Effect of Cultivation and Timing of Nitrogen Fertilization on Large Patch Disease of Zoysiagrass

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Abstract

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Large patch of zoysiagrass (*Zoysia* spp.) is caused by *Rhizoctonia solani* anastomosis group 2-2 LP. The effects of summer cultivation (core-aerification, verticutting, and sand topdressing) and spring and fall versus summer nitrogen (N) fertilization on large patch in fairway height 'Meyer' zoysiagrass were investigated from 2008 to 2011 in Manhattan, Haysville, and Olathe, KS. Disease was assessed by measuring patch diameters or analyzing digital images of affected plot areas to determine the percentage of non-green turfgrass within patches. Cultivation did not affect thatch temperature, soil temperature, soil water content, or turf recovery from large patch in early summer. Furthermore, cultivation did not result in overall significant reductions in patch diameters or average weekly rate of patch diameter increase among plots at the three experimental locations. In some site–year combinations, spring and fall N fertility was associated with lower percentages of non-green turf within affected plot areas in Manhattan and Haysville. In some cases, applications of N during spring and fall may alleviate large patch symptoms.

Zoysiagrass (*Zoysia japonica* Steud. and *Z. matrella* (L.) Merr.) is a warm-season (C4), perennial turfgrass that is widely used on golf courses in the "transition-zone" of the United States, a region that includes Kansas and states eastward to Virginia and North Carolina. Compared with certain cool-season (C3) turfgrass species used on fairways, such as creeping bentgrass (*Agrostis stolonifera* L.), zoysiagrass has lower water, fertilizer, and pesticide requirements while maintaining a high-quality surface (16). Large patch, caused by *Rhizoctonia solani* Kühn anastomosis group (AG) 2-2 LP, is the most common and most severe disease of zoysiagrass.

In Kansas, symptoms of large patch appear during spring (April to May) and autumn (September to October) as roughly circular, slightly matted tan patches with bright orange margins (17,40,45). Patches can range from 1 to 6 m or more in diameter (17,45), with healthy turf sometimes scattered within the patches. Symptoms on individual plants occur as reddish-brown to black lesions on the basal leaf sheaths. During summer conditions, regrowth within patches often results in full turf recovery, though weeds can encroach while the zoysiagrass is thinned.

In laboratory tests, a temperature range of 15 to 25°C was optimum for infection of zoysiagrass by the fungus (17). It has been suggested that the lack of symptoms during summer months is because thatch and soil temperatures often exceed 30°C, potentially hindering growth of the fungus while, at the same time, favoring zoysiagrass root and shoot growth (17). Additionally, soil moisture and leaf wetness are important factors in large patch development and severity. Severe large patch symptoms on zoysiagrass have been associated with compacted and poorly drained soils as well as with periods of excessive rain (17). Brown patch, a common disease of creeping bentgrass and tall fescue, caused by the related fungus *R. solani* AG 2-2 IIIB, has also been reported to be more severe in poorly drained soils (14,20,35).

Currently, management of large patch is primarily by fungicide applications, and there have been few studies of the effects of cultural practices on the disease. In one study, lower mowing heights resulted in more severe disease symptoms (18). Large patch was not affected by nitrogen (N) source (urea, urea formaldehyde, poultry litter, sewage sludge, and bovine waste) or rate (N at 74 and 148 kg/ha/year). The study did not, however, evaluate the effect of different fertilization timings. In an effort to promote faster emergence from winter dormancy, some turfgrass managers apply N fertilizer early in the spring. Similarly, N fertility is applied by some turfgrass managers during late fall to extend the duration of green color retention by zoysia, thereby delaying the onset of dormancy (16).

In the turfgrass industry, cultivation refers to aerification (punching solid or hollow tines into the soil to create holes) and verticutting (slicing into the turf canopy and roots with vertical blades), both of which improve root zone conditions (10). On zoysiagrass turf, aerating and verticutting fairways when large patch is active in spring has been anecdotally reported to result in the development of new satellite areas of infection by removal and deposition of infected cores in healthy areas of turf (42) but the effects of cultivation in summer is not known.

Additionally, the effect of N fertilization timing on large patch development and severity in zoysiagrass is not known, although turfgrass managers have associated severe large patch outbreaks with excessive N fertilization (18). Similarly, high N applications are also associated with increased susceptibility of cool-season turfgrasses to Rhizoctonia brown patch (11,39). Furthermore, the influence of the interplay between cultivation and timing of fertilization, if any, on large patch remains to be determined. The objectives of this study were to (i) evaluate the effect of cultivation on soil moisture and soil and thatch temperatures, (ii) evaluate the effects of cultivation (core-aerification, verticutting, and sand topdressing) on large patch development, and (iii) evaluate the effect of timing of fertilization on large patch development.

Materials and Methods

Pathogen isolation, storage, and inoculation. *R. solani* AG 2-2 LP isolates were recovered from large patch-infected zoysiagrass

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samples from Kansas. Leaf sheath sections measuring 1 to 2 cm with blight symptoms were removed from infected plants, surfaced disinfected with 0.5% NaOCl for about 2 min, blotted dry, and placed in a 9-cm-diameter petri plate containing potato dextrose agar at 6 g/liter amended with chloramphenicol (10 mg/liter) and streptomycin (10 mg/liter) (¼ PDA^{++}) (Biotech Research Grade). Cultures were maintained at 23°C in the dark. Identification of *R. solani* from hyphal-tipped cultures was based on hyphal characteristics, nuclear conditions (nuclear counts) (26), hyphal anastomosis with a known tester isolate belonging to the anastomosis group AG-2-2 LP on agar-coated glass slides (8,27), and polymerase chain reaction (PCR) using the AG 2-2 LP-specific primer P22-LP (9). One large patch isolate was used to infest oat kernels as previously described (46). In all, 150 g of dry oat kernels were mixed with 150 ml of distilled water in a glass jar and autoclaved twice at 121°C for 30 min. After cooling, several plugs from an actively growing *R. solani* AG 2-2 LP culture on ¼ PDA^{++} were placed into each jar. The glass jars containing the inoculated oat kernels were shaken every 1 to 3 days to ensure even colonization. After about 14 days of incubation, the infested oat kernels were subsequently used, without drying, for inoculation of field plots.

Experimental field plots of established 'Meyer' zoysiagrass stands were inoculated at Manhattan on 25 September 2008, Olathe on 2 October 2008, and Haysville on 3 October 2008 (Table 1). Stands were divided into 3.7-by-3.1-m plots that were each subdivided into four quadrants. The center of each quadrant was inoculated by placing 8 to 10 g of infested oat kernels in a small furrow about 5 to 7 cm in diameter, made using a hand trowel, between the turf and thatch layer. Plots were provided 5.0 cm of irrigation daily for 10 days following inoculation to promote the establishment of disease. Subsequently, irrigation output was adjusted to prevent stress and supplement rainfall to provide 2.5 cm of water per week. In addition to the inoculated patches, the turf area at the Manhattan site had some disease symptoms arise from preexisting natural large patch infection.

Summer cultivation and fertility treatments. The effects of cultivation (core-aerification, verticutting and sand topdressing) and timing of N applications on large patch development were examined in 2008 at Manhattan using natural infection and again in 2009, 2010, and 2011 at Manhattan, Olathe, and Haysville using inoculated patches. At each site, treatment plots were arranged in a randomized complete-block split-plot design with cultivation (versus noncultivation) as the whole plot (3.7 by 6.2 m) and timing of fertilization (spring and fall versus summer) as the split-plot (3.7 by 3.1 m). There were four blocks, leading to four replicate plots per treatment combination at each of the three sites. There was a 0.6-m alley between the blocks. Each 3.7-by-3.1-m split-plot contained four inoculation foci, as described above. Cultivation was carried out once every year (Table 1) at each of the three experimental locations. In Haysville, a Ryan Greensaire 24 aerator (Ryan) with a core spacing of 5 cm and core depth of 7.6 cm was used. At Olathe, a Plugr PL800 aerifier (SourceOne, Inc.) with a core spacing of 20.3 cm and core depth of 5.7 cm was used and, at Manhattan, a John Deere aerifier (Deere & Company) with a core spacing of 5.1 cm and core depth of 3.8 cm was used. At each location, around 640 core holes/m^2 were made during aerification. After aerification, the cores were allowed to air dry for several hours and subsequently broken up using a BlueBird verticutter (BlueBird). Approximately 0.7 cm of dry sand was then applied as top-dressing and incorporated into the turf canopy with a cart-mounted brush driven across the plot. At the end of the study in 2011, soil cores were collected from the cultivated plots to a depth of about 15 cm and examined to measure the depth of accumulated sand.

All plots received N at a total of 90 kg/ha/year. Dates are shown in Table 1. In Olathe and Haysville, plots receiving the spring/fall timing were treated with urea (46-0-0) in two separate N applications of 45 kg/ha. In Manhattan, the spring and fall treatments were each split into two N applications of 22.5 kg/ha. Plots receiving summer fertilization were treated with polymer-coated urea (41-0-0) (Pursell Technologies Inc.) in a single midsummer application of N at 90 kg/ha. Turfgrass stands were mowed twice per week with a triplex reel mower at a mowing height of 14.3 mm in Manhattan and Wichita and 19.5 mm in Olathe.

Disease assessment. During spring and fall, when patch symptoms were visible with distinct margins, patch diameters were measured weekly, to the nearest centimeter and expressed as the average patch diameter along two consistent perpendicular axes. The individual patches served as subsamples in each plot. In Manhattan, patch diameters were adjusted due to the unequal sizes of the preexisting naturally occurring patches within experimental plots. Adjustments were similarly made for Manhattan in subsequent years to include patches from inoculation carried out in fall 2008. The patch diameters were rescaled to reflect a common percentage size origin (100%) at the start of each season. Increases thereafter were reflected as percentage increases over the initial adjusted size of 100% and expressed as "relative patch diameters". The rate of increase in patch diameters, estimated as the average weekly increases in patch diameters, was determined at all three locations in 2009.

Table 1. Experiment locations with schedule of cultural practices

Year, activity[z]	Manhattan	Olathe	Haysville
2008			
Spring fertilization	28 April & 8 May	1 May	29 April
Summer fertilization	27 June	8 July	14 July
Fall fertilization	22 September & 23 October	24 September	25 September
Cultivation	27 June	8 August	14 August
Plot inoculation	25 September	2 October	3 October
2009			
Spring fertilization	27 April & 28 May	30 April	4 May
Summer fertilization	23 June	24 June	26 June
Fall fertilization	26 Aug. & 25 September	28 August	4 September
Cultivation	22 June	24 June	26 June
2010			
Spring fertilization	30 April & 1 June	3 May	5 May
Summer fertilization	30 June	21 June	22 June
Fall fertilization	1 September & 4 October	16 September	15 September
Cultivation	8 July	21 June	22 June
2011			
Spring fertilization	28 April & 27 May	26 April	27 April
Summer fertilization	6 June	2 June	3 June

[z] Cultivation included core-aerification, verticutting, and sand topdressing. Spring and fall fertility was applied as urea (46-0-0) with nitrogen (N) at a rate of 45 kg/ha each season for an annual total N of 90 kg/ha. In Manhattan, the spring and fall applications were each split into two applications of N at 22.5 kg/ha/season. Summer fertility was applied once per year as polymer-coated urea (41-0-0) with N at 90 kg/ha.

During early summer, patches become less visible with indistinct margins, making patch diameter measurements difficult. During this period and whenever patch margins were not clearly defined, starting in spring 2009, disease was estimated by digital image analysis of the patches to quantify the percentage of non-green turf in a subsection of each plot. Patch symptoms within a 65-by-75-cm area in the center of each plot were photographed weekly using the automatic settings of a Nikon D70s digital camera (Nikon Inc.) at 1.2 m above the turf canopy. Plots were manually brushed and air blown with a motorized leaf blower to remove dead grass clippings and fallen leaves prior to being photographed. If cultural practices were performed on the same date, photographs were taken first. Digital images were analyzed with SigmaScan Pro software (version 5.0; SPSS) using a SigmaScan Pro macro named "Turf Analysis" (22) for batch analysis of the digital images. The threshold settings (22) were adjusted to hue = 0 to 53 and saturation = 0 to 57. These threshold settings allowed for estimation of pixels (expressed as percentages) that represented non-green turf relative to healthy (green) turf. The data obtained allowed for quantification of turf recovery in the different treatment plots during early summer as well as in early fall while the disease was active but patch margins were not clearly defined.

Data analysis. Statistical analysis of data for whole-plot, split-plot, and interaction effects were performed with Minitab statistical software (version 16; Minitab Inc.). Data obtained for patch diameter measurements and digital image analysis were tested for normality and subjected to analysis of variance (ANOVA). Treatment means were compared using Fisher's individual error rate at $P \leq 0.05$. The overall effect of each treatment combination on large patch, for a given set of data points, was calculated and expressed as the area under the disease progress curve (AUDPC). The AUDPC values for both patch diameters and percentage of non-green turf were calculated with the formula $\Sigma[(y_i + y_{i+1})/2][t_{i+1} - t_i]$, where $i = 1,2,3,\ldots,n - 1$, y_i is the amount of disease (patch diameters or percentage of non-green turf), and t_i is the time of the ith rating (25).

Microclimate measurements. To evaluate the effect of cultivation practices on soil moisture, soil temperature, and thatch temperature, dual probes and thermocouple sensors connected to an analog data logger were installed in a total of eight plots, four in cultivated (two in spring/fall- and two in summer-fertilized plots) and four in noncultivated plots (two in spring/fall- and two in summer-fertilized plots), at the Manhattan site in September 2008 and left in place through fall 2010. The soil-encapsulated thermocouple sensors were assembled as previously described (19) and installed in the thatch layer of the turf to measure thatch temperatures at hourly intervals. The volumetric soil water content (ratio of water volume to soil volume: cubic meters of water per cubic meter of soil) at 13 cm below the thatch layer was measured using the dual-probe heat-pulse (DPHP) technique (7,41,44). DPHP sensors were fabricated as previously described (2,4). Measurements were automated and logged once daily at 0626 CST. All data acquisition and controls were accomplished with a micrologger and accessories (CR10x, two AM16/32s, and one AM25T; Campbell Scientific). Dual probes and thermocouples were installed in four replicate cultivated and noncultivated whole plots. Data were collected, tested for normality, and subjected to ANOVA using Minitab statistical software. Mean comparisons were performed using the Fisher's individual error rate function in Minitab. Volumetric soil water content and soil and thatch temperature data were collected in the treatment plots from May to September and April to July 2009 and 2010, respectively.

Results

Effects of summer cultivation on soil and thatch temperatures and volumetric soil water content. Temperatures in the soil and thatch were very similar, with no significant differences. In 2009, the soil and thatch temperatures ranged from a low of 16.2°C on 1 May to a high of 29.2°C on 26 June, with a season-long average of 22.7°C for the cultivated and 22.6°C for the noncultivated plots. In 2010, the soil and thatch temperatures ranged from 13.6 to

Table 2. Effect of summer cultivation and timing of nitrogen (N) application on large patch symptoms as measured by size and digital image analysis during 2008, 2009, 2010, and spring 2011 at Manhattan, KS

	Patch diameter (cm)[y]		Diseased turf (%)[x]					
			2010					2011
	AUDPC		June				October	June
Treatment[z]	2008	2009	4	19	24	AUDPC	25	1
Whole plot								
Cultivated	880.5	928.6	60.4	26.2	16.1	363.3	47.6	24.6
Noncultivated	696.1	1,065.1	60.5	30.3	22.7	382.6	47.7	28.6
Split plot								
Summer	527.4	958.9	58.8	24.6	22.9	364.1	53.4 a	33.8 a
Spring & fall	1,049.3	1,034.7	62.1	31.9	15.8	381.9	41.9 b	19.3 b
Whole-plot × split-plot								
Cultivated summer N	856.1	899.6	62.9	23.3	17.4	368.5	53.2	30.9 ab
Noncultivated summer N	198.6	1,018.3	54.6	25.9	28.5	359.6	53.5	36.7 a
Cultivated spring & fall N	904.9	957.5	57.8	29.0	14.8	358.1	41.9	18.2 c
Noncultivated spring & fall N	1,193.6	1,111.8	66.4	34.7	16.8	405.6	41.9	20.4 bc
ANOVA								
Whole-plot	NS	NS	NS	NS	NS	NS	NS	NS
Split-plot	NS	NS	NS	NS	NS	NS	*	*
Whole-plot × split-plot	NS	NS	NS	NS	NS	NS	NS	*

[x] Percent non-green turf was estimated by analysis of digital images within a 65-by-75-cm grid within plots using SPSS 5 image analysis software. For each treatment, each value represents the average of 16 patches total from four replicate plots. Values in a column followed by the same letters are not statistically different ($P = 0.05$). AUDPC = area under disease progress curve, calculated as $\Sigma[(y_i + y_{i+1})/2][t_{i+1} - t_i]$, where $i = 1,2,3,\ldots,n - 1$, y_i is the amount of disease (relative patch diameters for 2008 and 2009 data and percent diseased turf for 2010), and t_i is the time of the ith rating.

[y] Values estimated from patches from four replicate plots per treatment.

[z] In 2008, spring fertility was applied as urea with N at 22.5 kg/ha on 28 April and 8 May and fall fertility on 2 September and 23 October, for an annual total N of 90 kg/ha. Summer fertility was applied on 27 June as polymer-coated urea with N at 90 kg/ha. In 2009, spring fertility was applied as urea with N at 22.5 kg/ha on 27 April and 28 May. Summer fertility was applied on 23 June as polymer-coated urea with N at 90 kg/ha. Note that cultivation and N treatments had been conducted since June 2008. Summer cultivation was performed on 22 June. In 2010, spring fertility was applied as urea with N at 22.5 kg/ha on 30 April and 1 June. Summer fertility was applied on 30 June as polymer-coated urea with N at 90 kg/ha and fall fertility was applied as urea with N at 22.5 kg/ha on 1 September and 4 October for an annual total N of 90 kg/ha. In 2011, spring fertility was applied as urea with N at 22.5 kg/ha on 28 April and 27 May. Summer fertility was applied on 6 June as polymer-coated urea with N at 90 kg/ha. Note that cultivation and N treatments had been conducted since 2008. ANOVA = analysis of variance: NS = not significant and * = significant.

27.9°C, with a season-long average of 19.4°C for the cultivated and 18.9°C for the noncultivated plots. In both years, measurements in cultivated and noncultivated plots never differed by more than 2°C. Differences were not significant ($P = 0.05$) in either year.

In 2009, volumetric water content in the cultivated plots ranged from 0.35 to 0.48, with a season-long average of 0.42, compared with a range of 0.40 to 0.50 and a season-long average of 0.43 in the noncultivated plots. In 2010, the volumetric water content in the cultivated plots ranged from 0.24 to 0.46, with a season-long average of 0.35, compared with a range of 0.30 to 0.46 and a season-long average of 0.37 in the noncultivated plots. There were no significant ($P = 0.05$) differences in either year.

Effects of summer cultivation and timing of N application on large patch. *Manhattan.* In 2008, the whole-plot cultivation treatment, split-plot N fertilization timing, and their interaction had no significant effect on weekly relative patch diameters (*data not shown*) or AUDPC values for the May-to-July and October 2008 periods of disease activity (Table 2). Patch diameters in October were 40 to 78 cm larger than were last recorded in mid-July, indicating pathogen growth over summer when the turf was symptomless (*data not shown*).

In spring 2009, in addition to natural infection, all experimental plots had fairly uniform large patch symptoms following inoculation of plots in fall 2008. Cultivation, fertility, and their interaction had no significant effect on weekly relative patch diameter (*data not shown*), AUDPC based on patch diameter (Table 2), or patch severity as measured by digital image analysis (*data not shown*). The average rate of increase in patch diameters averaged 6.4 and 10.3 cm per week in the cultivated and noncultivated plots, respectively, but were not significantly ($P = 0.05$) different. Cool, dry weather conditions during the fall resulted in scarce development of disease symptoms as well as the early onset of turf dormancy and, therefore, fall large patch data could not be collected in 2009.

In 2010, after the zoysiagrass greened up and disease symptoms were visible in spring, patches within most of the experimental plots had enlarged and coalesced to cover most of the plots, so that patch diameter measurement was no longer feasible. Therefore, weekly assessment of treatment effects on large patch was carried out solely through analysis of digital images of the plots. Cultivation, fertility, and their interaction did not have any significant effect on percent non-green turf based on weekly measures or the AUDPC (Table 2). Following the onset of fall large patch symptoms, plots that received spring and fall applications of N had significantly lower percentages of non-green turf compared with those that received summer applications (Table 2).

In 2011, fertility and the fertility–cultivation interaction had a significant effect on large patch based on digital image analysis. Plots which received spring and fall applications of N had significantly higher percentages of non-green turf compared with those that received summer applications (Table 2).

Haysville. In spring 2009, cultivation, fertility, and their interaction had no significant effect on weekly patch diameters, measured from 19 May to 25 June, or AUDPC (Table 3). Additionally, there was no significant difference in the percentage of non-green turf measured by image analysis among the treatment plots on 26 June, the single date on which that method was used (Table 3). The average rate of increase in patch diameters averaged 18.2 and 13.4 cm per week in the cultivated and noncultivated plots, respectively, and were not significantly ($P = 0.05$) different. Similar to Manhattan, cool and dry weather conditions during the fall resulted in poor establishment of disease symptoms, as well as the early onset of turf dormancy.

The 2009–10 winter delayed emergence from dormancy due to prolonged cool temperatures but did not create any winter-kill. Patch diameter data could not be collected due to poorly defined margins, and data were collected solely by analysis of digital images of plots on 22 June and 7 July. Spring and fall applications of N resulted in significantly lower percentages of non-green turf on 22 June compared with those that received summer applications of N (Table 3). In 2011, patch data were collected only on 3 June,

Table 3. Effect of summer cultivation and timing of nitrogen (N) application on large patch symptoms as measured by patch diameters (2009) and digital image analysis (26 June 2009, 2010, and 2011) at Haysville, KS

	Large patch symptoms										
	Patch diameter (cm)								Non-green turf (%)[w]		
	2009[x]								2010		2011
	May		June						June	July	June
Treatment[y]	19	26	2	9	15	25	26	AUDPC[z]	22	7	3
Whole plot											
Cultivated	50.8	53.6	55.4	69.4	74.6	100.3	32.9	328.6	29.4	27.3	55.6
Noncultivated	42.6	42.2	48.5	61.0	73.4	99.5	28.5	296.2	27.2	18.6	63.9
Split plot											
Summer	44.3	47.2	50.4	64.9	76.1	103.3	29.7	312.5	36.3 a	27.0	64.1 a
Spring & fall	49.2	48.6	53.5	65.5	71.9	96.4	31.7	312.3	20.6 b	18.8	55.4 b
Whole-plot × split-plot											
Cultivated summer N	55.0	63.1	59.1	75.6	83.2	104.4	30.6	360.7	38.1	30.3	60.3
Noncultivated summer N	33.5	31.3	41.6	54.1	68.9	102.2	28.7	263.8	33.9	23.7	67.8
Cultivated spring & fall N	46.6	44.1	51.6	63.1	66.0	96.1	35.1	296.2	20.7	24.2	50.9
Noncultivated spring & fall N	51.7	53.1	55.3	67.9	77.8	96.7	28.2	328.3	20.5	13.4	59.9
ANOVA											
Whole-plot	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Split-plot	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*
Whole-plot × split-plot	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[w] Percent diseased (non-green) turf was estimated by analysis of digital images within a 65-by-75-cm grid within plots using SPSS 5 image analysis software. Each value represents the average of 16 patches from four replicate plots. Values in a column followed by the same letters are not statistically different ($P = 0.05$).

[x] Each value except for 26 June 2009 represents the average diameter (in centimeters) of 16 patches from four replicate plots. On 26 June 2009, large patch was estimated from the plots as percent diseased turf by analysis of digital images within a 65-by-75-cm grid.

[y] In 2009, spring and fall fertility was applied as urea with N at 45 kg/ha on 4 May and 4 September, while summer fertility was applied on 26 June as polymer-coated urea with N at 90 kg/ha. In 2010, spring and fall fertility was applied as urea with N at 45 kg/ha on 5 May and 15 September, while summer fertility was applied on 22 June as polymer-coated urea with N at 90 kg/ha. In 2011, spring fertility was applied as urea with N at 45 kg/ha on 27 April, while summer fertility was applied on 3 June as polymer-coated urea with N at 90 kg/ha. Note that cultivation and N treatments had been conducted since 2008. ANOVA = analysis of variance: NS = not significant and * = significant.

[z] AUDPC = area under disease progress curve based on patch diameters from 11 May to 25 June 2009, calculated as $\Sigma[(y_i + y_{i+1})/2][t_{i+1} - t_i]$, where $i = 1,2,3,\ldots,n - 1$, y_i is the amount of disease (patch diameter), and t_i is the time of the ith rating.

when symptoms were most visible. The cultivated plots that received spring and fall applications of N had significantly lower percentages of non-green turf compared with the noncultivated plots that were summer fertilized (Table 3).

Olathe. In spring 2009, cultivation, fertility, and their interaction had no significant effect on large patch (Table 4). The average rate of increase in patch diameters averaged 3.7 and 5.1 cm per week in the cultivated and noncultivated plots, respectively, and were not significantly ($P = 0.05$) different.

In 2010, the percentage of non-green turf on 1 June was significantly lower in the noncultivated plots compared with their corresponding cultivated plots (Table 4). This difference was no longer significant by 21 June. In 2011, cultivation, fertility, and their interaction had no significant effect on large patch (Table 4).

Discussion

The effects of N fertility were not consistent from year to year or site to site. In 2010 and 2011, soluble N applied as urea in spring and fall was associated with lower percentages of non-green turf at two of the three sites. Applications of N during spring and fall, when the growth of zoysiagrass is not optimal, might have promoted more turfgrass growth within affected areas, masking the effects of disease rather than suppressing pathogen growth. Indeed, in the years when patch size measurements were taken, fertility had no effect on the rate of patch size increase. Even when statistically significant, the differences in percentage of non-green turf were always less than 16% and may not be of much practical significance, depending on the tolerance for damage at a given site. The effects on disease of N fertilizer applied very early or very late in the season, such as at first emergence from dormancy in spring or when the turfgrass is nearly dormant in fall, were not examined. Along with the disease implications, fall fertilization may also have implications for winter hardiness (15). Winter damage was not directly measured in this study, and no patterns related to fertility were observed anecdotally; however, Meyer is a particularly cold-tolerant variety (31,32). Therefore, spring or fall N applications to encourage recovery from large patch are limited in their potential efficacy for reducing large patch symptoms, and fall applications should be approached with caution regarding possible effects on winter hardiness.

The decrease in symptoms associated with spring and fall N applications in some years was unexpected. Increased N has been shown to increase severity of brown patch in cool-season turfgrass (6,12), with quick-release N more favorable to disease than slow-release (13), but brown patch is caused by a different *R. solani* AG group and affects different turf species. The influence of N on *Rhizoctonia* diseases is variable (21,47) and it is difficult to make generalizations. For example, in cereals, applying N led to reduced bare patch (root rot) in one set of experiments (24) but not in another (36).

Though cultivation and sand topdressing is standard on golf course putting greens, it is not standard for zoysiagrass fairways. Some superintendents cultivate zoysiagrass fairways to promote overall root growth and encourage the degradation of thatch. Cultivating zoysiagrass fairways when large patch is active has been observed to spread large patch, presumably through the dispersal of infected turf cores (43). However, the effect of summer cultivation on large patch development and severity has not been reported. Poor drainage has been associated with more severe large patch damage in the field. In this study, 3 years of cultivation and sand topdressing did not contribute to significantly improved drainage (as measured by volumetric water content at 5 cm below the thatch) in the cultivated plots. Water content at the thatch level was not measured, nor was leaf wetness, which may be more important indicators of microclimate favorability to large patch development. Soil moisture can also influence soil temperatures. For example, wetter soils may be slower to warm up in spring and in summer, and wetter soils retain heat and do not cool as much overnight. In our study, cultivation did not result in any detectable change in soil and thatch temperatures. Studies of in vitro mycelial growth of *R. solani* AG 2-2 LP have shown that the fungus is capable of near-optimum growth at 30°C (17). According to soil microclimate data collected through summer 2009 and 2010, the maximum soil and thatch temperatures recorded were around 29 and 28°C, respectively, despite air temperatures well above 32°C. Therefore, the

Table 4. Effect of summer cultivation and timing of nitrogen (N) application on large patch as measured by patch diameters (2009) and digital image analysis (2010 and 2011) at Olathe, KS

	Large patch (percent non-green turf)									
	2009[v]						2010[w]			2011[w]
	June			July			May[x]	June		June
Treatment[y]	11	17	24	1	8	AUDPC[z]	26	1	21	7
Whole plot										
Cultivated	47.5	50.7	49.1	56.4	56.2	208.0	64.9	56.0 a	19.2	22.9
Noncultivated	45.5	47.9	46.6	47.2	47.4	188.1	72.2	45.6 b	22.4	24.7
Split plot										
Summer	50.6	55.6	50.7	56.9	56.2	216.5	64.9	51.5	23.3	21.7
Spring & fall	42.4	43.0	45.2	46.8	47.4	179.6	72.3	50.2	18.3	26.0
Whole-plot × split-plot										
Cultivated summer N	47.3	50.9	51.1	62.1	60.4	218.0	64.1	57.0	23.3	22.5
Noncultivated summer N	53.9	60.3	50.2	51.6	51.9	215.0	65.6	45.9	23.2	20.9
Cultivated spring & fall N	47.7	50.4	47.1	50.7	51.9	198.0	65.8	55.0	15.1	23.5
Noncultivated spring & fall N	37.0	35.5	42.9	42.8	42.8	161.1	78.8	45.3	21.5	28.5
ANOVA										
Whole-plot	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
Split-plot	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Whole-plot × split-plot	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[v] Each value represents the average diameter (in centimeters) of 16 patches from four replicate plots.

[w] Percent diseased (non-green) turf was estimated by analysis of digital images within a 65-by-75-cm grid within plots using SPSS 5 image analysis software. Each value represents the average of 16 patches from four replicate plots. Values in a column followed by the same letters are not statistically different ($P = 0.05$). Plot digital images were collected before cultivation was performed on 21 June 2010.

[x] On this date, percentage of each plot affected by large patch was determined by visual estimation.

[y] In 2009, spring fertility was applied as urea with N at 45 kg/ha on 1 May, while summer fertility was applied on 8 July as polymer-coated urea with N at 90 kg/ha. In 2010, spring fertility was applied as urea with N at 45 kg/ha on 3 May while summer fertility was applied on 21 June as polymer-coated urea with N at 90 kg/ha. In 2011, spring fertility was applied as urea with N at 45 kg/ha on 26 April, while summer fertility was applied on 2 June as polymer-coated urea with N at 90 kg/ha. Note that cultivation and N treatments had been done since 2008. ANOVA = analysis of variance: NS = not significant and * = significant.

[z] AUDPC = area under disease progress curve, calculated as $\Sigma[(y_i + y_{i+1})/2][t_{i+1} - t_i]$, where $i = 1,2,3,\ldots,n - 1$, y_i is the amount of disease (patch diameter), and t_i is the time of the ith rating.

absence of disease symptoms during summer during the study period was not directly due to suppressive soil or thatch temperatures. It is not clear at this point what other factors might be involved in disease suppression during summer. Like other C4 grasses, zoysiagrass has optimum growth at higher temperatures and, in hot summer conditions, it may outgrow the pathogen. Alterations in zoysiagrass gene expression at high temperatures could also influence disease development.

Although zoysiagrass recovers from the disease during summer, it is also possible for symptoms to persist during the summer months in shaded and moist areas, especially during unusually cool summer weather (17). It has been suggested that high summer temperatures suppress large patch, and that the fungus survives the summer in thatch or on stolons as sclerotia (46). However, based on our findings, it appears unlikely that the lack of symptoms during summer is a direct consequence of summer temperatures. Indeed, earlier reports (1,23) involving the isolation of *R. solani* AG 2-2 LP from sheath tissues with no obvious symptoms suggested that the fungus is present in zoysiagrass at all times but disease symptoms only occur when climatic conditions are favorable to the pathogen but not favorable to the plant. The patch diameter data for fall 2008 in Manhattan suggests that, although symptoms were not visible during the summer of that year, pathogen growth likely continued through the thatch or soil, resulting in the larger patch diameters when symptoms reappeared in the fall, following the onset of favorable environmental conditions for disease development. Across the sites, the estimated weekly rate of patch diameter increase in 2009 was 3.7 to 18.2 cm/per week, similar to an earlier reported rate (1) of 1.5 cm/day, under optimum temperature for *R. solani* AG 2-2 LP. Although the rate of patch diameter increase varied somewhat from site to site, there were no differences between cultivated and noncultivated plots.

In our study, cultivation played no role in disease development, in contrast to several studies on the effects of cultivation on Rhizoctonia diseases in other crops. For example, in several studies of bare patch (root rot) of wheat, caused by *R. solani*, reduced tillage led to an increase in severity (24,36,37). It has been suggested that tillage may physically disrupt the fungal hyphae (3,34) or alter the soil microbial community to be suppressive to disease (34). Similarly, root and crown rot in sugar beet, caused by *R. solani* AG 2-2 IIIB severity, was higher in noncultivated compared with cultivated treatments (5). In another study of the bare patch pathosystem, the effects of cultivation were more complex. *R. solani* AG-8 exhibited similar radial growth rates in soil cores from conventionally tilled and direct-seeded fields (38). However, the differences in the extent of colonization varied with the site, including the time since disturbance. It has been suggested that the decrease in soil temperatures and increased soil moisture as a result of no-till might favor Rhizoctonia root rot of barley (38). The large patch pathosystem has some important differences from the above examples of annual cropping systems. First, zoysiagrass is a perennial plant with constant contact with the pathogen, no fallow period, and no opportunity for rotation. In zoysiagrass, *R. solani* AG 2-2 LP is a sheath blighting pathogen, not a root pathogen; therefore, hyphal networks in soil may be less important than dynamics in the thatch layer and lower plant canopy. Furthermore, cultivation practices in turfgrass (core aeration and verticutting) are less disruptive to soil structure than most tillage systems in crops.

Two aspects of large patch disease symptoms, patch diameters and percentage of non-green turf within a patch, were evaluated to better understand the effects of cultivation and timing of fertilization of zoysiagrass on large patch disease. In a practical sense, reductions in patch diameters and increases in green tissue within the patch area would be beneficial and relevant to turfgrass managers. A large-diameter patch with a high percentage of green tissue that recovers quickly may be less objectionable than a small patch in which most of the turfgrass is blighted, potentially requiring a longer time to recover.

The cultural practices were conducted over several years to examine the potential impact over time. However, it is possible that even longer-term research may be needed to investigate changes in soil structure, microbial communities, or other factors. In the wheat and barley bare patch system, some responses of *R. solani* to cultivation differed between sites with 3 versus 20 years of direct seeding (no cultivation) (38). Another variable in disease dynamics is host cultivar. This large patch study included only one zoysiagrass cultivar, Meyer. Different varieties and germplasm lines have varying rates of establishment (29), cold tolerance and green-up after winter dormancy (31,33), recovery from damage or sod harvest (30), and susceptibility to and ability to recover from large patch (28,32). Different cultivars may respond differently to various combinations of disease and cultural practices, including N fertility. Additional research using different cultivars and possibly different isolates of the pathogen could be insightful. More precise scheduling of spring and fall N applications based on soil temperature thresholds or degree-day models could also be valuable to optimize N fertility practices. In particular, spring-only N applications to promote recovery from epidemics may be interesting to examine. Finally, though the effect of N source in summer has been examined (18), the effect on large patch of different N sources applied in spring and fall may be a valuable area of future research as well.

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