025
PV222	*Pythium vanterpooli*	Bluegrass	A. F. Schmitthenner	0.021
6-1C, 6-1X	*Rhizoctonia cerealis*	Turf grass	Dept. Pl. Pathol., Ohio State Univ.	0.018, 0.013
6-3A, 6-3B, 6-3D	*Rhizoctonia solani*	Turf grass	Dept. Pl. Pathol., Ohio State Univ.	0.015, 0.008, 0.013
TRIC	*Trichoderma* spp.	Turf grass	W. W. Shane	0.011
GH1	Kentucky bluegrass leaf tissue	Greenhouse	...	0.030
G-LA	Kentucky bluegrass leaf tissue	Healthy lawn	...	0.028
PDB	Potato dextrose broth	...	Difco	0.007
ATCC 56289	*Leptosphaeria korrae*	Kentucky bluegrass	R. M. Smiley	1.477

[a] Mean values of 2 experiments, each with 2 replicate wells per antigen. Plates were coated with 77.4 μg antigen in 350 μm per well, blocked with 1% bovine serum album, washed, and treated with an 1:16 dilution of MAb from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an antimouse IgG.

(Cambridge Technology, Inc., Cambridge, MA). MAb subtypes were determined by ELISA using an isotyping kit (Zymed Labs, San Francisco, CA).

One clone with the desired properties, LKc50, was derived from one of 18 original clones showing reaction against ATCC 56289. Optimum concentrations for I-ELISA assay were determined by titration of both the homogenized mycelia of *L. korrae* and the culture supernatant. Dilutions for the MAb supernatant ranged from 1/4 to 1/512 and 77.4, 7.74, and 0.774 μg per well of the homogenized mycelia of *L. korrae*.

Reaction of LKc50 supernatant against isolates of *L. korrae* and non-*L. korrae* isolates. The specificity of antibody from LKc50 was tested against 24 isolates of *L. korrae* from six states and against four non-*L. korrae* antigens. Plates were coated with 77.4 μg antigen in 350 μl per well, blocked with 1% bovine serum albumin, washed, and treated with an 1:16 dilution of MAb from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an antimouse IgG. Each treatment was replicated six times, and the experiment was repeated. The same protocol was used to test the antibody against 42 isolates of related and nonrelated fungi, two sources of grass, and PDB. Each treatment was replicated twice, and the experiment was repeated.

TABLE 3. Reaction of monoclonal antibody LKc50 against lyophilized mycelia extracts of *Leptosphaeria korrae* strain ATCC 56289 using indirect enzyme-linked immunosorbent assay[a]

L. korrae antigen concentration per well (μg)	Absorbance (405 nm) for dilutions of MAb supernatant							
	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
77.4	0.882	0.894	0.869	0.669	0.504	0.340	0.285	0.123
7.74	0.838	0.982	0.907	0.689	0.499	0.379	0.175	0.141
0.774	0.157	0.219	0.193	0.233	0.221	0.112	0.069	0.043

[a] Plates were coated with 360 μl of sample, blocked with 1% bovine serum albumin, washed, and treated with 1:2 dilutions of MAb from clone LKc50. Samples were probed with peroxidase-labeled antimouse antibody using 2, 2′-azino-di-[3-ethyl-benzthiazoline sulfonate] as the substrate. Reading of buffer-treated microtiter well was set at 0.000.

TABLE 4. Reaction of monoclonal antibody LKc50 against *Leptosphaeria korrae* and *Ophiosphaerella herpotricha* in agar cultures using indirect enzyme-linked immunosorbent assay

Fungus and isolate designation	Geographical origin	Source	Absorbance (405 nm)
Trial 1			
L. korrae			
13-300	Michigan	J. C. Stier	0.518[a]
13-501	Ohio	J. C. Stier	0.437
13-500	Ohio	J. C. Stier	0.652
13-502	Ohio	J. C. Stier	0.339
O. herpotricha			
#27	Kansas	N. Tisserat	0.051
#67	Kansas	N. Tisserat	0.064
Positive control[b]			0.680
Agar control			0.000
Trial 2			
L. korrae			
Bowman #2	Ohio	W. W. Shane	0.339
13-500	Ohio	J. C. Stier	0.328
D. Dean	Idaho	D. C. Thompson	0.261
D. Sod Vickens	Idaho	D. C. Thompson	0.266
Positive control[b]			0.495
Agar control			0.000

[a] Each number represents the mean of two replicates. An agar plug (7 mm) from each culture was ground in 5 ml of buffer and strained through 1 layer of Kimwipe tissue paper. Plates were coated with 350 μl of sample, blocked with 1% bovine serum albumin, washed, and treated with an 1/16 dilution of MAb from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an antimouse IgG.

[b] Homogenized, lyophilized mycelia of *L. korrae* strain ATCC 56289 used at a concentration of 77.4 μg per well.

MAb assay for *L. korrae* in agar cultures. The efficacy of LKc50 for verification of *L. korrae* in cultures was evaluated. Plugs (7 mm in diameter) were excised with a No. 3 cork borer from 10- to 20-day-old cultures of *L. korrae*. The top 3 mm of three plugs per fungal colony was ground between two pieces of sandpaper, rinsed with 5 ml of sensitization buffer, and filtered through one layer of tissue paper, and the filtrate was added to the ELISA plate as previously described. The identical procedure was conducted using *Ophiosphaerella herpotricha* (Fr. ex Fr.) J. C. Walker, a pathogen of turfgrass roots (12), for purposes of comparison.

Detection of *L. korrae* in infected plant material. Infected plant material was collected from NRS patches in Kentucky bluegrass lawns in Washington, Idaho, and Ohio. For each sample, 20 basal stems, crowns, and roots (1.5 cm in length) were washed with water and ground in 5 ml of carbonate sensitization buffer with a mortar and pestle. The suspension was filtered through one layer of Kimwipe tissue paper (Kimberly-Clark Corp., Roswell, GA). The resulting filtrate was used to sensitize polystyrene ELISA plates as previously described. Apparently healthy grass samples were collected and processed similarly.

The presence of *L. korrae* on the samples was verified by microscopic examination of plant tissue and by isolations. Isolations were made from three plants per patch. Basal stem sections were washed for 5 min under running tap water, followed by 0.26% NaOCl for 1 min, rinsed under running tap water for 5 min, and dipped in sterile distilled water for 15 sec. The pieces were air-dried on filter paper for 2–4 hr under sterile conditions. Grass sections, five per plant, were placed on acidified PDA (pH 4) in petri plates (9 cm) and incubated at 22–26 C under fluorescent

TABLE 5. Reaction of *Leptosphaeria korrae* specific monoclonal antibody (MAb) LKc50 in indirect enzyme-linked immunsorbant assay to field samples of necrotic ring spot infected and healthy Kentucky bluegrass

Sample	Turf stand symptoms	Basal stem signs	*L. korrae* isolated	Absorbance (405 nm)
Ohio samples (Trial 1)				
diseased				
1	patch	moderate[a]	yes	0.250[b]
2	patch	slight	yes	0.210
3	patch	moderate	yes	0.173
healthy[c]				
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[d]	none	none	no	0.096
...	buffer	...	...	0.000

[a] Rating system for ectotrophic colonization (possibly *L. korrae*) of basal stem, crown and roots is as follows — intense: colonization with hyphal 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.

[b] Average value of four subsamples. For each sample, 20 washed basal stems plus roots (1.5 cm length) were ground in 5 ml of buffer, filtered through 1 layer of Kimwipe tissue paper, and used to sensitize plates. Plates were coated with 350 μl of sample, blocked with 1% bovine serum albumin, and treated with a 1/16 dilution of MAb from clone LKc50. Samples were probed with alkaline phosphatase conjugated to an antimouse IgG.

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

[d] Noninfected 1-mo-old Kentucky bluegrass plants grown in greenhouse.

light. Plates were examined at weekly intervals for fungal colonies with characteristics of *L. korrae* or other turf pathogens. Putative colonies of *L. korrae* were tested for teleomorph production on autoclaved hard fescue seed, as described previously.

RESULTS

MAb production and cross-reactivity tests. In cross-reactivity tests using I-ELISA, MAb LKc50 reacted positively to all of the 24 isolates of *L. korrae* screened (Table 1). In the same test, a negative reaction was obtained against four other species of fungi. Readings for the antigens of *L. korrae* ranged from 25 to 44 times greater than those for the four non-*L. korrae* fungi. LKc50 was Ig-typed and determined to be an IgM.

MAb LKc50 reacted negatively to 38 of 42 isolates of related and nonrelated fungi, PDB, and two grass samples (Table 2). Reaction of MAb LKc50 was mildly positive to 25-1A (a *Drechslera erythrospila* sp.), moderately positive to two isolates, 3375 (an *Ascochyta* sp.) and CURV1 (a *Curvularia* sp.), and appreciably positive to 40-1A (an *Exserohilum holmii* strain) (Table 2).

Sensitivity of assay. In sensitivity assays using MAb LKc50 in I-ELISA, antigen could be detected at 7.7 μg dry weight *L. korrae* per microtiter well. Optimum MAb dilution was 1/16 for the 7.7 μg dry weight sample. Absorbance readings dropped off rapidly at more dilute MAb concentrations (Table 3).

Detection of *L. korrae* in pure culture. Using MAb LKc50 in I-ELISA, ample fungal mycelia were present in agar plugs from cultures to allow identification of *L. korrae* (Table 4). Reaction to *L. korrae* was 5–11 times greater than that for *O. herpotricha.*

Detection of *L. korrae* in infected plant material. *L. korrae* was detected by I-ELISA in all turf samples showing symptoms (patches) and signs (runner hyphae and mycelial plates) typical of necrotic ringspot. The presence of *L. korrae* in these samples was confirmed by isolations and induction of ascospores in vitro (Table 5). *L. korrae* was not detected by isolations or by I-ELISA assay in samples from nonsymptomatic areas (Table 5).

Absorbance readings from the *L. korrae* patches averaged 3 times greater than those from healthy turf at the Ohio site. Readings from the Washington and Idaho necrotic ring spot patches averaged 3.6 times greater than the healthy turf control.

DISCUSSION

Antibodies have proven useful for detection and monitoring of *Pythium* spp. on turfgrass (5,7) and of other major fungal pathogens, including *Phytophthora, Rhizoctonia, Lanzia*, and *Moellerodiscus* spp. (Agri-Diagnostics and Associates, Cinnaminson, NJ). Direct application of this technology to the problems involved in diagnosing necrotic ringspot would aid research in studies of etiology, biology, and control and help to reduce management costs for turfgrass by guiding chemical control measures.

Studies on the ecology and management of necrotic ring spot have been hampered by difficulties in detecting the pathogen. The ability to identify nonfruiting fungal cultures by I-ELISA could be a useful tool in these studies. Approximately 50% of the cultures in our collection that resemble *L. korrae* in growth habit did not produce ascospores within 3 mo, although they elicited a strong I-ELISA reaction with the LKc50 antibody. We feel that a positive I-ELISA test using the antibody specific to *L. korrae* provides good evidence that these cultures are *L. korrae.* Other techniques, such as protein band or restriction enzyme fragment profiles, have been used successfully for identification of fungi but are relatively slow and can not be used to directly diagnose diseased plants (1,4,11).

Antibody from LKc50 reacted positively with all isolates of *L. korrae* tested to date. Our tests indicate that false positive results may occur with the current form of the assay with a small number of nontarget organisms when using high antigen concentrations from axenic cultures. Verification of antibody assays by traditional isolation techniques is advised.

Antibody from LKc50 was effective in detecting *L. korrae* on naturally infected Kentucky bluegrass samples. The samples probably represent a relatively intense infestation of *L. korrae* as judged by the prominent ectotrophic hyphae signs and the relative ease with which the fungus could be isolated. On occasion we have been able to isolate *L. korrae* from naturally infected Kentucky bluegrass that exhibited a weak reaction in our I-ELISA test (W. Shane and J. Stier, *unpublished data*). We attribute this weak reaction to a low concentration of *L. korrae* on the sample, as evidenced by low levels of ectotrophic hyphae and infrequent isolations of *L. korrae* from such samples. Incubation of infected plant tissue on standard isolation media will sometimes increase the *L. korrae* biomass to concentrations sufficient for detection by I-ELISA. Tests are in progress to determine the reactivity of LKc50 antibody to mycelia of *L. korrae* grown under a variety of conditions.

The *L. korrae* antibody could prove to be a useful tool for studying the ecology and epidemiology of *L. korrae* and for diagnosing necrotic ring spot.

LITERATURE CITED

1. Abbott, L. K., and Holland, A. A. 1975. Electrophoretic patterns of soluble proteins and isoenzymes of *Gaeumannomyces graminis.* Aust. J. Bot. 23:1-12.
2. Crahay, J. N., Dernoeden, P. H., and O'Neill, N. R. 1988. Growth and pathogenicity of *Leptosphaeria korrae* in bermudagrass. Plant Dis. 72:945-949.
3. Endo, R. M., Ohr, H. D., and Krausman, E. M. 1985. *Leptosphaeria korrae*, a cause of the spring dead spot disease of bermudagrass in California. Plant Dis. 69:235-237.
4. Hawkes, N. J., and Harding, H. W. J. 1981. Isoelectric focusing as an aid to the identification of *Leptosphaeria narmari*, a cause of spring dead spot. Australas. Plant Pathol. 14:72-76.
5. Miller, S. A., Grothaus, G. D., Petersen, F. P., and Papa, S. L. 1986. Detection of Pythium blight in turfgrass using a monoclonal antibody-based diagnostic test. Phytopathology 76:1057.
6. Oi, V. T., and Herzenberg, L. A. 1980. Immunoglobulin-producing hybrid cell lines. Pages 351-372 in: Selected Methods in Cellular Immunology. B. B. Mishell and S. M. Shiigi, eds. W. H. Freeman, San Francisco, CA.
7. Shane, W. W. 1988. Evaluation of Pythium blight prediction models using an antibody-aided detection technique. (Abstr.) Phytopathology 78:1612.
8. Shane, W. W., and Nameth, S. T. 1988. Monoclonal antibodies for diagnosis of necrotic ring spot of turfgrass. (Abstr.) Phytopathology 78:1521.
9. Smiley, R. W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, MN. 102 pp.
10. Smiley, R. W., and Craven-Fowler, M. 1984. *Leptosphaeria korrae* and *Phialophora graminicola* associated with Fusarium blight syndrome of *Poa pratensis* in New York. Plant Dis. 68:440-442.
11. Tisserat, N. 1988. Differentiation of *Ophiosphaerella herpotricha* and *Leptosphaeria korrae* by restriction fragment length polymorphism analysis. (Abstr.) Phytopathology 78:1613.
12. Tisserat, N. A., Pair, J. C., and Nus, A. 1989. *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermudagrass in Kansas. Plant Dis. 73:933-937.
13. Walker, J., and Smith, A. M. 1972. *Leptosphaeria narmari* and *L. korrae* spp. nov., two long-spored pathogens of grasses in Australia. Trans. Br. Mycol. Soc. 58:459-466.
14. Worf, G. L., Stewart, J. S., and Avenius, R. C. 1986. Necrotic ring spot disease of turfgrass in Wisconsin. Plant Dis. 70:453-458.