Evaluation of an Enzyme-linked Immunosorbent Assay Method for Predicting Brown Patch Infection in Turf

Michael A. Fidanza and Peter H. Dernoeden

Department of Agronomy, University of Maryland, College Park, MD 20742-5821

Additional index words. *Rhizoctonia solani*, perennial ryegrass, *Lolium perenne*, turfgrass

Abstract. A field investigation was conducted during 1991 and 1992 to determine the effectiveness of enzyme-linked immunosorbent assay (ELISA) to predict brown patch (*Rhizoctonia solani* Kühn) infection events in 'Caravelle' perennial ryegrass (*Lolium perenne* L.). Turfgrass samples were collected either between 7:00 and 8:00 AM or 4:00 and 5:00 PM, and from plots mowed to a height of either 1.7 or 4.5 cm. Pathogen detection levels were generally higher in AM-sampled turf and in plots mowed to a height of 4.5 cm. During 2 years, only 7 of 15 infection events were predicted from samples collected from high-cut turf and only three from samples collected from low-cut turf. While this technology is useful for confirming the presence of *R. solani*, it was unreliable for predicting infection events.

Molecular biology and biotechnology research has led to the development of antibodies that detect proteins or nucleic acids of plant pathogens (Clark, 1981). As a result, enzyme-linked immunosorbent assay (ELISA) methods were developed for plant disease diagnosis and detection by a double-sandwich antibody reaction (Miller and Martin, 1988). Miller and Martin (1988) discuss the potential of ELISA to detect plant pathogens and quantify their populations before the appearance of disease symptoms. Plant pathogen levels, as determined from ELISA techniques, can be compared to established disease thresholds and facilitate disease management decisions (Miller, 1982).

Commercial ELISA-based turfgrass disease detection kits are available for several turfgrass pathogens (Rittenberg et al., 1988). Turfgrass disease diagnosis and detection using ELISA was demonstrated by Shane (1991), Baldwin (1993), and Schumann et al. (1994). Shane (1991) concluded that pathogen quantification was not consistent; however, ELISA was useful to confirm pythium blight diagnosis. Baldwin (1993) used ELISA technology to support fungicide spray decisions for dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett). In that study, less fungicide was applied when ELISA detection results were used to guide treatment, when compared to fungicide sprays based on visual disease assessment. For brown patch, fewer fungicide sprays were applied and acceptable brown patch (*Rhizoctonia*

solani Kühn) control was achieved by combining weather-based disease forecasts with ELISA-based confirmation of pathogen levels (Schumann et al., 1994).

While ELISA technology has been shown to improve fungicide use strategies for turfgrass disease management, the influence of mowing height and sampling time on pathogen detection levels by ELISA has not been investigated. Therefore, the objectives of this study were to monitor *R. solani* levels in 'Caravelle' perennial ryegrass using leaf tissue samples collected from turf mowed at two heights of cut and two collection timings. The ELISA system is sold under the trade name of Reveal Turf Disease Detection Kit. The assays were made available by Agri-Diagnostic Associates (Cinnaminson, N.J.), but are now sold by Neogen Corp., Lansing, Mich.

Materials and Methods

The ELISA method was evaluated using leaf samples from a mature stand of 'Caravelle' perennial ryegrass. Field experiments were conducted in 1991 and 1992 at the Univ. of Maryland Cherry Hill Turfgrass Research and Education Facility in Silver Spring. The study site was subjected to two mowing heights (1.7 or 4.5 cm), and plot size measured 1.5 × 6.0 m. The plots were randomly dispersed over a 5.5 × 10.0-m area and were mowed three times weekly; clippings always were removed.

From 21 June to 19 July 1991 (i.e., 29 sampling days), two assays were conducted using hand-clipped leaf samples from 1.7- and 4.5-cm-high plots, and samples were collected between 7:00 and 8:00 AM or between 4:00 and 5:00 PM. About 2 g of primarily symptomless turfgrass clippings were randomly sampled at four locations per plot (i.e., four subsamples) from two replicate plots at both mowing heights. The four subsamples were combined before analysis. The study area received no fertilizer or fungicide during the study.

Turfgrass clippings were sampled and extractions prepared according to the instructions provided by Agri-Diagnostic Associates. Briefly, plant tissue sap was extracted by grinding leaf blades on abrasive pads and placed in an extraction solution. Several drops from the extraction solution were placed on the surface of the detector, which is a porous device that contains specific antibodies attached to its surface. Next, the detection, rinse, color, and finishing solutions were added to the detector in sequence, resulting in a color reaction on the surface of the detector. The color reaction was quantified with a portable, battery-powered reflectometer (AgriMeter II, Agri-Diagnostic Associates).

Based on the intensity of the color reaction on the detector, the AgriMeter II provided a numerical value (i.e., pathogen level) determined by Agri-Diagnostic Associates that corresponds to a disease risk threshold. Disease risk was determined according to the Reveal Interpretation Guide, which defines risk levels: 0 to 19 (low); 20 to 29 (caution); 30 to 37 (danger); and >38 (extreme). A low range value indicates that the pathogen was not detected or that the pathogen was present at a low population level. The caution range indicates that the pathogen was present and disease development is possible if favorable weather conditions occur. The danger range indicates that the pathogen is present and disease symptoms are likely, and a preventive disease management program is suggested. The extreme range indicates that disease symptoms are likely, and a curative disease management program is suggested.

The ability of ELISA to forecast brown patch outbreaks was determined by comparing observed events when foliar *R. solani* mycelium was either present or absent vs. the daily risk levels detected. Detector levels corresponding to the danger (i.e., 30 to 37) or extreme (i.e., >38) risk ranges were considered necessary to forecast a brown patch outbreak or *R. solani* infection event. Periodically throughout the study, turfgrass clippings with disease symptoms were examined microscopically to confirm the presence of *R. solani*. The *R. solani* biotype at the study site was identified as belonging to anastomosis group AG-1-IA.

Because detection levels were typically greater from AM- vs. PM-sampled turf in 1991, leaves were only collected between 7:00 and 8:00 AM in 1992. From 6 June to 31 Aug. 1992 (i.e., 87 sampling days), two assays were conducted on samples from plots maintained at 1.7- and 4.5-cm mowing heights. The assay methods were the same as previously described.

To compare the four levels of disease risk statistically, the results from the ELISA tests were standardized according to the midpoint rule: 0 to 19 (low range) = 10; 20 to 29 (caution range) = 25; 30 to 37 (danger range) = 34; and >38 (extreme range) = 59 (Campbell and Madden, 1991). All data were subjected to repeated measures analysis of variance to evaluate the treatment effects over time (Rowell and Walters, 1976; SAS Institute, 1985). Significantly different means were separated us-

Received for publication 17 Apr. 1995. Accepted for publication 3 July 1995. Maryland Agriculture Experiment Station Scientific Article no. A7745 and Contribution no. 9066, College Park. We are grateful to James Adams of Agri-Diagnostic Associates (Cinnaminson, N.J.) for providing the ELISA test kits. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

ing Fisher's protected least significant difference test at $P \leq 0.05$ (Steel and Torrie, 1980).

Results

AM and PM sampling (1991). The assay consistently tested positive for *R. solani* throughout the duration of the study (Table 1), but data collected before 30 June are not shown. Pathogen levels were influenced by mowing height and sampling time (data not shown). Detection levels were higher from plots maintained at 4.5 (25) vs. 1.7 cm (19). When comparing AM and PM sampling times averaged over mowing height, higher detection levels were observed from AM-sampled turf on 22 of 29 days (Table 1). Mean detection levels were 26 for AM and 18 for the PM sampling (data not shown).

Brown patch outbreaks, as determined by microscopic observation of foliar *R. solani* mycelium, occurred on 3, 8, and 11 July (Table 1). Successful detection of a brown patch outbreak was considered one that was predicted when detection levels ≥ 30 were recorded within 72 h of an observed infection event. Pathogen levels ≥ 30 were evident in plots maintained at the 4.5-cm mowing height before the infection events on 3 and 11 July, but not on 8 July. Samples collected from 1.7-cm-high turf predicted the infection events of 3 July (AM sample on 1 July) and 11 July. The 11 July infection event was predicted from samples collected from both timings and heights of cut on 10 July. In low-cut turf, pathogen levels were in the danger range on 24 and 29 June (data not shown) and 17 to 18 July, but no foliar mycelium was evident within 72 h. Hence, these were false predictions.

The ELISA method was somewhat more effective in predicting brown patch disease outbreaks using high-cut rather than low-cut turf samples (Table 1). On high-cut turf, detection levels reached and maintained the danger/extreme ranges for 96 h before the appearance of foliar mycelium on 3 July, and 48 h before the disease outbreak on 11 July. Detection levels, however, steadily declined below the danger range before the disease outbreak on 8 July. There also were five false predictions (25, 26, and 29 June and 18 and 19 July) from samples collected from high-cut turf. When considering all detections ≥ 30 , there were nine false predictions (low- plus high-cut plots) and two out of three possible correct predictions were achieved in 1991.

AM sampling only (1992). Samples were collected only between 7:00 and 8:00 AM from 6 June to 31 Aug. in 1992 (Table 2), and data collected before 28 June are not shown. Detection levels were generally higher from samples collected from plots mowed to a height of 4.5 cm (i.e., 27 of 87 days) when compared to low-cut samples, but detection levels were in the same risk range regardless of mowing height on 43 of 87 days. For data averaged over the season, detection levels were significantly higher in turf mowed to a height of 4.5 cm when compared to low-cut turf (17 vs. 13).

Foliar mycelium was detected on 1, 2, 8, 14, 15, 22, 24, 28, and 29 July and 18, 19, and

Table 1. Mean pathogen detection levels in perennial ryegrass as influenced by mowing height and sampling time in 1991.

Date	Detector level ²				<i>Rhizoctonia solani</i> mycelium ³
	Mowing ht (cm)				
	1.7		4.5		
	Time of assay				
June/July	AM ^y	PM	AM	PM	
30	27 ^w	15	33	36	—
1	30	29	38	60	—
2	26	28	32	49	—
3	25	26	34	44	+
4	20	22	28	31	—
5	7	20	20	24	—
6	14	9	12	12	—
7	19	17	10	10	—
8	23	28	7	8	+
9	30	13	43	10	—
10	35	45	41	39	—
11	33	12	38	6	+
12	24	9	27	11	—
13	10	7	18	5	—
14	8	6	9	2	—
15	21	5	29	16	—
16	15	13	10	10	—
17	34	3	21	18	—
18	37	19	37	30	—
19	21	14	35	14	—

²Detector level indicates brown patch risk as follows: 0 to 19 (low), 20 to 29 (caution), 30 to 37 (danger), and >38 (extreme).

³Sampling time was either 7:00 to 8:00 AM or 4:00 to 5:00 PM.

⁴Response indicates mycelium seen (+) or not seen (—) on turfgrass foliage.

^wData are a mean of two replicate bulk samples from each height of cut and each time of assay.

26 Aug. (Table 2). Quantifying pathogen levels with the ELISA method in 1992 resulted in more successful brown patch forecasts, and no false predictions, from high-cut turf samples. In high-cut turf, disease risk values ≥ 30 coincided with brown patch outbreaks on 1, 2, 8, 14, 15, 22, and 24 July. On 1 and 8 July, however, the prediction (i.e., detection level ≥ 30) came on the day foliar mycelium was detected, but detection levels ≥ 30 were not recorded for the previous 72 h. In high-cut turf, assay values in the danger or extreme range were observed 24 h before a disease outbreak on 2 July 1992 and 48 h before brown patch outbreaks on 14, 15, 22, and 24 July. Therefore, only five of 12 infection events were predicted within 72 h before the appearance of foliar mycelium in high-cut turf in 1992.

Disease risk in the danger and extreme range was recorded on 22 and 23 July from low-cut turf samples, coinciding with infection events on 22 and 24 July. Hence, only the 24 July infection event was predicted before the disease outbreak. There were false predictions from 1.7-cm sampled plots on 15 June (data not shown) and 31 July. The ELISA method failed to predict infection events within 72 h on five dates (i.e., 28 and 29 July and 18, 19, and 26 Aug.). Although detection levels in high-cut turf ≥ 30 were recorded on 1 and 8 July when foliar mycelium was evident, the risk levels 72 h before these dates were low.

Discussion

Over 2 years, seven of 15 infection events were predicted within 72 h before the appearance of foliar *R. solani* mycelium. In both

years, detection levels were higher from samples collected in turf mowed to a height of 4.5 cm, when compared to turf maintained at 1.7 cm. Of the seven predicted infection events, only three in both years were successfully determined from samples collected from plots mowed to a height of 1.7 cm. Golf course fairways, tees, and greens are mowed ≤ 1.7 cm. Hence, these data indicated that there would be little value in using the Reveal kits for predicting brown patch outbreaks on golf courses, at least according to the forecast guidelines imposed in this study. It should be noted that Schumann et al. (1994) used a prediction threshold risk value of ≥ 23 . This threshold would have increased prediction accuracy in this study, but it also would have resulted in 17 and 24 false disease predictions in 1991 and 1992, respectively.

These kits may be of limited value on higher-cut turfgrass sites, but the 47% success rate among samples from 4.5-cm plots is not satisfactory for disease management on commercial turfgrass sites. These results were similar to the findings of Shane (1991), who, monitoring pythium blight epidemics, reported that increases in detection levels coincided with, but typically did not precede, the development of disease symptoms. Shane (1991) concluded that, while advanced detection of disease development was unsatisfactory, the ELISA method was useful for verifying the diagnosis of the disease. The greatest value of the *R. solani* assay would be its ability to effectively identify *R. solani* as the pathogen from infected tissues. This is important, because the foliar mycelium produced by *Pythium* spp. is not distinguishable from *R. solani* without the aid of a microscope.

Table 2. Mean pathogen detection levels in perennial ryegrass as influenced by mowing height in 1992.

Date June/July/August	Detector level ²		<i>Rhizoctonia solani</i> mycelium ³
	Mowing ht (cm)		
	1.7	4.5	
28	5*	1	—
29	9	14	—
30	4	6	—
1	21	30	+
2	17	31	+
3	9	2	—
4	4	29	—
5	9	25	—
6	19	27	—
7	12	29	—
8	19	34	+
9	16	27	—
10	32	22	—
11	22	29	—
12	12	38	—
13	10	49	—
14	13	31	+
15	10	24	+
16	5	11	—
17	10	16	—
18	10	19	—
19	16	28	—
20	25	41	—
21	21	34	—
22	54	30	+
23	39	32	—
24	27	22	+
25	27	23	—
26	25	20	—
27	29	18	—
28	19	16	+
29	29	18	+
30	18	17	—
31	30	18	—
1	2	7	—
2	4	13	—
3	4	25	—
4	13	13	—
5	7	3	—
6	5	3	—
7	4	1	—
8	3	2	—
9	4	3	—
10	3	2	—
11	1	5	—
12	13	3	—
13	5	0	—
14	1	1	—
15	4	6	—
16	13	17	—
17	21	25	—
18	11	17	+
19	4	10	+
20	1	1	—
21	6	4	—
22	2	2	—
23	3	3	—
24	5	11	—
25	5	6	—
26	18	21	+
27	15	20	—
28	9	26	—
29	9	15	—
30	6	13	—
31	4	13	—

²Detector level indicates brown patch risk as follows: 0 to 19 (low), 20 to 29 (caution), 30 to 37 (danger), and >38 (extreme).

³Response indicates mycelium seen (+) or not seen (—) on turfgrass foliage.

⁴Data are a mean of two replicate bulk samples from each height of cut.

Literature Cited

- Baldwin, N.A. 1993. Evaluation of turfgrass disease diagnostic test kits in Europe. *Intl. Turfgrass Soc. Res. J.* 7:342–347.
- Campbell, C.L. and L.V. Madden. 1991. *Introduction to plant disease epidemiology*. Wiley, New York.
- Clark, M.F. 1981. Immunosorbent assays in plant pathology. *Annu. Rev. Phytopathol.* 19:83–106.
- Miller, S.A. 1982. Biotechnology-based disease diagnosis. *Plant Dis.* 72:188.
- Miller, S.A. and R.R. Martin. 1988. Molecular diagnosis of plant disease. *Annu. Rev. Phytopathol.* 26:409–432.
- Rittenberg, J.H., F.P. Petersen, G.D. Grothans, and S.A. Miller. 1988. Development of a rapid field usable immunoassay format for detection and quantification of *Pythium*, *Rhizoctonia*, and *Sclerotinia* spp. in plant tissue. *Phytopathology* 78:1516.
- Rowell, J.G. and D.E. Walters. 1976. Analyzing data with repeated observations on each experimental unit. *J. Agr. Sci.* 87:423–432.
- SAS Institute. 1985. *SAS user's guide: Statistics*. 5th ed. SAS Inst., Cary, N.C.
- Schumann, G.L., B.B. Clarke, L.V. Rowley, and L.L. Burpee. 1994. Use of environmental parameters and immunoassays to predict rhizoctonia blight and schedule fungicide applications on creeping bentgrass. *Crop Protection* 13:211–218.
- Shane, W.W. 1991. Prospects for early detection of pythium blight epidemics on turfgrass by antibody-aided monitoring. *Plant Dis.* 75:921–925.
- Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics*. 2nd ed. McGraw-Hill, New York.