



# Large patch (*Rhizoctonia solani* AG 2-2LP) severity on Japanese lawngrass (*Zoysia japonica*) influenced by fungicide and application target site

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## ABSTRACT

Large patch, caused by *Rhizoctonia solani* AG 2-2LP, affects the sheaths and stems of Japanese lawngrass (JLG; *Zoysia japonica*) in the transition zone. Large patch is difficult to control with traditional fungicide sprays because much of the applied fungicide solution remains on the leaf away from the infection court. Our objective was to determine the amount of protection provided by fungicides deposited on the leaf, sheath, or stem of JLG. Growth chamber experiments were conducted in 2015 in Knoxville, TN, to evaluate large patch control using fungicides deposited on three target sites of JLG (leaf, sheath, and stem). Azoxystrobin, flutolanil, tebuconazole, and chlorothalonil were applied with a pipette as 2.5- $\mu$ l droplets that were dispensed singly on the leaf, sheath, or stem. Measurements of percent visual disease severity and photochemical efficiency ( $F_v/F_m$ ) were collected every seven days. In both experimental runs, JLG treated with fungicides applied on the sheath or stem significantly reduced large patch severity and had higher  $F_v/F_m$  values compared to JLG receiving leaf applications on most rating dates. Azoxystrobin, flutolanil, and tebuconazole applied on the leaf resulted in a range of 35–75% large patch severity between the two experimental runs. When these fungicides were applied on the stem and sheath, large patch severity ranged from 2 to 30%. Chlorothalonil, a contact fungicide, was least affected by the target site of application on most rating dates. This experiment demonstrated that the target site of fungicide application was critical in large patch management. Developing novel spray strategies that result in greater penetration of the fungicide solution into the thatch, and deposition closer to the site of infection is warranted.

## 1. Introduction

Large patch, *Rhizoctonia solani* Kühn AG-2-2LP, is the most severe disease of Japanese lawngrass (JLG; *Zoysia japonica* Steud.), also known as zoysiagrass, in humid-temperate regions of the world (Green et al., 1993; Hyakumachi et al., 1998). Large patch development is favored by mild air temperatures (20–25 °C) and high relative humidity (> 90%). Symptoms include black, water-soaked lesions located on the stem or sheath. Damage from large patch often persists until the onset of warmer temperatures that are more favorable for JLG growth. Control of this disease is warranted because, once established, large patch often returns in the same location each year and becomes progressively more severe (Spurlock, 2009).

Large patch is managed primarily with fungicide applications on golf courses, sod farms, and other public landscapes. This is due, in part, to the lack of identifiable cultural control programs such as mowing

height, fertilization, cultivation, or germplasm resistance that effectively reduce large patch (Green et al., 1994; Obasa et al., 2012, 2013; Miller et al., 2016). Sites with a prior history of large patch may require two-to-three fungicide applications during the fall and spring to limit the spread of this disease. However, turfgrass managers have reported variable and often poor large patch control with fungicides that have demonstrated effectiveness in field trials by university researchers.

There are several possible reasons for control failure such as spray deposition factors (application rates, surface coverage, and nozzle pressure), disease pressure, environmental factors, and the residual efficacy of fungicides are among the most documented in turfgrass systems (Couch, 1984; Kennelly and Wolf, 2009; Latin, 2005; McDonald et al., 2006; Vincelli and Dixon, 2007). In cropping systems, adequate fungicide deposition near the infection court is critical in managing plant disease. Csinos (1989) reported that targeting fungicide applications to the main stems of peanut (*Arachis hypogaea* L.), with a narrow-

Abbreviations: JLG, Japanese lawngrass; DAIT, Days after initial treatment

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**Table 1**Fungicide treatments evaluated for large patch (*Rhizoctonia solani* AG 2-2LP) control.

| Active ingredient | Trade name     | Manufacturer                                            | Fungicide class                   | Phytomobility <sup>a</sup> | Rate (g a.i. L <sup>-1</sup> ) <sup>b</sup> |
|-------------------|----------------|---------------------------------------------------------|-----------------------------------|----------------------------|---------------------------------------------|
| Azoxystrobin      | Heritage WG    | Syngenta Crop Protection LLC., Greensboro, NC           | Quinone outside inhibitor         | Xylem systemicity          | 0.76                                        |
| Tebuconazole      | Torque         | Nufarm Americas Inc., Burr Ridge, IL                    | Demethylation inhibitor           | Xylem systemicity          | 1.03                                        |
| Flutolanil        | Prostar 70 WG  | Bayer Environmental Science, Research Triangle Park, NC | Succinate dehydrogenase inhibitor | Xylem systemicity          | 5.81                                        |
| Chlorothalonil    | Daconil Ultrex | Syngenta Crop Protection LLC., Greensboro, NC           | Benzonitrile                      | No systemicity             | 9.98                                        |

<sup>a</sup> Phytomobility information was referenced from Latin (2011).<sup>b</sup> Fungicides were applied with a pipette to dispense a single 2.5-μl droplet of the fungicide solution onto the stem, sheath, or leaf of Japanese lawngress (*Zoysia japonica*). This application mimics an ultra-coarse spray droplet landing on leaf, sheath, or stem from a spray mixture containing the labeled high rate of fungicide applied at a spray rate volume of 815 L ha<sup>-1</sup>.

band width application, resulted in significantly lower incidence of southern stem rot (*Sclerotium rolfsii* Sacc.) compared to a wide-band spray application. Similarly, Butzler et al. (1998) observed increased control of *Sclerotinia* blight (*Sclerotinia minor* Jagger) in peanut when mechanical pruning was administered prior to a fungicide application. The authors suggested that pruning allowed for more fungicide deposition to the site of penetration. Similarly, penetration of fungicides into the lower plant canopy in peanut stands resulted in better stem rot control and yield (Augusto et al., 2010).

*Rhizoctonia solani* AG 2-2LP infects and colonizes the sheaths and stems of JLG plants (Aoyagi et al., 1998). Most fungicides are xylem mobile, localized penetrant or contact (Latin, 2011). For xylem mobile fungicides, quality fungicide deposition should target the lower canopy depending on the physical and chemical properties of the fungicide. For most fungicides to be effective, the active ingredient must be deposited in close proximity to the infection court or be able to translocate towards the infection court. Fungicide solution that is deposited on the upper leaf canopy may not provide sufficient protection against large patch. The objective of this research was to evaluate large patch development in response to fungicides applied on three separate plant parts of JLG including the leaf, sheath, and stem.

## 2. Materials and methods

### 2.1. Plant culture

Plugs of JLG (c.v. 'Meyer'), 10-cm in diameter, were collected in July of 2014 from a golf course fairway in Knoxville, TN. The plugs were washed using a pressurized washer to remove soil and other contaminants. Individual stolons were separated by hand and rinsed three times with tap water. Stolons were singly propagated in potting medium (Fafard Professional Potting Mix, Sun Gro Horticulture, Agawam, MA) contained in 3.8-cm diameter 'conetainers' (Steuwe and Sons, Tangent, OR) and maintained in a greenhouse at 28 °C. During the 10-week establishment period, plants were trimmed to a height of 3 cm, and irrigated twice daily with an overhead irrigation system. Fertilizer was applied every 14 days at a rate of 49 kg N ha<sup>-1</sup> with a complete 24-8-16 fertilizer (All Purpose Plant Food, The Scotts Company, Marysville, OH). After establishment, the JLG plants were thinned to two individual tillers per conetainer.

### 2.2. Pathogen isolation and inoculation

Recovery and isolation of *R. solani* were similar to the methods of Obasa et al. (2012). In brief, an isolate of *R. solani* AG 2-2LP was recovered from a JLG fairway at Gettysvue Golf and Polo Club in Knoxville, TN, in April 2014. Plant samples, which exhibited characteristic black water-soaked lesions, were collected from the margin of a large patch outbreak. Plant material was rinsed with tap water, cut into < 1.0 cm sections and surface sterilized with 0.5% NaOCl for 1 min before being placed onto ¼ strength potato dextrose agar (PDA) amended with tetracycline (5 mg L<sup>-1</sup>) and streptomycin (10 mg L<sup>-1</sup>). After 24 h, the tips of mycelial fragments growing from the leaf section were

transferred to new amended ¼ strength PDA culture plates. After three days, five plugs of *R. solani* were placed in a 1000 ml flask containing 300 g of twice autoclaved oat kernels. The oat kernels were previously soaked in distilled water for 24 h prior to being autoclaved. After 20 and 14 days of incubation, in the 1st and 2nd experimental run, respectively, six infested kernels were placed in each conetainer. The inoculated JLG plants were placed inside a large plastic tub with a transparent lid. Moistened paper was placed throughout the plastic tub to ensure high (> 90%) relative humidity. The plastic tub was placed in a growth chamber (Convion Adaptis, Controlled Environment Ltd., Winnipeg, Canada) maintained at 24 °C (day) and 20 °C (night), with a 12-hr photoperiod.

### 2.3. Treatments

Treatments were arranged as a 4 × 3 factorial in a randomized complete block design with five replications. The fungicides evaluated were three systemic fungicides (azoxystrobin, tebuconazole, and flutolanil) and one contact fungicide (chlorothalonil; Table 1). Azoxystrobin, tebuconazole, and flutolanil were chosen as they are commonly used in the turfgrass industry to control large patch. Chlorothalonil was chosen as the contact fungicide as it represents a different chemical class to control large patch. The fungicides were applied onto three application target sites of JLG (leaf, sheath, and stem). Azoxystrobin, tebuconazole, flutolanil, and chlorothalonil were applied at a concentration of 0.76, 1.03, 5.81, and 9.98 g a.i. L<sup>-1</sup>, respectively, with a pipette that delivered a 2.5-μl droplet (1684 μm in diameter) of fungicide solution that was dispensed singly on the leaf, sheath, or stem. This application mimics an ultra-coarse spray droplet (ASABE, 2013) landing on leaf, sheath, or stem from a spray mixture containing the labeled high rate of fungicide applied at a spray rate volume of 815 L ha<sup>-1</sup>. The fungicide droplets were allowed to dry on the plant for 24 h before inoculation. Conetainers were discarded and replaced if the fungicide droplet moved off the intended plant part. Two nontreated controls were added, but were not included in the statistical analysis. One of the nontreated controls was inoculated and the other was not. The nontreated controls were added to reference large patch development when no fungicide was applied and to monitor turfgrass health in the experimental environment. Each nontreated control had four replications. Unless otherwise stated, mention of the nontreated control, hereafter, refers to the inoculated nontreated control.

### 2.4. Data collection

#### 2.4.1. Disease severity

Large patch severity was assessed visually using a modified nearest percent estimate (NPE) method. The following were the indices of the NPE assessment: 0, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% disease severity. This rating method was chosen to provide equal interval assessments between 10 and 100%. Other, more categorical estimates (similar to the Horsfall-Barratt scale) have recently been shown to be a less reliable measure of disease severity compared to NPE measurements and may result in a higher probability of a type II error

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