# Research Reports

# Evaluation of Chitinase Activity in Tall Fescue Cultivars Inoculated with *Rhizoctonia solani*[1]

**Matthew Cutulle[2], Jeffrey Derr[3], David McCall[4], Adam Nichols[3], Brandon Horvath[5], and Tatiana Sanchez[6]**

## Abstract

Tall fescue has great utility as a low maintenance turfgrass in the northern and transition zone regions of the United States. A factor limiting tall fescue utility is its susceptibility to the pathogen *Rhizoctonia solani* Kuhn, the causal agent of foliar brown patch. Chitinase activity has been positively correlated with resistance to *R. solani* in other plant species. A chitinase assay was developed for tall fescue. Three tall fescue cultivars with differing agronomic qualities and brown patch susceptibility as well as a resistant hybrid bluegrass cultivar were inoculated with *R. solani* in a greenhouse humidity chamber. Chitinase activity 48 hours after inoculation was negatively correlated with percent brown patch severity 10 days after inoculation. 'Jaguar' tall fescue was the most tolerant to R. *solani* and exhibited the highest chitinase activity before and after inoculation. No significant increase in chitinase activity was observed in the other tall fescue cultivars following *R. solani* inoculation. Identifying tall fescue cultivars expressing high amounts of chitinase activity could be important for developing brown patch-tolerant tall fescue cultivars.

**Index words:** brown patch, turf disease, turf varieties, plant defense mechanisms.

**Species used in this study:** 'Jaguar', 'Matador', and 'Kentucky 31' tall fescue [*Festuca arundinacea* Schreb synonym *Schedonorus phoenix* (Scop.)], 'Thermal Blue Blaze' hybrid bluegrass (Poa pratensis L. × Poa arachnifera Torr.).

### Significance to the Horticulture Industry

Tall fescue is a commonly planted turf species for home lawns and other turf areas. Following inoculation, less brown patch development was noted on 'Jaguar' than 'Kentucky 31' or 'Matador' tall fescue, possibly due to Jaguar's higher level of chitinase activity. Kentucky 31 tall fescue had similar levels of brown patch severity as Matador in the greenhouse, although Kentucky 31 proved less susceptible in field trials, perhaps due to its anatomy of wide or coarse textured leaf blades and lower leaf density. Brown patch did not develop on hybrid bluegrass and chitinase activity on the latter turfgrass and Jaguar tall fescue were similar. While resistance may be due to multiple factors, breeding for elevated chitinase activity may improve brown patch tolerance in tall fescue. The chitinase screen described in this paper would be an excellent preliminary screen for brown patch resistance in tall fescue breeding lines as well as prescreening prodigy from crosses for resistance.

### Introduction

Tall fescue is one of the most commonly used turfgrasses for home lawns and common areas in North America, Europe, Asia and North Africa; additionally it serves as a feed for livestock and is used for soil stabilization (Buckner et al. 1979). This economically-important grass species is aesthetically-pleasing, quick to establish, and requires less inputs when compared to some other cool-season grass species (Cutulle et al. 2013, Turgeon 1999). Susceptibility to brown patch, which is caused by the fungal pathogen *Rhizoctonia solani* Kuhn (Dong et al. 2007, Couch 1995, Piper and Coe 1919), is a major issue in many tall fescue lawns.

Brown patch is the most severe and widespread disease in turf-type tall fescue. Brown patch symptomology on infected turfgrass plants consists of cream-colored, water soaked lesions on the shoots and stem. The causal agent *R. solani* Kuhn (Piper and Coe 1919) is a basidiomycete soilborne fungus, consisting of multiple anastomosis groupings or strains with a wide host range (Couch, 1995). Typically, tall fescue lawns in the summer are subject to concomitant infection by *R. solani* and environmental stress, which can lead to a thinning of the turf stand. Foliar infection is initiated when daytime air temperature reached about 30 C (86 F) (Dickson 1930). Additionally, high humidity and nighttime temperatures of no lower than 16 C (61 F) are required for foliar infection (Rowell 1951; Fidanza et al. 1996). Upon contact with the plant, rounded *R. solani* hyphae grow over the plant surface. Actual infection begins when the hyphae flatten out and press into the epidermal cells of the plant (Armentrout and Downer 1987, Christou 1962). Hyphal internodes shorten and form side branches. Additionally, branching of hyphae can form tightly-packed, dome-shaped infection cushions that adhere tightly to leaf and sheath surfaces. *R. solani* penetrates the plant using thin infection pegs. These infection pegs are formed from closely-adhered swollen hyphal tips (Fukotomi and Takada 1979). It is thought that the hyphal tips seek out weak areas of the plant cell wall before initiating penetration (Matsuura 1986). Throughout penetration, carbohydrates from the fungal cell wall are introduced into the plant, which can induce defense responses (Kaku et al. 2006).

Chitinase enzymes are important in plant defense for their ability to catalyze the degradation of chitin (Salzman et al 2005, Kaku et al. 2006). Chitin is a polymer of N-acetylg-

[1]Received for publication September 11, 2015; in revised form October 16, 2015. We thank Dr. Sue Tolin and Dr. Chuan Hong for use of their labs as well as their contributing expertise, and Kelley McCall, Patricia Richardson and Jason Focht for their assistance with the greenhouse chitinase assays.

[2]Syngenta Crop Protection, 7145 58th Avenue Vero Beach, FL 32967. Correspondence should be addressed to: matt.cutulle@syngenta.com.

[3]Virginia Tech, Hampton Roads Ag. Res. & Ext. Center, Virginia Beach, VA 23455.

[4]Virginia Tech, Glade Road Research Center, Blacksburg, VA 24061.

[5]Plant Science Department, 252 Ellington Plant Science Building, Joe Johnson Drive, Knoxville, TN 37996.

[6]Plant Pathology Department, University of Florida, 2550 Hull Rd, Gainesville, FL 32611.

  

lucosamine $(C_8H_{13}O_5N)_n$ and a major cell wall component. Not only do chitinases initiate fungal cell wall lysis but also reduce fungal growth by binding physically to the chitin in the fungal cell wall. Furthermore chitin oligomers formed after fungal cell wall lysis can act as elicitors by inducing a change in a receptor protein, which then can lead to a kinase cascade and defense response (Salzman et al. 2005). Specifically, the chitin oligosaccharide elicitor-binding protein (CEBiP) is a membrane protein in rice and has been shown to be an important receptor and transduction component in chitin recognition (Kaku et al. 2006). The membrane protein-kinase CERK1, described by Miya et al. (2007) in *Arabidopsis thaliana* (L.) Heynh., is able to autophosphorylate and initiate a defense response in the presence of chitin. The defense processes triggered by oligomeric chitin may include hypersensitivity, increased glucanase and chitinase activity, lignification of plant cell walls and increase in phytoalexins. *FaChit1* is a class 1 chitinase from tall fescue that is activated in the presence of oligomeric chitin, thus tall fescue should express more chitinase activity when it is being parasitized by *R. solani* (Wang et al. 2009). Chitinase activity has been positively correlated with resistance to *R. solani* in other plant species (Metraux 1991). Kern et al. (2010) overexpressed a chitinase gene from the entomopathogenic fungus *Metarhizium anisopliae* in tobacco, which resulted in increased resistance to *R. solani*. In one study, rice cultivars that were moderately resistant to *R. solani* expressed more chitinase at a basal level and after the plants were inoculated when compared to cultivars that were more susceptible to *R. solani* (Shrestha et al. 2007).

Plants that are more metabolically efficient would likely have the largest increase in chitinase activity when challenged with a fungal pathogen (personal communication with Dr. John McDowell, Virginia Tech). Quantifying chitinase activity in tall fescue cultivars with different anatomical or morphological characteristics that vary in susceptibility to brown patch when infected by *R. solani* will help clarify the relationship between pathogen infection, plant enzymatic defense, and plant morphological characteristics.

Research was conducted to evaluate chitinase activity in four grasses: 1) 'Jaguar' tall fescue, a turf-type cultivar with thinner leaf blades, 2) 'Kentucky 31' tall fescue, which is a pasture-type, wide leaf blade tall fescue frequently used in turf applications that is typically not as susceptible to brown patch as most turf-type tall fescues, 3) 'Matador' tall fescue, which has desirable agronomic qualities for turf applications but is very susceptible to brown patch and 4) 'Thermal Blue Blaze' hybrid bluegrass, which is tolerant to *R. solani*. Chitinase levels were determined in both inoculated and non-inoculated plants to determine if the presence of *R. solani* increases chitinase activity in tall fescue.

## Materials and Methods

Approximately 10 seeds of Kentucky 31, Jaguar, and Matador tall fescue and 50 seeds of Thermal Blue Blaze hybrid bluegrass were planted in 3.8 cm diameter (1.5 in), 20 cm (8 in) deep Conetainers™ (Steuwe & Son, Corvallis, OR) filled with profile medium (Turface, Profile Products LLC, 750 Lake Cook Rd., Suite 440, Buffalo Grove, IL 60089). Plants were grown in a greenhouse for 12 weeks prior to being placed in a humidity chamber with the following dimensions: 90 cm (35 in) height, 90 cm depth, and 270 cm (106 in) width. Two separate chambers were constructed for inoculated plants and non-inoculated plants. The greenhouse was equipped with cooling fans and light shutters in order to moderate temperatures, which ranged from 20 to 30 C (68 to 86 F). Plants were irrigated daily from an overhead mist system. A 20-20-20 NPK fertilizer (Scotts-Sierra Horticultural Products 14111 Scottslawn Rd. Marysville, OH 43041) was applied at a rate of 96 $kg \cdot ha^{-1}$ during establishment. The fertilizer contained 4% ammonical nitrogen, 6% nitrate nitrogen, 10% urea nitrogen, 20% $P_2O_5$ and 20% $K_2O$. Scissors were used to maintain plants at a height of 7 cm (3 in). Cutting occurred weekly and clippings were removed Inoculum was prepared by incubating 250 g of sterilized Kentucky 31 tall fescue seed with five 5-mm plugs of *R. solani* from anastomosis grouping AG2- 2IIIB grown on potato dextrose agar (BD™ Difco™ Dehydrated Culture Media**,** Fischer Scientific**,** 300 Industry Drive, Pittsburgh, PA 15275) at 27 C for 1 week. The isolate used for inoculations was collected from tall fescue and were provided by Dr. Sajeewa Amaradassa (USDA, Beltsville, MD). Plants were transferred to a sealed humidity chamber in order to promote environmental conditions that would favor the infection process by *R. solani*. The experimental design within the chamber was a randomized block with 5 replications and three samples per experimental unit. An experimental unit consisted of 3 containers 4 cm in diameter. The treatments were arranged as a factorial consisting of cultivars and two inoculation treatments (inoculated or non-inoculated). Each experimental unit was planted with Kentucky 31, Jaguar, Matador tall fescue or Thermal Blue Blaze hybrid bluegrass and was treated with 1 gram of sterilized seed placed onto the lower foliage with or without inoculum. Chitinase activity was recorded 48 hours after inoculation. Percent disease severity was recorded 10 and 30 days after inoculation, which was determined as a visual assessment of the amount of foliage covered by brown patch lesions. The entire experiment was repeated and data were pooled across experiments because there was no significant treatment by experimental run interaction.

*Protein quantification and chitinase assay.* A 400 mg (0.9 lb) sample of grass tissue was collected from all 15 pots per treatment from the top 4 cm (1.6 in) of the foliage and homogenized in 800 ul of 0.2 M sodium acetate buffer (pH 5.0) and 0.1 mM of phenyl methyl sulphonyl fluoride using a customized drill bit and mesh bags. The tissue extract was then centrifuged at 13,000xg for 10 min at 4 C with a micro-centrifuge (Model 2358 Fisher Scientific, Pittsburg, PA). Subsequently, the supernatant was decanted and centrifuged again under the same conditions. The remaining clear supernatant was then used for both total protein quantification and chitinase assays. Protein concentrations were determined based on colormetric calculations using a Pierce BCA Protein Assay kit and an albumin protein standard (Pierce Protein Research Products. 3747 N Meridian Rd, Rockford, IL, 61101). Chitinase activity was measured by incubating tissue extracts with Remazol Brilliant Violet-labeled chitin and measuring absorbance at 550 nm (Wirth and Wolf 1990). Chitinase activity per sample was determined by the following equation: (sample absorbance RBV chitin – absorbance RBV chitin with deionized water) / (total protein of sample) = chitinase activity per mg protein (Wirth and Wolf 1990).

*Data analysis.* All data were subjected to analysis of variance ($\alpha = 0.05$) using Proc Mixed model methodology in

SAS (Statistical Analysis software (SAS) v. 9.1, Cary, NC). Cultivar type and inoculum treatments were considered fixed effects and replication of experimental units were considered random. Data for all visual ratings were subjected to arcsine transformation before ANOVA. Interpretations were not different from non-transformed data and normality was acceptable based on the Shapiro-Wilk diagnostic; thus, non-transformed means are presented for clarity. Brown patch severity and chitinase activity were correlated using Proc Corr in SAS.

## Results and Discussion

Cultivars responded differently to inoculation with *R. solani* in terms of chitinase activity (Fig. 1). Of the 3 tall fescue cultivars, Jaguar tall fescue had the highest chitinase activity at both a constitutive level and when challenged with *R. solani*. Chitinase activity in tissue extract from Jaguar was 0.57 units·mg$^{-1}$ protein in non-inoculated plants, which increased to 0.64 units·mg$^{-1}$ protein in plants inoculated with *R. solani* (Fig. 1). Chitinase activity in Thermal Blue Blaze hybrid bluegrass was approximately 0.57 units·mg$^{-1}$ protein regardless of inoculation treatment.

Brown patch severity differed among the turfgrass cultivars (p-value < 0.05) (Table 1). Chitinase activity in Jaguar was statistically higher vs. Kentucky 31. For the inoculated plants, Matador and Kentucky 31 tall fescue had brown patch ratings of 16 and 17%, respectively, which was significantly greater than the the values recorded for Jaguar tall fescue or Thermal Blue Blaze hybrid bluegrass. Jaguar tall fescue suffered 10% leaf blighting, which was less than the other two tall fescue cultivars but higher than Thermal Blue Blaze hybrid bluegrass, which had approximately 1% of the leaf area blighted. Brown patch severity ratings were negligible for the non-inoculated plants, which indictates that minimal *R. solani* contamination occurred. At 30 DAI, brown patch severity was > than 50% leaf blighting on all three tall fescue cultivars. Constant moisture on leaf surfaces and high inoculum levels in the humidty chamber provided optimum conditions for disease development that would likely not

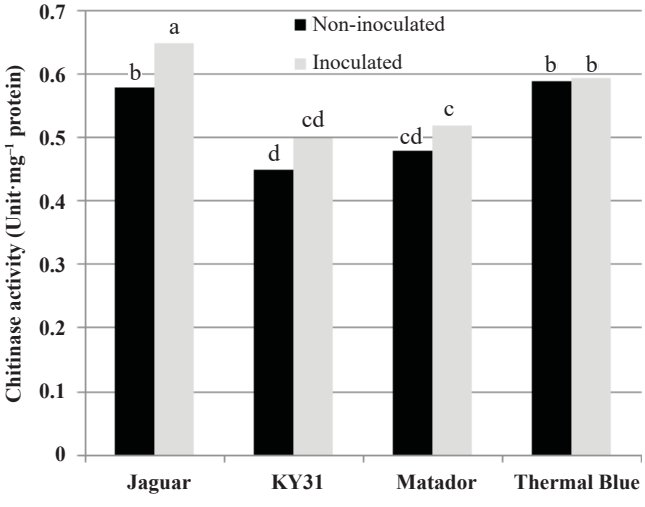


**Fig. 1. Chitinase activity in 'Jaguar', 'Kentucky 31' and 'Matador' tall fescue and 'Thermal Blue Blaze' hybrid bluegrass 48 hours after inoculation with *Rhizoctonia solani* in a greenhouse setting. Means with the same letter are not different based on Fisher Protected LSD at the 0.05 level.**

**Table 1. Impact of grass cultivar on percent foliar brown patch severity determined visually 10 and 30 days after inoculation (DAI) with *Rhizoctonia solani* in a greenhouse setting[z].**

| Grass type | Brown patch 10 DAI (%) | Brown patch 30 DAI (%) |
|---|---|---|
| 'Jaguar' tall fescue | 9b[y] | 55b |
| 'Kentucky 31' tall fescue | 16a | 75a |
| 'Matador' tall fescue | 17a | 80a |
| 'Thermal Blue Blaze' hybrid bluegrass | 1c | 1c |

[z]Rating system consist of a value of 100 for the entire canopy covered in brow patch lesion. Non-inoculated plants contained no lesions and were used to calibrate the 0 value.

[y]Means within a column followed by the same letter group are not significantly different according to Fishers Protected LSD at the $P < 0.05$ level.

be observed in the field. Brown patch was not observed on hybrid bluegrass after four weeks incubation. Tall fescue cultivars with high basal chitinases activity may be more resistant to brown patch under low levels of inoculum compared low-chitinase cultivars. However, all tall fescue cultivars are susceptible to brown patch and other physiological and anatomical components such as glucanase activity, leaf blade width, turf stand density, and recuperative ability are important in plant defense.

Chitinases constitute an important component of plant defense against *R. solani* and other fungal pathogens. Chitinase activity in tissue extracts from tall fescue was negatively correlated with brown patch severity 10 days after inoculation. The Pearsons coefficent R-value in the simple correlation was –0.72 and the p-value was less than 0.05, indicating that increased chitinase activity resulted in less disease and that the relationship was significant. These results complement the findings of Shrestha et al. (2008) who evaluated chitinase activity in different rice cultivars that were inoculated with *R. solani*. Correlating rice chitinase activity with the number of infection cushions per sample resulted in an R-value of –0.92. Two important characterisitcs associated with resistance to *R. solani* may be high basal level of chitinase activity and an efficient increase in chitinase activity during the infection process. Rice cultivars that were more resistant to *R. solani* expressed more chitinase activity when no pathogen was present and increased chitianse activity after inoculation compared to the more sensitive cultivars. We observed similar trends in our study.

Jaguar tall fescue, which was the more resistant of the three tall fescue cultivars to *R. solani*, had the greatest amount of constitutive chitinase activity and the greatest increase in chitinase activity 48 hours after inoculation (Fig. 1). Hybrid bluegrass had minimal brown patch and relatively high basal levels of chitinase, which did not increase after inoculation. Hybrid bluegrass is typically not a host for *R. solani*, thus if penetration did not occur, chitin fragments would not elicit a defense response. Though no tall fescue cultivar is highly resistant to *R. solani*, Kentucky 31 tall fescue is considered to be one of the less susceptible tall fescue cultivars to this pathogen. However, this response may be due to leaf architecture characteristics such as its wider leaf blades, which result in a less dense canopy rather than physiological characteristics. This would explain why Kentucky 31 and the more susceptible Matador tall fescue had similar levels of chitinase activity. Alternatively, Kentucky 31 tall fescue could be expressing more enzymes involved in lignification

of the cell wall or other defense mechanisms. This theory would warrant further study.

Tall fescue cultivars with desirable agronomic traits such as narrow blades and dense canopies are associated with decreased resistance to brown patch (Giesler et al. 1996). Tall fescue canopy density is believed to have a direct impact on disease incidence (Giesler et al. 1996). A denser leaf canopy increases relative humidity within the canopy and leaf wetness duration, thereby creating an optimal environment for *R. solani* infection. Because this study was conducted in a humidity chamber, the potential advantage of Kentucky 31 tall fescue's wide leaf anatomy on mitigating infection by *R. solani* was negated. Matador tall fescue has the most desirable agronomic characteristics of the tall fescue cultivars evaluated in this study. Grass blades from Matador tall fescue were thinner and greener when compared to Kentucky 31 and darker green when compared to Jaguar (personal observation, data not shown). However, Jaguar has adequate agronomic qualities such as dark green color and thinner leaf blades and was less susceptible to brown patch, thus making it a useful cultivar for the southern region of the tall fescue growing region when combined with appropriate cultural practices. Also of importance may be the recuperative ability of the tall fescue cultivar. High basal levels of chitinase activity may reduce metabolic resources for growth and plant recovery. Therefore, an ideal tall fescue cultivar would have low basal levels of chitinase but efficiently express high amounts of chitinase after being challenged by a pathogen.

Chitinase activity in rice observed 48 hours after inoculation in *R. solani* was greater than was observed in our study (Shrestha et al. 2008). Possible reasons for reduced chitinase response in tall fescue compared to rice may be due to the presence of virulence proteins in the *R. solani* AG2-2IIIB anastomosis grouping that are analogous to Avr4 and EcP6 from *Cladosporium fulvum* Cooke. Avr4 is an effector protein that protects fungal cell walls by binding to long chains of chitin and preventing hydrolysis (Van Esse et al. 2007). Specifically, in tomatoes Avr4 appeared to reduce the effectiveness of plant chitinases on fungal chitin in the apoplast. Another *C. fulvum* protein that reduces chitinase effectiveness is Ecp6. Ecp6 sequesters chitin oligosaccharides and prevents them from eliciting a defense response in the host plant (De Jonge et al. 2010). Though *C. fulvum* is an ascomycete and *R. solani* is a basidiomycete, the existence of chitinase perturbing proteins in nature necessitates further exploration of virulence proteins in *R. solani*. If proteins similar to Avr4 and Ecp6 are present in the *R. solani*, then chitinase activity would not be expected to increase much in tall fescue after inoculation. The conserved tall fescue chitinase gene *FaChit1* increases expression in the presence of chitin (Wang et al. 2009) Therefore, perturbation of interactions between tall fescue receptor proteins and chitin from *R. solani* may be the reason chitinase activity does not increase in tall fescue as much as rice as opposed to inferior perception by tall fescue receptor proteins.

Future research should focus on identifying tall fescue cultivars that constitutively express a high amount of chitinase and effectively increase chitinase levels after infection by *R. solani*. Screening for effector proteins in anastomosis groupings that impact tall fescue would further elucidate the interaction between *R. solani* and tall fescue. Selection of tall fescue cultivars that produces high levels of chitinase in response to *R. solani* infection in combination with proper cultural practices such as correct mowing height, optimal fertility application, and proper irrigation, may help reduce severity of brown patch.

## Literature Cited


Armentrout, V.N. and A.J. Downer. 1987. Infection cushion development by *Rhizoctonia solani* on cotton. Phytopathology. 77:619–623.

Buckner, R., J. Powell, and R. Frakes. 1979. Historical development. *In*: R. Buckner, L.P Bush (Eds.), Tall Fescue, Agronomy Monograph # 20, American Society of Agronomy, Madison, WI, p. 1–8.

Christou, T. 1962. Penetration and host-plant relationships of *Rhizoctonia solani* in the bean plant. Phytopathology. 52:291.

Couch, H.B. 1995. Diseases of Turfgrasses. 3rd ed. Krieger Publishing Company, Malabar, FL. 434 pp.

Cutulle, M, J. Derr, D. McCall, B. Horvath, and A. Nichols. 2013. Impact of hybrid bluegrass and tall fescue seeding combinations on brown patch severity and weed encroachment. HortScience 48:493–500.

De Jonge, R., H. Peter van Esse, A. Kombrink, T. Shinya, Y. Desaki, R. Bours, S. Van der Krol, N. Shibuya, M.H.A.J. Joosten, and B.P.H.J. Thomma. 2010. Conserved fungal Lys M effector Ecp6 prevents chitin-triggered immunity in plants. Science 329:953–955.

Dickson, L.S. 1930. The effect of air temperatures on the pathogenicity of *Rhizoctonia solani* parasitizing grasses on putting green turf. Phytopathology 20:507–608.

Dong, S., L. Treadway, H.D. Shew, G.L. Wang, E. Sivamani, and R. Qu. 2007. Resistance of transgenic tall fescue to two major fungal diseases. Plant Sci. 173:501–509.

Fidanza, M.A., P.H. Dernoeden, and A.P. Grybauskas. 1996. Development and field validation of a brown patch warning model for perennial ryegrass turf. Phytopathology 86:385–390.

Fukutomi, M. and H. Takada. 1979. Ultrastructure of infection process of *Rhizoctonia solani* Kuhn in cucumber hypocotyls. Ann. Phytopath. Soc. Jpn 45:453–462.

Giesler, L.J., G.Y. Yuen, and G.L. Horst. 1996. Tall fescue canopy density effects on brown patch disease. Plant Dis. 80:384–88.

Kaku, H., Y. Nishizwa, N. Ishii-Minami, C. Akimoto-Tomiyama, N. Dohmae, K. Takio, E. Minami, and N. Shibuya. 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc. Natl. Acad. Sci. USA 103:11086–11091.

Kern, M.F., S.F. Maraschin, D.V. Endt, A. Schrank, M.H. Vainstein, and G. Pasquali. 2010. Expression of a chitinase gene from *Metarhizium anisopliae* in tobacco plants confers resistance against *Rhizoctonia solani*. Appl. Biochem. Biotechnol. 160:1933–1946.

Matsuura, K. 1986. Scanning electron microscopy of the infection process of *Rhizoctonia solani* in leaf sheaths of rice plants. Phytopathology 76:811–814.

Metraux J.P., P.A. Goy, T. Staub, J. Speich, A. Steinmann, J. Ryals, and E. Ward. 1991. Induced systemic resistance in cucumber in response to 2,6-dichloro-isonicotinic acid and pathogens. *In*: Hennecke H. and D.P.S. Verma, eds. Advances in Molecular Genetics of Plant-Microbe Interactions Vol. 1. Dordrecht: Kluwer Academic Publishers p. 432–439.

Miya, A., P. Albert, T. Shinya, Y. Desaki, K. Ichimura, K. Sirasu, Y. Narusaka, N. Kawkami, H. Kaku, and N. Shibuya. 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA., 104:19613–19618.

Piper, C.V. and H.S. Coe. 1919. *Rhizoctonia* in lawns and pastures. Phytopathology 9:89–92.

Rowell, J.B. 1951. Observation on the pathogenicity of *Rhizoctonia solani* on bentgrass. Plant Dis. Reptr. 35:240–242.

Salzman, R.A., J.A. Brady, and S.A. Finlayson. 2005. Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. Plant Physiolo. 138:352–368.

Shrestha, C.L., I. Ona, S. Muthukrishnan, and T.W. Mew. 2008. Chitinase levels in rice cultivars correlate with resistance to the sheath blight pathogen *Rhizoctonia solani*. Eur. J. Plant Pathol. 20:69–77.

Turgeon, A.J. 1999. Turfgrass Management. Prentice Hall Pub. Co., Upper Saddle River, NJ. 392 pp.

Van Esse, P.H., M.D. Bolton, I. Stergiopoulos, P.J.G.M. De Wit, B.P.H.J.T Thomma. 2007. The chitin-binding *Cladosporium fulvum* effector protein Avr4 is a virulence factor. Mol. Plant Microbe Interact. 20:1092.

Wang, J., N. Tian, X. Huang, L.Y. Chen, M. Schlappi, and Z.Q. Xu. 2009. The tall fescue turf grass class I chitinase gene *FaChit1* is activated by fungal elicitors, dehydration, ethylene, and mechanical wounding. Plant Mol. Biol. Rep. 27:305–314.

Wirth, S.J. and G.A. Wolf. 1990. Dye labeled substrates for the assay and detection of chitinase and lysozyme activity. J. Microbiol. Methods 12:197–205.