Preventative Control of Pythium Root Rot in Creeping Bentgrass Greens and *in vitro* Sensitivity of *Pythium* spp. to Fungicides

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Introduction

Pythium root rot (PRR) has become a major problem for golf course superintendents managing creeping bentgrass (CBG) putting greens in the southeast US. Since 2008, the Turf Disease Diagnostics Lab at NC State has received roughly 3,500 samples, of those samples 18% were diagnosed with PRR. The disease is characterized by noticeably reduced, necrotic roots, and foliar dieback. In 1994, Abad et al., identified numerous *Pythium* species associated with Pythium root rot on CBG in North Carolina. Although prevalent and very problematic, little data exists on the management of this devastating disease.

Objectives

- Evaluate fungicides for preventative control of PRR in field experiments.

- Determine *in vitro* sensitivity of aggressive *Pythium* spp. to fungicides labeled for PRR control.

Materials and Methods

In vitro sensitivity

Potato dextrose agar (PDA) was amended with six concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10 µg ml^{-1}) of commercial fungicides.

Three day old hyphal plugs (6mm) were placed in the center of the amended media and incubated in the dark at 23°C. Colony diameters were measured every 24 hours for three days or until the plate was filled.

Salicyclhydroxamic acid (SHAM, 100µg/ml) was added to QoI-amended media.

EC_{50} values were estimated by linear regression (PROC REG, SAS version 9.3).

Preventative control of PRR

In spring 2013 Grass-leaf cultures containing *Pythium aphanidermatum*, *P. arrhenomanes*, and *P. ultimum* var. *ultimum* (Figure 1) plugs were grown for three days under light.

Two soil cores (5cm depth) were removed in each plot of the trial. One grass-leaf culture was placed in each hole and the turf core was replaced on top of the inoculum.

11 fungicides (Table 1) labeled for controlling PRR were sprayed at high rates every 21 days and watered in.

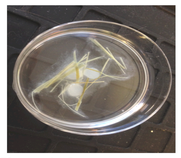

Figure 1. Grass-leaf culture.

Table 1. Fungicides used in preventative PRR trial. Sprayed every 21 days at highest recommended rates.

Fungicide	Use Rate of product
cyazofamid	0.9 fl. Oz. * 1000 $ft.^{-2}$
mefenoxam	1.0 fl. Oz * 1000 $ft.^{-2}$
propamocarb	2.0 fl. Oz. * 1000 $ft.^{-2}$
propamocarb + fluopicolide	1.2 fl. Oz. * 1000 $ft.^{-2}$
etridazole	3.0 fl. Oz. * 1000 $ft.^{-2}$
azoxystrobin	0.4 oz. Wt. * 1000 $ft.^{-2}$
pyraclostrobin	0.7 fl. Oz. * 1000 $ft.^{-2}$
fluoxastrobin	0.36 fl. Oz. * 1000 $ft.^{-2}$
cyazofamid + azoxystrobin	0.9 fl. Oz. * 1000 $ft.^{-2}$ + 0.4 oz. Wt. * 1000 $ft.^{-2}$
cyazofamid + fluoxastrobin	0.9 fl. Oz. * 1000 $ft.^{-2}$ + 0.36 fl. Oz. * 1000 $ft.^{-2}$
cyazofamid + pyraclostrobin	0.9 fl. Oz. * 1000 $ft.^{-2}$ + 0.7 fl. Oz. * 1000 $ft.^{-2}$
Non-Treated	

Results – *in vitro* sensitivity

Table 2. *In vitro* sensitivity of *Pythium aphanidermatum*, *P. ultimum* var. *ultimum*, *P. arrhenomanes*, *P. vanterpoolii*, *P. volutum*, *P. irregulare, and P. graminicola* species to pyraclostrobin, trifloxystrobin, azoxystrobin, fluoxastrobin, cyazofamid, etridazole, mefenoxam, chlorothalonil and fluazinam.

	EC_{50} concentration (µg ml^{-1}) for fungicide class and material used[z]								
Species	pyraclostrobin	trifloxystrobin	azoxystrobin	fluoxastrobin	cyazofamid	etridazole	mefenoxam	chlorothalonil	fluazinam
P. aphanidermatum	>10	>10	>10	>10	7.9	>10	>10	>10	>10
P. ultimum var. *ultimum*	>10	>10	>10	>10	>10	>10	>10	>10	>10
P. arrhenomanes	>10	>10	>10	>10	0.0005	>10	>10	>10	>10
P. vanterpoolii	>10	>10	>10	>10	0.015	>10	>10	>10	>10
P. volutum	0.001	>10	0.02	0.005	0.004	>10	>10	>10	>10
P. irregulare	>10	>10	>10	>10	>10	>10	>10	>10	>10
P. graminicola	>10	>10	>10	>10	>10	>10	>10	>10	>10

[z] EC_{50} = 50% effective concentration of fungicide to limit fungal growth by 50%.

Pythium arrhenomanes, *P. volutum*, *P. aphanidermatum* and *P. vanterpoolii* were most sensitive to cyazofamid.

QoI fungicides, azoxystrobin, fluoxastrobin and pyraclostrobin effectively reduced growth of *P. volutum.*

Other fungicides did not reduce growth at the concentrations tested.

Results – Preventative control of PRR

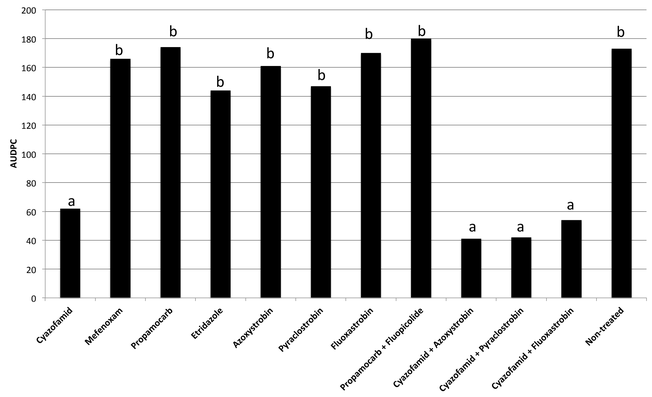

Figure 2. Disease severity observed in the summer of 2014 expressed in area under disease progress curve (AUDPC). AUDPC values were calculated from disease severity data collected every two weeks from July to August 2014. Bars followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio *t* test (*k*=100, P=0.05).

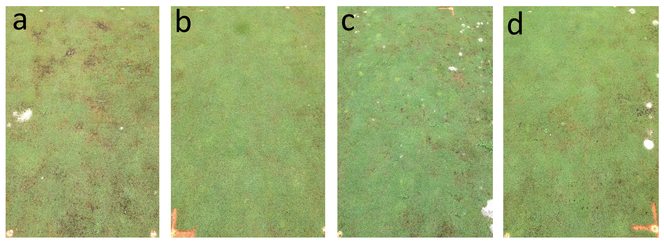

Figure 3. Plots from CBG green representing **a**) Non-treated control ; **b**) Cyazofamid + Azoxystrobin; **c**) Cyazofamid; **d**) Cyazofamid + Pyraclostrobin.

Suppression of PRR was only achieved with applications of cyazofamid alone or in combination with a QoI fungicide (Figure 2).

All other treatments were similar to the non-treated control.

Turf treated with cyazofamid and a combination of either pyraclostrobin or azoxystrobin had more dense turf distribution and had more aesthetically pleasing color (Figure 3 a-d).

Conclusions

The highly aggressive *Pythium* species were most sensitive to cyazofamid.

Cyazofamid with QoI fungicides, azoxystrobin and pyraclostrobin, suppressed PRR in CBG green.

Preventative applications of cyazofamid with azoxystrobin and pyraclostrobin in the early summer will reduce PRR severity.

Acknowledgements

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References

Abad, Z. G., Shew, H. D., and Lucas, L. T. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology 84:913-921.

Kerns, J. P., Soika, M. D., and Tredway, L. P. 2009. Preventive control of Pythium root dysfunction in creeping bentgrass putting greens and sensitivity of *Pythium volutum* to fungicides. Plant Dis. 93:1275-1280.