Prospects for Early Detection of Pythium Blight Epidemics on Turfgrass by Antibody-Aided Monitoring

W. W. SHANE, Assistant Professor, Department of Plant Pathology, The Ohio State University, Columbus 43210-1087

ABSTRACT

Shane, W. W. 1991. Prospects for early detection of Pythium blight epidemics on turfgrass by antibody-aided monitoring. Plant Dis. 75:921-925.

Methods for sampling turfgrass tissue were compared for their effectiveness in monitoring Pythium blight epidemics with enzyme-linked immunosorbent assay (ELISA). Sample areas consisted of marked strips on golf course fairways and tees with bentgrass and annual bluegrass naturally infested by *Pythium aphanidermatum*. Samples consisted of 1) whole plants picked by hand and assayed as whole plants; 2) whole plants sectioned into lower, middle, and upper strata components; and 3) leaf clippings collected with a reel mower set at a 1.2-cm cutting height. ELISA readings for mowed samples generally matched those for whole-plucked samples (r^2 values ranging from 0.457 to 0.601). Fluctuations in detectable *Pythium* antigens were most pronounced on the uppermost stratum compared with moderate to very little change in ELISA readings for the two lower strata. Several episodes of *Pythium* antigen increase were detected by ELISA assays of mowed samples, although signs and symptoms of Pythium blight were not evident. However, increases in ELISA readings for Pythium coincided with, but did not generally precede, the onset of blight symptoms with a 2- to 3-day sampling interval. Antibody-aided detection is useful for verification of diagnosis and determination of general *Pythium* population fluctuations, but current methodology is not satisfactory for advanced detection of blight epidemics.

Additional keyword: epidemiology

The primary causal agent of Pythium blight, *Pythium aphanidermatum* (Edson) Fitzp. (10), is sporadic in occurrence on highly managed turf but potentially devastating in its effects. Under optimum conditions, *P. aphanidermatum* can quickly damage and kill bentgrass (*Agrostis palustris* Hudson), perennial ryegrass (*Lolium perenne* L.), and annual bluegrass (*Poa annua* L.). The pathogen overwinters as oospores in soil or thatch material (2,5). In Ohio, overwintering oospores in thatch germinate in March, April, and May (Julian days [JD] 60–151), at the start of a new infection cycle (4). Onset of blight symptoms generally occurs during June or July (JD 152–212), depending on the weather. Dispersal of the pathogen occurs by mycelial growth, sporangia, mechanical movement of infected tissue, and zoospores. Blight symptoms are most severe at temperatures of 30–36 C (1).

Salaries and research support were provided by state and federal funds appropriated to Ohio Agricultural Research and Development Center (OARDC) and OSU. Additional support was provided by the Ohio Turfgrass Foundation.

OARDC Journal Article 197-89.

Accepted for publication 11 March 1991 (submitted for electronic processing).

Concern about the impact of pesticides on the environment and high costs for fungicide sprays for *Pythium* on fairways (average of 12–24 ha for 18-hole course) have led to the development of Pythium blight prediction models (2,3,9). Development and evaluation of such models has been hindered by the difficulties encountered in monitoring pathogen activity on the host. Studies on Pythium blight of turfgrass have relied on visual observation of symptoms and signs in the field (2,3,9). However, symptoms may not be a true measure of *Pythium* populations in turfgrass foliage. Colonization of plant tissue in the early stages of an epidemic may proceed without obvious blight symptoms. Pythium blight symptoms and signs may be confused with those caused by other pathogens, especially under conditions not ideal for disease development. In addition, symptoms attributable to a Pythium blight epidemic may persist in a turfgrass stand even though *Pythium* biomass may be decreasing.

A promising approach to the detection of pathogens in plant tissue is the use of enzyme-linked immunosorbent assays (ELISA) for fungal antigens in plant extracts. Pathogen-specific antibodies have been developed for *Leptosphaeria korrae* J. C. Walker & A. M. Sm. (8), *Pythium*, *Rhizoctonia*, and *Lanzia/Moellerodiscus* spp. (Agri-Diagnostics Associates, Cinnaminson, NJ), which are common pathogens on turfgrass and other plants. ELISA has been used to detect *Pythium* in symptomless areas of a turfgrass stand with Pythium blight symptoms (7). An attractive prospect is the use of ELISA to detect the onset of a Pythium blight epidemic in sufficient time to allow the successful application of fungicides. Further applications of ELISA may be to monitor the effects of management actions and weather on pathogen growth and survival. However, very little information is currently available on methods for collecting and processing plant samples from large turfgrass stands for the purpose of disease detection by ELISA. In this study, the objective was to evaluate the use of ELISA for monitoring Pythium blight epidemics. A preliminary report has been published (12).

MATERIALS AND METHODS

Monitoring sites. Two fairways (12S and 17G) and one tee (17S) in 1987, four fairways (12S, 17G, 16L, and 16H) in 1988, and two fairways (17G and 16L) in 1989 were monitored at the Ohio State University Golf Club, Columbus. All sites were on turf established more than 20 yr ago, and contained mixtures of bentgrass, annual bluegrass, and small amounts of perennial ryegrass. The sites were in low areas adjacent to a small creek.

The fairways and tees were irrigated on alternate days, except when rainfall provided adequate moisture. The sites were highly conducive to Pythium blight, as evidenced by severe disease in preceding seasons. Test areas were not treated with fungicides known to be toxic to *Pythium*.

Sampling. Monitoring and sampling were done in a marked 2-m-wide strip across the lowest point in each tee or fairway. Observations were made and samples were collected at 8 a.m., before the fairways and tees were cut by gang or triplex mowers in routine maintenance operations.

In 1987, 1988, and 1989, leaf clippings from the marked areas were collected with a hand-powered reel mower set at a 1.2-cm cutting height. Two to four adjacent swaths were made with the mower across a fairway site (approximately 25 m wide) or tee (15 m wide) to obtain a bulk sample of approximately 2 kg. Bulk samples were blotted on

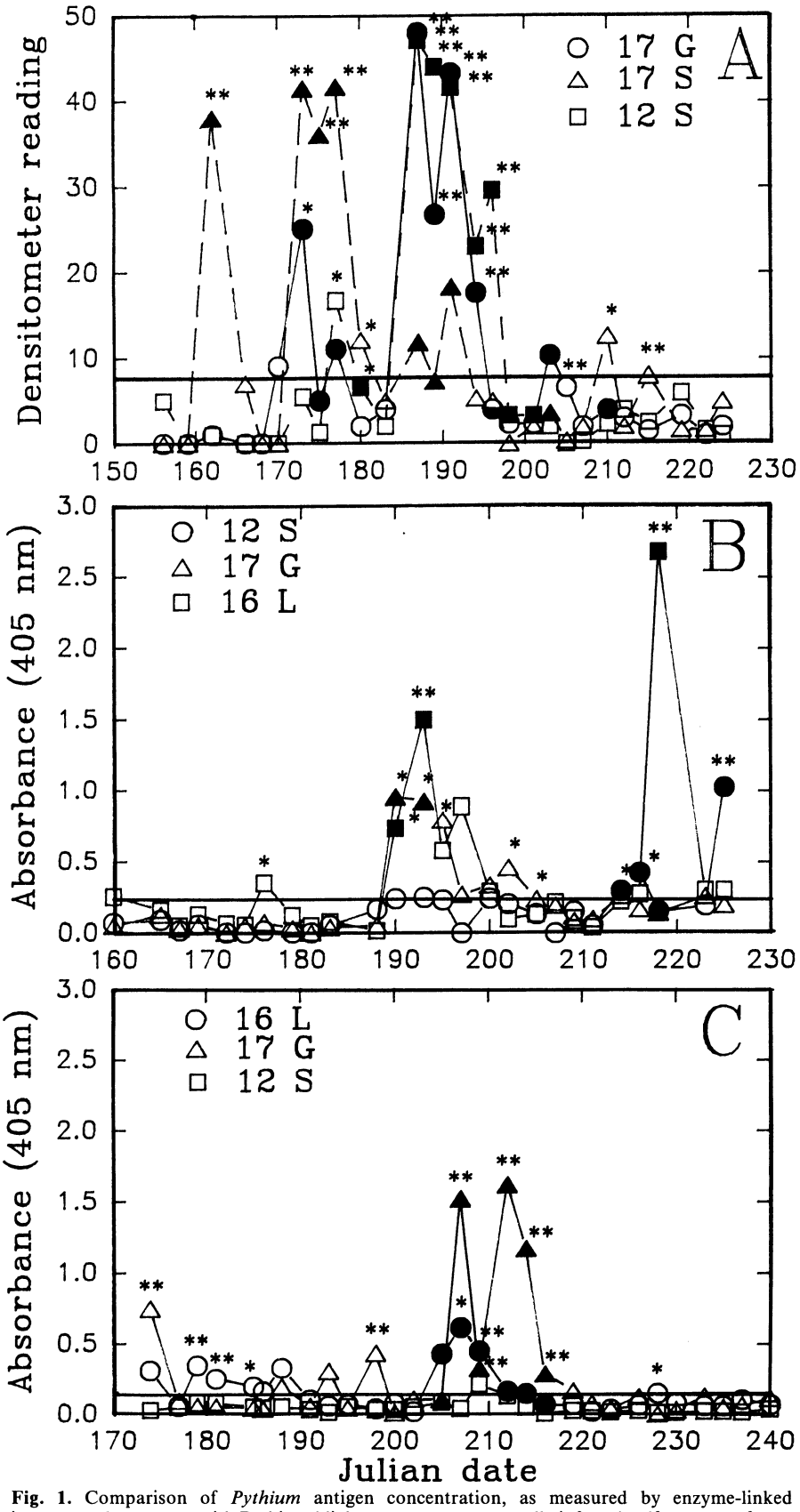

Fig. 1. Comparison of *Pythium* antigen concentration, as measured by enzyme-linked immunosorbent assay, with Pythium blight symptoms on naturally infected golf course turfgrass in **(A)** 1987, **(B)** 1988, and **(C)** 1989. A dipstick assay was used in 1987 and a multiwell assay was used in 1988 and 1989. Sample sites were marked areas of mixed annual bluegrass, bentgrass, and perennial ryegrass in fairways (17G, 12S, and 16L) or a tee (17S). Samples were grass clippings collected with a hand-powered reel mower set at a 1.2-cm cutting height. Horizontal lines indicate positive-negative threshold established as the season mean of the negative controls (healthy grass) plus two standard deviations. Values shown are the average of three replications. Solid symbols indicate Pythium blight symptoms or signs could be detected without a microscope. Means marked with an asterisk indicate values significantly greater than the negative control for the date (*, $P = 0.05$; **, $P = 0.01$) using Dunnett's one-tailed t test (13).

several layers of paper towel to remove free water, and three 10-g subsamples were taken. Samples were collected weekly on Mondays, Wednesdays, and Fridays. The presence of water-soaked, dark, slimy leaves with white mycelium, typical of Pythium blight symptoms and signs, were noted.

Disease observations were made by golf course personnel for the remaining days of each week. Tentative diagnoses of Pythium blight in the sites were verified by microscopic examination of symptomatic tissue for mycelium, oospores, and sporangia typical of *Pythium*. Identification of *Pythium* species was based on oospore and sporangia characteristics (6). Grass tissue with symptoms but no signs of Pythium blight was plated on a selective medium for *Pythium* (11) to induce oospore production for disease diagnoses.

In 1987, 15 leaves per site at each sampling date were selected from the lower canopy and plated on a selective medium (11) to ascertain the presence and identities of *Pythium* species. Routine plating of leaves was discontinued after 1987 when it was discovered that *P. aphanidermatum* could be isolated from the lower canopy throughout the 1987 season.

In 1988 and 1989, samples were collected by hand-plucking 2 × 2 cm bunches of grass shoots plus stolons and shallow roots (to 1.5 cm below crown) at random across the strip areas before mower samples were taken. Approximately 100 g of hand-plucked samples were collected from each site. The samples were blotted by hand on several layers of paper towel to remove free water. In 1988, three 10-g whole plant subsamples from the 12S, 16L, and 17L were analyzed and compared with mowed samples as described previously.

In 1989, plucked plant samples from the marked areas of the 16L and 17G fairway sites were cut into three subsections: upper stratum (tissue 0.5 cm above the crown), middle stratum (0.5 above to 0.5 cm below the crown), and lower stratum (0.5–1.5 cm below the crown). Weights for upper, middle, and lower strata samples were 5, 5, and 3 g, respectively. Three samples for each strata were analyzed for each site.

Additional grass clipping samples were collected from turfgrass exhibiting Pythium blight symptoms and signs to determine *Pythium* antigen concentrations in "hot spots." "Directed" samples were collected within 15 m of marked areas only from turfgrass with Pythium blight symptoms. A single bulk sample of approximately 2 kg was collected with a hand-powered reel mower set at a 1.2-cm cutting height. A bulk sample was blotted on several layers of paper towel to remove free water, and three 10-g subsamples were taken. Directed samples were collected during

4 days in 1987 and 5 days in 1988.

Assay for *Pythium*. Each 10-g grass sample was ground in 100 ml of distilled water for 1 min in a blender, and the resultant brei was filtered through a double layer of tissue paper. Smaller samples were processed similarly but with correspondingly less water. Relative concentrations of *Pythium* antigen in the extracts were determined with *Pythium*-specific ELISA tests (Agri-Diagnostics, Cinnaminson, NJ) that were sensitive to major species of *Pythium*, including *P. aphanidermatum*, *P. ultimum* Trow, and *P. graminicola* Subramanian, but relatively insensitive to *P. torulosum* Coker & F. Patterson and *P. vanterpoolii* V. Kouyeas & H. Kouyeas (7).

The positive control for the assays was an extract from *P. aphanidermatum*, strain PA4, from a 2-wk-old potato-dextrose broth shake culture incubated at approximately 25 C and room light. The mycelia were washed with distilled water on filter paper in a Büchner funnel. The mycelia were lyophilized, ground in liquid nitrogen, rehydrated in distilled water, and filtered (0.25 μm). The filtrate was lyophilized and stored at 4 C.

In 1987, the Agri-Diagnostic dipstick assay for *Pythium* was used to detect *Pythium* antigens in grass samples (7). The dipstick supported a *Pythium*-specific antibody immobilized on a membrane. The dipstick was placed for 60 min in a vial containing 1 ml of grass extract, 4 ml of kit-supplied buffer, and 2 ml of soluble conjugated *Pythium*-specific antibody. The dipstick was rinsed for 5 s and placed in a solution containing a substrate specific for the conjugated antibody. The dipstick membrane was rinsed for 5 s and air-dried for 30 min before reading. Color intensity of the membrane was read with the Agri-Diagnostics reflectance meter. Reflectance values were adjusted for background color based on antibody-free membranes subjected to the same plant extracts and assay reagents.

In 1988 and 1989, the Agri-Diagnostic *Pythium* multiwell ELISA kit C was used. Grass extract (25 μl) was mixed with 75 μl of extraction buffer and added to a multiwell precoated with a *Pythium*-specific antibody. The multiwell plate was agitated on a shaker for 10 min at room temperature (24–27 C). The wells were aspirated, rinsed five times with wash solution, and 100 μl of a solution containing a *Pythium*-specific antibody-peroxidase conjugate was added. The plate was again agitated for 10 min, rinsed five times with a wash solution, and 100 μl of a peroxidase-specific substrate was added and shaken for 10 min, followed by 50 μl of stop solution. No significant reaction was detected with assays of uninfected grass from various sources. Healthy grass sap at the concentrations employed in these tests did not interfere with the assay process (W. W. Shane, *unpublished*). Absorbance values were determined at 405 nm with a model 700 microplate reader (Cambridge Technology, Inc., Cambridge, MA). Negative controls consisted of nonsensitized wells and uninfected grass tissue.

The positive-negative thresholds for mowed samples were based on apparently healthy grass from a bentgrass green at the Ohio State University Turf Plots (1987) and from a bentgrass-annual bluegrass fairway at the Ohio State Golf Club (1988 and 1989). The two areas had no history of Pythium blight, had good air and water drainage (which discouraged *Pythium* activity), and displayed no symptoms of Pythium blight during the test period. Mowed grass samples from the negative control sites were collected and processed as described previously.

Positive-negative thresholds for ELISA were established as the mean of the negative controls (healthy grass) for all sample dates plus two standard deviations. Dunnett's one-tailed *t* test (13) was used to compare readings for the sample sites against the negative control for each sampling date.

RESULTS

P. aphanidermatum was the most prevalent species isolated from turfgrass leaves displaying cottony blight and water-soaking in the test areas during the months of June through August in all 3 yr. *P. torulosum* was occasionally found and *P. ultimum* was not detected. Lobed sporangia typical of *P. aphanidermatum* were occasionally seen on grass with blight symptoms. *P. aphanidermatum* was also isolated from asymptomatic tissue. Pythium spp. were isolated from leaf samples taken from the lower canopy at all sampling dates from June through August in 1987 (*data not shown*). Routine isolations from leaf tissue were discontinued after 1987 because of the widespread occurrence of *Pythium* in the lower canopy in the marked test areas.

Pythium antigen concentration in the mowed samples from tee and fairway grass strips, as measured by ELISA, fluctuated greatly during the 3 yr (Fig. 1). *Pythium* antigen maxima and fluctuations differed among the sites within each year. For example, the 17S site had greater Pythium antigen early in the season and less in the midseason of 1987 compared with 12S and 17G (Fig. 1A). Likewise, sites 17G and 16L had high *Pythium* antigen concentrations 1988 compared with 12S (Fig. 1B). Blight epidemics were more frequent in 1987 than 1988 and 1989.

Blight symptoms correlated well with high ELISA readings for *Pythium* (Fig. 1). Blight symptoms were always detected in marked areas when ELISA values for mowed samples were above 20 (dipsticks in 1987) or 1.0 (multiwell plates in 1988 and 1989).

On occasion, Pythium blight symptoms were present in the strip sites, although ELISA readings were below the positive-negative threshold (Fig. 1A, JD 176–210). Samples from strip sites were taken without regard to symptoms and included both diseased and healthy tissue. ELISA values for blighted grass collected adjacent to the strip sites were 2.6–14.6 times greater than those for strip

Table 1. Comparison of *Pythium* antigen concentrations in turfgrass clippings from strip and directed sample areas on bentgrass/annual bluegrass fairways on selected sample dates and sites at the Ohio State University Golf Course in 1987 and 1988[a]

Sample date[b] Julian date	Fairway	Relative *Pythium* antigen concentration	
		Strip	Directed[c]
1987			
205	17G	6.6**[d]	37.3**
208	17G	2.0	23.3**
210	17G	4.0	39.3**
212	17G	3.0	10.0*
1988			
193	17G	0.923*	2.757**
216	12S	0.423*	2.570**
218	17G	0.142	2.078**
218	12S	0.152	1.941**
225	12S	1.024**	2.672**

[a] Leaf clippings from strip and directed areas were collected with a hand-powered reel mower set at a 1.2-cm cutting height. Strip samples were taken without regard to disease symptoms from a marked 2-m-wide strip across a Pythium blight-prone fairway. Directed samples were taken solely from turfgrass with Pythium blight signs and/or symptoms in areas immediately adjacent to marked areas.

[b] Values in 1987 are densitometer readings determined by enzyme-linked immunosorbent assay (ELISA) in a dipstick format. Values in 1988 are absorbance at 405 nm determined by ELISA in a multiwell format. Values are the average of three samples.

[c] All directed samples were significantly greater than the strip samples for the same date ($P = 0.05$) using a one-tailed *t* test (13).

[d] Means marked with an asterisk indicate ELISA values significantly greater than the healthy grass control for the date (*, $P = 0.05$; **, $P = 0.01$) using a one-tailed *t* test (13).

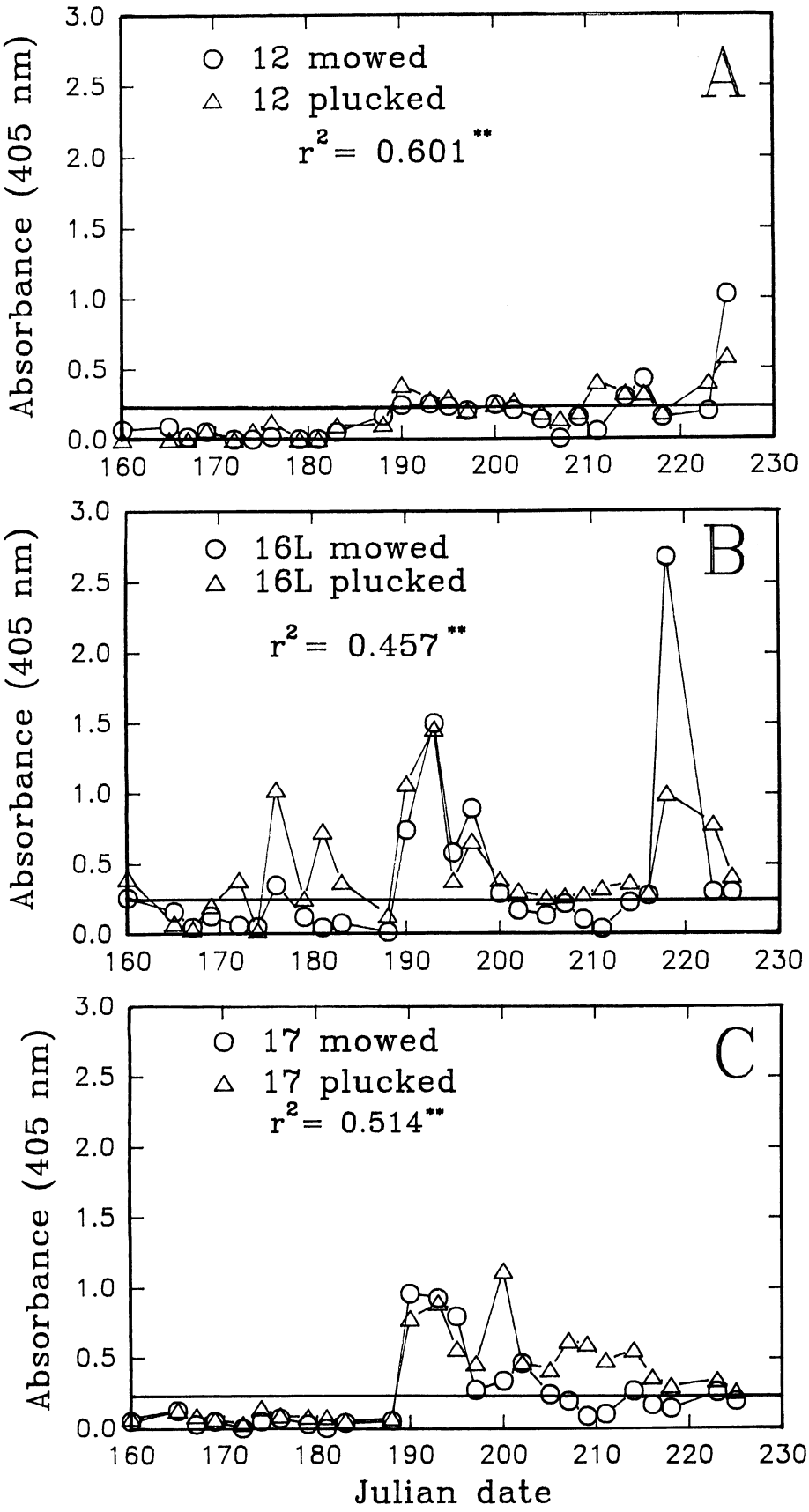

Fig. 2. Comparison of *Pythium* antigen concentrations, as measured by enzyme-linked immunosorbent assay, in hand-plucked whole plants with leaf clippings (1.2-cm mowing height) from three naturally infected golf course fairways in Columbus, OH, during 1988. **(A)** Site 12S, **(B)** site 16L, and **(C)** site 17G. All sites were mixtures of bentgrass, annual bluegrass, and ryegrass. Samples were collected in the same 2-m-wide marked areas across the fairways on each collection date. Values shown are the average of three replications. Horizontal lines indicate positive-negative threshold established as the season mean of the negative controls (healthy grass) plus two standard deviations.

samples (Table 1). Several slight but significant increases in *Pythium* antigen concentrations were detected without corresponding macroscopic signs or symptoms of Pythium blight on the day or within several days of sampling, e.g., JD 187–200 in 1989 for 17G (Fig. 1C).

Occasionally, ELISA readings increased immediately before the appearance of Pythium blight symptoms (Fig. 1A, site 17 G on JD 173). More often, increases in ELISA readings coincided with but did not precede the onset of blight symptoms in mowed samples at the 2- to 3-day sampling interval used in this test.

ELISA readings for mowed and plucked samples were strongly correlated, with highly significant ($P < 0.01$) r^2 values ranging from 0.457 to 0.601 (Fig. 2). Although differences were seen during some time periods, neither sampling method yielded consistently greater ELISA readings.

Hand-plucked plant samples from two sites in 1989 were sectioned into lower, middle, and upper strata sections to see if increases in *Pythium* antigen concentrations were apparent for specific plant parts. Prominent ELISA readings were apparent at times in upper but not in middle or lower stratum samples (Fig. 3). There was no evidence for the buildup of *Pythium* populations in the lower and middle strata in advance of Pythium blight episodes.

DISCUSSION

Antibody-aided detection is useful for verification of diagnoses and determination of general *Pythium* population fluctuations. Monitoring leaf tissue with ELISA revealed several episodes of apparent *Pythium* antigen increase, although no symptoms were obvious without a microscope. ELISA-aided monitoring provides a useful supplement to visual observation for epidemiological studies of Pythium blight epidemics.

ELISA readings, although indicative, may not be directly correlated with *Pythium* biomass in vivo. The reactivity of the assays to sporangia, zoospores, and oospores is unknown, as is the efficiency of *Pythium* extractions from the various plant parts. Unlike culture plate isolation techniques, ELISA does not distinguish between living and dead fungal tissues. However, highly managed turfgrass is mowed frequently, which ensures that *Pythium* antigens detected on new grass clippings are the result of relatively recent fungal growth.

ELISA assays of leaf tissue generally showed no marked increase in *Pythium* antigen concentrations 2–3 days in advance of a disease outbreak. A sampling interval of 2–3 days was perhaps too long to detect initial fungal growth, especially in view of the explosive nature of *Pythium* blight development. Stier and Shane (14) observed that more than

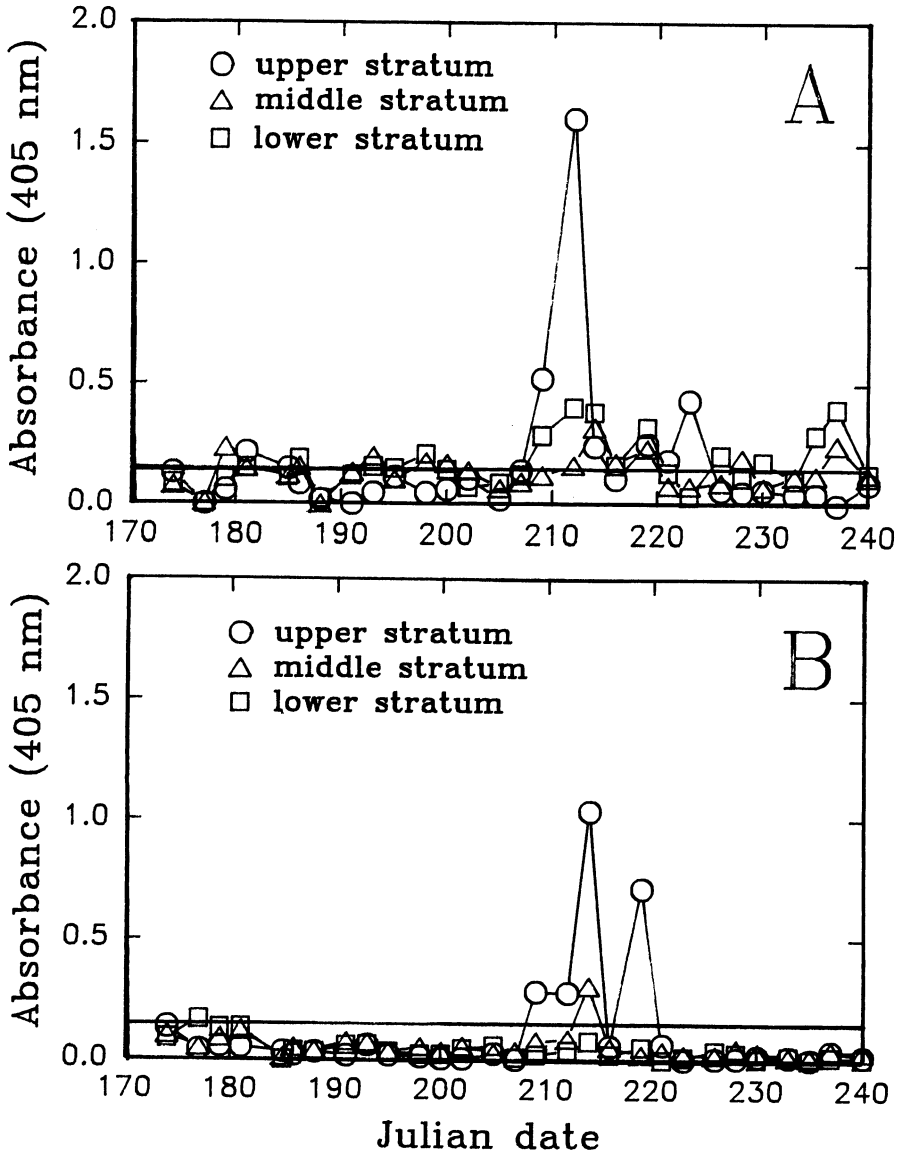

Fig. 3. Comparison of *Pythium* antigen concentrations, as measured by enzyme-linked immunosorbent assay, in plant subsections from two naturally infected fairways in 1989. Both fairways were mixtures of bentgrass, annual bluegrass, and perennial ryegrass. Samples were collected in the same 2-m-wide marked areas across the fairways on each collection date. Whole plant samples were cut into three subsections: upper stratum (tissue 0.5 cm above the crown, middle stratum (0.5 above to 0.5 cm below the crown), and lower stratum (0.5–1.5 cm below the crown). Values shown are the average of three replications. Horizontal lines indicate positive-negative threshold established as the season mean of the negative controls (healthy grass) plus two standard deviations.

24 hr was needed for appreciable symptom expression at 30.6, 26.7, and 21.1 C after inoculation of Kentucky bluegrass with *P. aphanidermatum* on agar plugs. However, subsequent work has shown that blight symptoms can be produced with this same system in less than 24 hr if *Pythium* cultures are grown at a high temperature (e.g., 30 C) before their use as inoculum (W. W. Shane, *unpublished*). The time between the onset of favorable environmental conditions and the appearance of blight symptoms would be less if the pathogen has already colonized the host. Daily sampling, or perhaps multiple samples per day, may reveal increases in *Pythium* antigens not detected in the present tests. The use of a model to predict *Pythium* population fluctuations based on environmental conditions may provide a guide for better timing of sample collection by antibody-aided detection.

Antibody-aided monitoring did not reveal an increase in *Pythium* antigens in the lower strata in the early stages of an epidemic, as expected initially. Samples from the lower two strata consisted primarily of stem, crown, and root tissue that are perhaps less accessible to colonization by *Pythium* than sheath or leaf tissue.

Monitoring Pythium blight epidemics by ELISA presents special problems because of the clustered distribution of infected plants. Small areas of obvious blight symptoms and signs could sometimes be seen in the marked areas even though average *Pythium* antigen concentrations in leaf tissue, as measured by ELISA, over the entire site were below the detection threshold. Prospects for presymptomatic detection of *Pythium* on turfgrass stands might be improved by increasing the concentration of plant material assayed by ELISA and/or by using an ELISA assay with greater sensitivity.

ACKNOWLEDGMENTS

I thank T. Joyner and P. Zarlengo for technical assistance and Agri-Diagnostics Associates, Cinnaminson, NJ, for donation of all immunological supplies used in these studies.

LITERATURE CITED

1. Freeman, T. E. 1960. Effects of temperature on cottony blight of ryegrass. (Abstr.) Phytopathology 50:575.
2. Hall, T. J. 1978. Survival of *Pythium aphanidermatum* on three naturally infested *Agrostis palustris-Poa annua* golf course turfs and the influence of air temperature and relative humidity on naturally occurring Pythium blight epiphytotics. M.S. thesis. The Ohio State University, Columbus. 122 pp.
3. Hall, T. J., Larsen, P. O., Madden, L. V., and McCormick, D. J. 1985. Forecasting turfgrass diseases—an update. Golf Course Manage. 53:74.
4. Hall, T. J., Larsen, P. O., and Schmitthenner, A. F. 1980. Survival of *Pythium aphanidermatum* in golf course turfs. Plant Dis. 64:1100-1103.
5. Hendrix, F. F., Jr., and Campbell, W. A. 1971. Pythiums as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.
6. Middleton, J. T. 1943. The taxonomy, host range, and geographical distribution of the genus *Pythium*. Mem. Torrey Bot. Club 20:1-171.
7. Miller, S. A., Grothaus, G. D., Petersen, F. P., Rittenburg, J. H., Plumley, K. A., and Lankow, R. K. 1989. Detection and monitoring of turfgrass pathogens by immunoassay. Pages 109-120 in: EPA Integrated Pest Management for Turfgrass and Ornamentals. A. R. Leslie and R. L. Metcalf, eds. Environmental Protection Agency, Washington, DC.
8. Nameth, S. T., Shane, W. W., and Stier, J. C. 1990. Development of a monoclonal antibody for detection of *Leptosphaeria korrae*, the causal agent of necrotic ringspot disease of turfgrass. Phytopathology 80:1208-1211.
9. Nutter, F. W., Cole, H., Jr., and Schein, R. D. 1983. Disease forecasting system for warm weather Pythium blight of turfgrass. Plant Dis. 67:1126-1128.
10. Saladini, J. L., Schmitthenner, A. F., and Larsen, P. O. 1983. Prevalence of *Pythium* species associated with cottony-blighted and healthy turfgrasses in Ohio. Plant Dis. 67:517-519.
11. Schmitthenner, A. F. 1973. Isolation and identification methods for *Phytophthora* and *Pythium*. Pages 94-100 in: Proc. Woody Ornamental Dis. Workshop, 1st.
12. Shane, W. W. 1988. Evaluation of Pythium blight prediction models using an antibody-aided detection technique. (Abstr.) Phytopathology 78:1612.
13. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw Hill Book Co., New York. 481 pp.
14. Stier, J. C., and Shane, W. W. 1988. Time course of Pythium blight development on turfgrass under controlled environmental conditions. (Abstr.) Phytopathology 78:1612.