2008-2009 GCSAA
Chapter Cooperative Research Program Progress Report

Project title: Management and biology of Brown Ring Patch on Annual Bluegrass Greens

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A. Report Abstract

Brown Ring Patch caused by *Waitea circinata* var *circinata* is a new, invasive pathogen of turfgrass in the U.S. Experiments were initiated in 2008 to address the key issues of chemical and cultural management, population structure and origin and the basis of resistance to certain classes of fungicides. In field experiments, it was shown that certain fungicides are most effective for the control of the disease. It appears that Headway and Endorse fungicides provide excellent curative activity against the disease, but have short residual activity. Certain DMI-fungicides (Trinity, Triton and Tourney) and ProStar appear to take a longer period of time to control the disease, but offer a high level of control with a long residual period. Repeated applications of fungicides also appear to give better control than single applications. Nitrogen appears to have a significant effect on decreasing disease development when 1 lb/1,000 sq ft was applied in nitrate, ammonium or urea form. Primo MAXX (0.125 fl oz/1,000 sq ft) appeared to cause a slight increase in disease in some cases. However, applications of nitrogen in combination with Primo MAXX appeared to provide the best disease control and turf color. Currently, population analyses using AFLP and determination of the molecular basis of fungicide resistance are being performed. Based on the results of 2008 field experiments, best management practices for brown ring patch include (i) application of nitrogen and Primo MAXX to reduce disease severity and improve turf color, (ii) application of Headway or Endorse for quick acting curative control, and (iii) application of certain DMI-fungicides or ProStar for long lasting control.

B. Project Rationale

Waitea circinata var *circinata* was recently discovered as a new, invasive pathogen of turfgrass in the U.S. affecting high value golf course putting greens (de la Cerda et al. 2007, Chen et al. 2007). Previously only found as a turfgrass pathogen in Japan causing “Brown Ring

Figure 1. Distribution of Brown Ring Patch in the U.S. on annual bluegrass (●) and rough bluegrass (○).

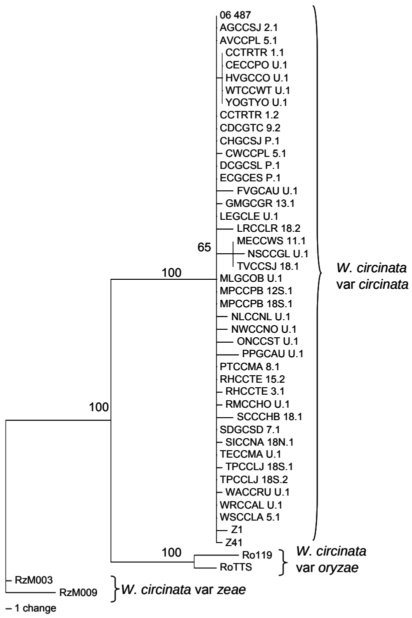


Figure 1. ITS-sequence similarity between *Waitea circinata* (*Rhizoctonia*)

Patch" (Toda et al. 2005), it was only first detected by the Turf Disease Diagnostic Lab at UC Riverside from golf courses in Washington and California beginning in 2003. Since then, it has been detected at over 50 locations in California, Oregon, Washington and Nevada and most recently in Illinois, Ohio, Pennsylvania, New York, New Jersey, Connecticut, Maine, Rhode Island and Massachusetts (Figure 1).

Little is known about the biology, origin or method of spread in the U.S. for this pathogen and there is very limited information on effective control measures. Recent work in the Douhan and Wong labs has confirmed its identity using morphological and molecular methodologies (de la Cerda *et al.* 2007). It appears that the pathogen is closely related to two known pathogens of turfgrass, *Rhizoctonia zeae* (telemorph: *Waitea circinata* var *zeae*) and *Rhizoctonia oryzae* (*Waitea circinata* var *oryzae*) (Figure 2), but appears to be a separate species that had not been found as a pathogen of turfgrass until now. *Waitea circinata* var *circinata* appears to be able to cause damage to turf over a wide range of temperatures, having been observed at approximately 15 to 35ºC daytime temperatures. Symptoms are manifested as circular rings several cm to a meter in size that causes yellowing and necrosis of turf that is often difficult to recover from (Figures 3 & 4). This wide temperature range is uncommon for many *Rhizoctonia* diseases (Burpee and Martin 1992) and this pathogen has been problematic for many golf courses as a year-round disease.

There is no information available on the origin or spread of the disease. Information on the basic biology of the pathogen (reproduction, temperature and humidity requirements) would greatly help forecast periods of disease susceptibility. Understanding the population structure of the pathogen would also help potentially determine its origin and method of spread (Milgroom 1996; Milgroom and Peever 2003). Recent work in the Douhan and Wong labs has shown that the pathogen is widespread in multiple states.

Figure 3. Symptoms of *Waitea circinata* var. *circinata* on annual bluegrass (*Poa annua*).

Figure 4. Long term damage caused by *W. circinata* var *circinata* on annual bluegrass.

Practically, fungicidal control of this pathogen has been problematic. Often, repeated fungicide applications are required to halt the disease. Recent work by the Wong Lab has already identified that this pathogen is completely resistant to benzimidazole fungicides and potentially QoI-resistant isolates have already been identified from California populations (Rios et al. 2006). Laboratory trials have shown that fungicide timing is crucial for the control of the pathogen. In these tests, preventive applications of fungicides provided near complete control while curative applications provided only 20 to 78% control (Wong and Kaminski 2007). The role of nitrogen in the management of this disease is unknown. Nitrogen is known to increase the severity of *R. solani* (Brown Patch) (Cubeta and Vilgalys 1997), but its effects are not well documented for other *Rhizoctonia* diseases (Couch 1995). Anecdotally, many of the locations with chronic Brown Ring Patch have been using low nitrogen fertility programs to increase ball roll and greens speed. Lack of recovery due to inadequate fertility seems to be a plausible reason for the increased severity of the disease at these locations. Also, the effect of plant growth regulators, such as Primo (trinexapac-ethyl) is unknown for Brown Ring Patch. Trinexapac-ethyl is commonly used on putting greens to reduce plant size in order to have increased ball roll on the greens surface (King et al. 1997) and its use has been observed in slowing recovery from Brown Ring Patch.

The overall objectives of this proposed multi-year study is to understand the biology and population structure of *W. circinata* var *circinata* and what management practices are most effective in controlling the disease. Based upon the results of the study, we would hope to implement sustainable best management strategies for golf course superintendents.

C. Materials and Methods

Chemical and Cultural Control of Brown Ring Patch

Mark Woodward and Jon Maddern, the directors at the course, made nine greens on the North Course of Torrey Pines Golf Course in La Jolla, CA, available for use from March to June of 2008. Fungicide, fertility and plant growth regulator trials were conducted here in lieu of the replicated locations originally proposed due to the availability of the site and significant disease pressure.

Fungicide Affects on the Development of Brown Ring Patch. Fungicide trials were conducted on three greens at various times from March to May. Trials were performed with manufacturer support and products and focus primarily on the effect of single curative applications. Previous work had shown that many fungicides worked very well preventively and these trials were more in line with curative applications made to greens by superintendents after the appearance of brown ring patch.

Generally, greens were scouted for the development of brown ring patch and trials conducted on portions of greens were the disease appeared to be developing uniformly. Applications were made to replicated plots using a hand-held CO_2-powered boom sprayer fitted with TeeJet 8002VS flat-fan nozzles. Applications were made at 35 psi in a water volume equivalent to 2 gal/1,000 ft^2. Plots were rated weekly for disease on a 0 to 10 scale reflecting 0 to 100% of the plot area affected by disease. Disease severity data was analyzed by ANOVA and means comparisons performed using

Fisher's Protected LSD (both with α = 0.05). For a description of materials under trial, please see Appendix A.

The first trial, conducted on green 3, focused on the effect of DMI-fungicides and their mixtures, and *Bacillus* spp.-based biological fungicides. The second was conducted on green 5 and focused on a range of chemicals typically used for Rhizoctonia disease control. The experiment on green 6 focused on the effect of repeated fungicide applications.

Effects of Nitrogen and Trinexapac-ethyl (Primo MAXX) on Brown Ring patch Development. In this experiment, the effect of three nitrogen sources (urea, nitrate and ammonium), alone and in combination with trinexapac-ethyl (Primo MAXX). Water soluble nitrogen was applied on 8 Apr and 22 Apr (trial 1) and 3 & 17 May (trial 2) at the rate of 0.5 lb/1,000 sq ft (1 lb total). Forms of nitrogen were: calcium nitrate (15-0-0), ammonium sulfate (21-0-0) and urea 46-0-0. Primo MAXX was applied at 0.125 fl oz/1,000 sq ft in combination with nitrogen to some plots. All applications were made as described above using a CO_2-powered sprayer and 2 gal/1,000 sq ft water volume.

Disease severity was rated by photographic analysis using SigmaScan software. Briefly, high resolution images of each plot were taken weekly using Canon D90 digital camera and tripod set at fixed height and angle. Images were cropped and processed to analyze a fixed, equal areas and software set to distinguish between yellow-brown symptomatic and non-symptomatic turf.

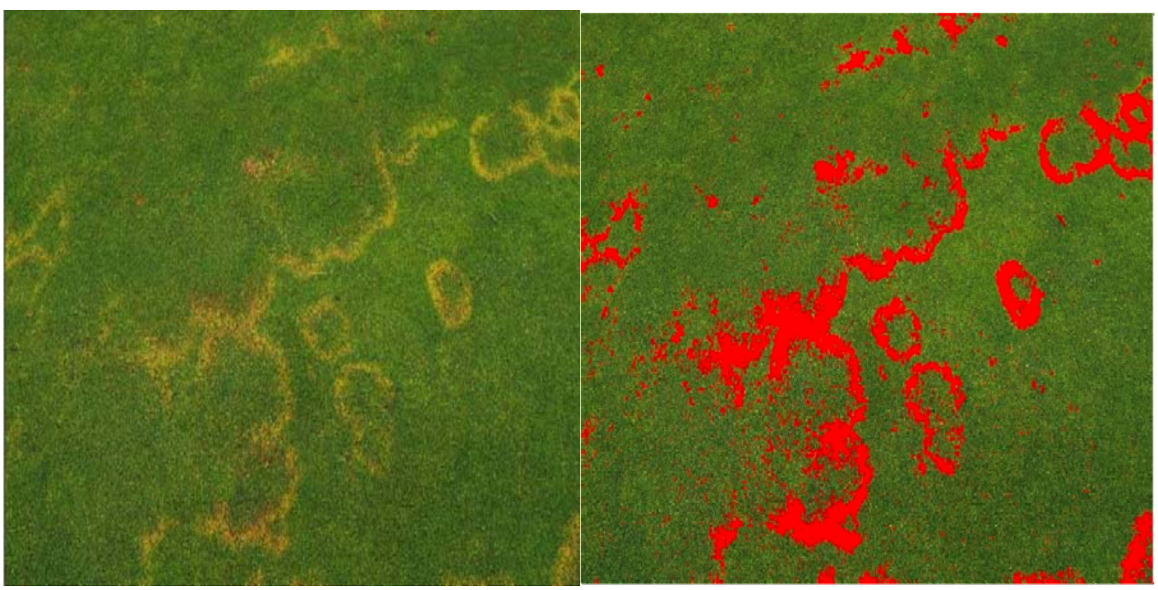


Figure 5. Digital analysis of disease severity using Sigma Scan software.

Turf color (quality) was measured using a CM-1000 chlorophyll meter, making six measurements per plot on a weekly basis. Both disease severity data and color were analyzed by ANOVA followed by Fisher's Protected LSD (α = 0.05).

Effects of Nitrogen, Trinexapac-ethyl and Fungicides on Brown Ring patch Development. The low rate of Heritage TL (1 fl oz) was applied alone or in combination with 0.5 lb/1,000 sq ft calcium nitrate and/or 0.125 fl oz Primo MAXX. The experiment was performed independently on greens 2 and 6. Disease severity was rated 5 times on a weekly basis from 25 Apr to 23 May and analyzed as described above.

Population and Molecular Analyses of *Waitea circinata* var. *circinata*

Isolates of the fungus were systematically sampled in March, April and June from three greens at Torrey Pines golf course to examine the population distribution within a single location. Additional isolates were collected through out the year from the disease diagnosis lab.

D. Results

The Effect of DMI mixtures and biological fungicides on brown ring patch.

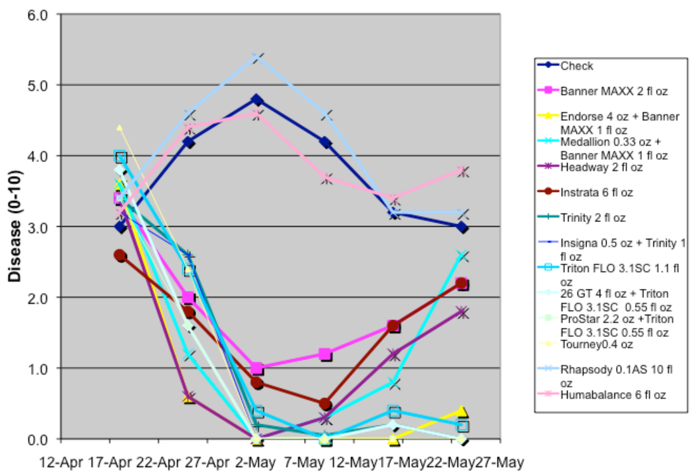


Figure 6. The Effect of DMI mixtures and biological fungicides on brown ring patch

In the first experiment, a curative application was made 18 Apr. By 25 Apr, all fungicides except the biologicals, had a significant effect on reducing disease, but applications of Headway or Endorse plus Banner MAXX appeared to give the most complete control (Table 1, Figure 6). All fungicides appeared to give very good or complete control by 14 days after application. By 16 May (3 weeks post-treatment), some applications (Banner MAXX, Medallion plus Banner MAXX, Instrata and Headway began to lose effectiveness. The remainder of the fungicides appeared to retain their effectiveness up to 5 weeks after treatment. In this trial, direct comparisons of DMI-fungicides suggest that Triton, Trinity and Tourney are more effective than Banner MAXX.

Table 1. The Effect of DMI mixtures and biological fungicides on brown ring patch

	Disease severity*							
Treatment and rate per 1000 sq ft	11 Apr	18 Apr	25 Apr	02 May	09 May	16 May	23 May	AUDPC**
Rhapsody 0.1AS 10 fl oz	1.5	4.3	5.8 a	6.8 a	5.8 a	4.0 a	4.0 ab	30.5 a
Humabalance L 6 fl oz	1.5	4.0	5.5 a	5.8 b	4.6 a	4.3 a	4.8 a	28.9 a
Check	1.8	3.8	5.3 a	6.0 ab	5.3 a	4.0 a	3.8 abc	28.0 a
Banner MAXX 1.3MEC 2.0 fl oz	2.5	4.3	2.5 bc	1.3 c	1.5 b	2.0 b	2.8 bc	14.3 b
Instrata 3.61SE 6.0 fl oz	1.8	3.3	2.3 bcd	1.0 cd	0.6 bc	2.0 b	2.8 bc	11.9 bc
Medallion 50WP 0.33 oz + Banner MAXX 1.3MEC 1.0 fl oz	1.5	4.5	1.5 cd	0.0 e	0.4 bc	1.0 bc	3.3 abc	10.6 bcd
Triton Flo 3.1SC 1.1 fl oz	2.5	5.0	3.0 bc	0.5 cde	0.0 c	0.5 bc	0.3 d	9.3 cde
Headway 1.39EC 2.0 fl oz	2.0	4.3	0.8 d	0.0 e	0.4 bc	1.5 bc	2.3 c	9.1 cde
Tourney 50WG 0.4 oz	1.8	5.5	3.0 bc	0.0 e	0.0 c	0.0 c	0.0 d	8.5 cde
Trinity 1.69SC 2.0 fl oz	1.8	4.3	3.3 b	0.3 de	0.1 c	0.3 c	0.0 d	8.1 cde
Insignia 20 WG 0.5 oz + Trinity 1.69SC 1.0 fl oz	1.8	4.0	3.3 b	0.0 e	0.0 c	0.3 c	0.0 d	7.5 cde
26 GT 2SC 4.0 fl oz + Triton Flo 3.1SC 0.55 fl oz	1.8	4.8	2.0 bcd	0.0 e	0.0 c	0.3 c	0.0 d	7.0 de
ProStar 70WG 2.2 oz + Triton Flo 3.1SC 0.55 fl oz	1.8	4.3	2.0 bcd	0.0 e	0.0 c	0.0 c	0.0 d	6.3 de
Endorse 2.5WP 4.0 oz + Banner MAXX 1.3MEC 1.0 fl oz ...	2.5	4.5	0.8 d	0.0 e	0.0 c	0.0 c	0.5 d	5.8 e
LSD Value (α = 0.05)			1.7	0.8	1.3	1.5	1.6	4.5
ANOVA *P*	0.93	0.88	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* Values reflect the mean of four replicated plots per treatment. Means followed by the same letter are statistically equivalent (Fisher's Protected LSD, α = 0.05)

** AUDPC values represent the mean sum of disease from 25 Apr to 23 May in four replicated plots per treatment.

The Effect of Fungicides for the Control of Brown Ring Patch. This trial was conducted on green 5 and focused on a range of fungicide generally considered effective against Rhizoctonia diseases. Also tested was the effect of Revolution soil surfactant that was hoped to increase fungicide effectiveness by increasing penetration into soil and thatch.

Table 2. Effect of Fungicides for the Control of Brown Ring Patch

	Disease severity*						
Treatment and rate per 100 sq ft	25 Apr	2 May	9 May	16 May	23 May	30 May	AUDPC**
Heritage TL 0.8ME 1.0 fl oz	5.0	3.8 cde	1.1 e-i	2.3 cd	4.0 abc	4.8 a	15.9 a-d
Disarm 480SC 0.27 fl oz + Daconil Ultrex 82.5WG 2.6 oz	6.3	5.8 abc	2.8 cd	3.3 abc	4.3 ab	3.8 ab	19.8 efg
Disarm 480SC 0.36 fl oz	4.8	4.5 bcd	3.3 bcd	3.5 abc	4.3 ab	3.5 ab	19.0 abc
Concert 4.3 SE 6.0 fl oz	6.8	4.3 bcd	2.3 de	3.3 abc	3.5 abc	3.0 abc	16.3 a-d
Insignia 20WG 0.50 oz + Revolution L 6.0 fl oz	4.8	5.8 abc	2.5 de	3.3 abc	4.8 a	3.0 abc	19.3 efg
Endorse 2.5WP 4.0 oz	4.3	1.3 e	0.5 ghi	1.3 def	2.3 cde	2.5 bc	7.8 fgh
Insignia 20WG 0.90 oz	5.5	4.5 bcd	2.3 de	2.5 bcd	3.0 a-d	2.5 bc	14.8 b-e
Headway 1.39EC 2.0 fl oz	5.5	2.3 de	0.6 f-i	1.4 def	3.0 a-d	2.3 bcd	9.5 e-h
Emerald 70WG 0.18 oz	6.5	5.3 abc	2.5 de	2.3 cd	3.0 a-d	2.3 bcd	15.3 a-e
Check	6.5	7.3 a	5.3 a	3.3 abc	2.3 cde	2.0 b-e	20.0 ab
Banner MAXX 1.3 MEC 2.0 fl oz	6.5	5.0 abc	1.8 d-h	2.0 cde	2.5 b-e	2.0 b-e	13.3 c-e
Chipco 26GT 2SC 6.0 fl oz	5.3	5.5 abc	4.5 ab	4.8 a	4.3 ab	2.0 b-e	21.0 a
PBI Exp 2 15WP 6.5 oz	6.3	6.3 abc	4.3 abc	4.0 ab	3.8 abc	2.0 b-e	20.3 ab
Insignia 20WG 0.90 oz + Revolution L 6.0 fl oz	4.8	4.3 bcd	2.1 def	2.3 cd	2.3 cde	1.5 c-f	12.4 def
Triton Flo 3.1SC 0.55 fl oz	6.3	6.8 ab	1.9 d-g	1.5 def	1.5 def	0.5 def	12.1 d-g
ProStar 70WG 2.2 oz	6.5	5.0 abc	0.5 ghi	0.0 f	0.0 f	0.3 ef	5.8 h
CX-09 45WP 1.0 oz	4.0	4.0 cd	0.0 i	0.0 f	0.0 f	0.0 f	4.0 h
ProStar 70WG 4.4 oz	7.0	6.3 abc	0.3 hi	0.0 f	0.0 f	0.0 f	6.5 gh
Triton Flo 3.1SC 0.55 fl oz + ProStar 70WG 2.2 oz	6.8	4.8 a-d	0.3 hi	0.0 f	0.0 f	0.0 f	5.0 h
Revolution L 6.0 fl oz	7.5	6.3 abc	3.3 bcd	2.5 bcd	1.3 def	0.0 f	13.3 c-e
PBI Exp 1 50WDG 1.6 oz	6.3	3.8 cde	0.3 hi	0.5 ef	1.0 ef	0.0 f	5.5 h
LSD (α=0.05)	2.4	2.5	1.6	1.5	1.9	1.9	5.9
ANOVA p-value	0.203	0.002	0.001	0.001	0.001	0.001	0.001

* Values reflect the mean of four replicated plots per treatment. Means followed by the same letter are statistically equivalent (Fisher's Protected LSD, α = 0.05)
** AUDPC values represent the mean sum of disease from 9 to 30 May in four replicated plots per treatment.

Disease in check plots increased from a mean 6.5 to 7.3 rating from 25 Apr to 2 May but from May 2 to May 30, disease decreased to a mean 2.0 rating due to the coalescing of expanding rings, suggesting potential antagonism between different genotypes of the pathogen (potential anastomosis and killing reactions between individuals). Similar decreases in disease in other treated plots were observed over the course of the study.

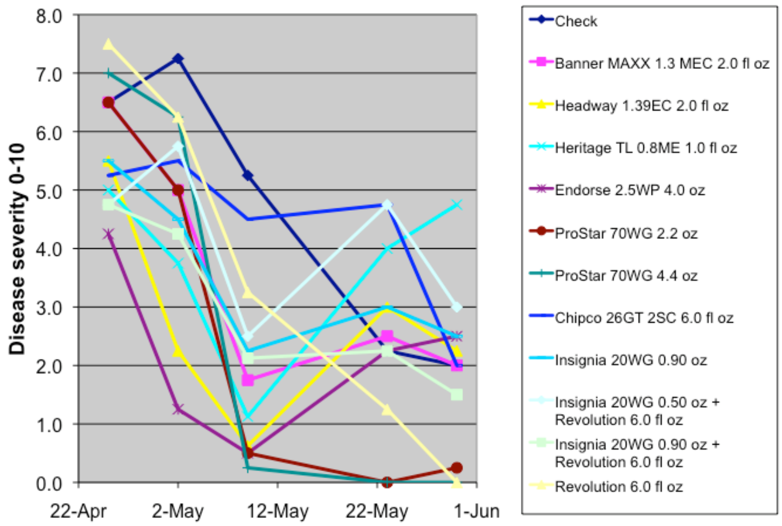


Figure 7. The Effect of fungicides on brown ring patch

One week after application, the single curative application of some fungicides significantly reduced disease, but effect was most pronounced for Headway and Endorse (Table 2, Figure 7). By 2 weeks after treatment, some additional treatments, most notably CX-9, ProStar and ProStar plus Triton FLO had almost no or little disease. The residual activity of Headway and Endorse began to dissipate 14 days after application, while those treated with CX-9, ProStar and ProStar plus Triton FLO continued to be disease free.

Of the DMI fungicides, Triton FLO was more efficacious than Banner MAXX. Compared to Banner MAXX, Concert performance was lower, likely due to the lower amount of propiconazole in the 6 fl oz rate of Concert as compared to the 2 fl oz rate of Banner MAXX. Control offered by 26GT and the QoI fungicides (Heritage, Insignia, Disarm) were good to moderate, and amongst the QoIs, Heritage appeared to be somewhat better than the others.

Applications with the surfactant Revolution had surprising results. When used in combination with Insignia, control did not appear to be improved, as compared with the 0.9 oz rate used alone. However, in Revolution only treated plots, there was a decline in disease, similar to that in the check plots, caused by the rapid expansion of rings that often coalesced and disappeared in these plots. It is possible that the increased soil moisture in these plots had an positive effect on pathogen growth, causing the aforementioned consequence. This needs to be examined in future studies.

Effects of Repeated QoI and Chlorothalonil Tank Mix Applications on Brown Ring Patch Development. In this trial, the effect of repeated QoI and chlorothalonil fungicide mixtures were made. Previous studies had shown that azoxystrobin (Heritage) was not as effective as other fungicides when used alone, and single applications were not effective. This objective of this trial was to examine the effect of the new azoxystrobin and chlorothalonil tank mix (Renown) and other premixed fungicides (Tartan, Concert and Headway) when repeated applications were made.

Seven days after the first application, all treatments except for Tartan and Concert had significantly less disease than the check treatment . Plots treated with Headway appeared to have the least amount of disease, although it was statistically equal to A15935 and Heritage plus Daconil. By May 16 (14 days after treatment), all fungicides had significant reductions in disease compared the check, with Renown, Headway, and Heritage plus Daconil giving better control than Tartan or Concert. By May 23, plots treated with a second application of Renown, Headway or Heritage plus

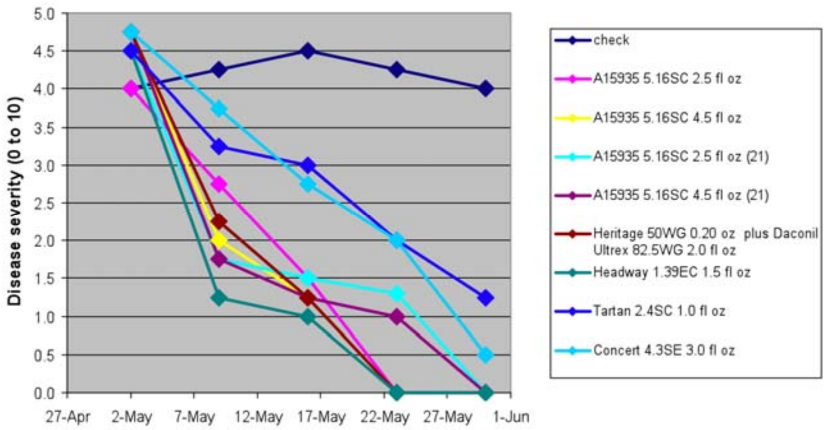


Figure 8. The Effect of Repeated Fungicide Applications on Brown Ring Patch

Daconil had no disease, while there continued to be disease in the plots treated only once with Renown or two times with Tartan or Concert. By May 30, all plots had received two fungicide applications and all treatments, except for Tartan had almost no disease.

There did not appear to be a dose response for differing rates of A15935 in this trial. No fungicides provided complete control after a single application; control was greatly increased after the second fungicide application.

Table 3. Effects of Repeated QoI and Chlorothalonil Tank Mix Applications on Brown Ring Patch Development.

	Disease severity rating*				
Treatment and rate per 1000 sq ft	2 May	9 May	16 May	23 May	30 May
Check	4.0	4.3 a	4.5 a	4.3 a	4.0 a
Renown 5.16SC 2.5 fl oz	4.0	2.8 bcd	1.5 c	0.0 c	0.0 c
Renown 5.16SC 4.5 fl oz	4.8	2.0 cde	1.3 c	0.0 c	0.0 c
Renown 5.16SC 2.5 fl oz**	4.5	1.8 de	1.5 c	1.3 bc	0.0 c
Renown 5.16SC 4.5 fl oz**	4.8	1.8 de	1.3 c	1.0 bc	0.0 c
Heritage 50WG 0.20 oz plus Daconil Ultrex 82.5WG 2.0 fl oz	4.8	2.3 cde	1.3 c	0.0 c	0.0 c
Headway 1.39EC 1.5 fl oz	4.5	1.3 e	1.0 c	0.0 c	0.0 c
Tartan 2.4SC 1.0 fl oz	4.5	3.3 abc	3.0 b	2.0 b	1.3 b
Concert 4.3SE 3.0 fl oz	4.8	3.8 ab	2.8 b	2.0 b	0.5 c
LSD Value		1.3	1.0	1.3	0.7
ANOVA *P*	1.0	<0.001	<0.001	<0.001	<0.001

* Disease severity was measured on a 0 to 10 scale with 10=100% of plot area covered by disease symptoms; means followed by the same letters are equal according to Fisher's Protected LSD (α=0.05).

** Applied at a 21-day interval, all other treatments applied at a 14-day interval

Nitrogen and Trinexapac-ethyl Effects on the Development of Brown Ring Patch

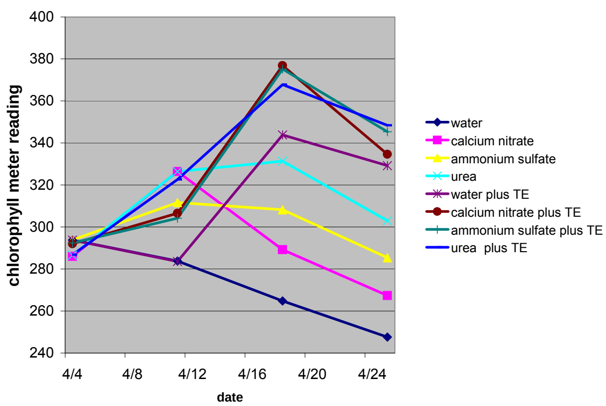
All nitrogen treatments had a positive effect on turf color in both experiments as compared to the water-treated plots (Table 4, Figure 9). Soil analysis of nitrogen was inconclusive for 2 samplings taken during the first trial on green 7, suggesting that the turf was absorbing nitrogen immediately.

Turf Color was taken as the primary measurement or indication that there was a physiological response from the turf. There was variation between experiments, but generally trends were consistent over the evaluation period. In most cases, there were not significant differences between nitrogen sources, but over all, urea did appear to give a stronger color response. Plots treated with Primo MAXX had higher color than those treated with nitrogen alone, and plots treated with Primo MAXX and nitrogen had the highest turf quality readings.

Table 4. Effect of Nitrogen and Trinexapac-ethyl on Turfgrass Color

	Chlorophyll meter rating*													
	Trial 1							Trial 2						
Treatment and rate per 1,000 sq ft	04 Apr	11 Apr		18 Apr		25 Apr		02 May	09 May		16 May		23 May	
water	294	284	c	265	e	248	e	212	219	d	199	c	231	d
1 lb calcium nitrate	286	326	a	289	d	267	d	210	222	d	209	bc	245	d
1 lb ammonium sulfate	294	312	b	308	c	285	c	213	231	c	211	bc	259	c
1 lb urea	287	326	a	331	b	303	b	211	240	c	212	bc	277	bc
0.125 fl oz Primo MAXX	294	284	c	344	b	329	ab	209	246	bc	223	ab	292	ab
0.125 fl oz Primo MAXX plus 1 lb calcium nitrate	292	307	b	377	a	335	ab	207	254	bc	230	a	304	a
0.125 fl oz Primo MAXX plus 1 lb ammonium sulfate	292	304	b	375	a	345	a	209	265	ab	226	ab	301	a
0.125 fl oz Primo MAXX plus 1 lb urea	287	323	a	368	a	348	a	214	269	a	232	a	299	a

* The mean of four replicated plots using six CM-1000 readings per plot; means followed by the same letters are equal according to Fisher's Protected LSD (α=0.05).



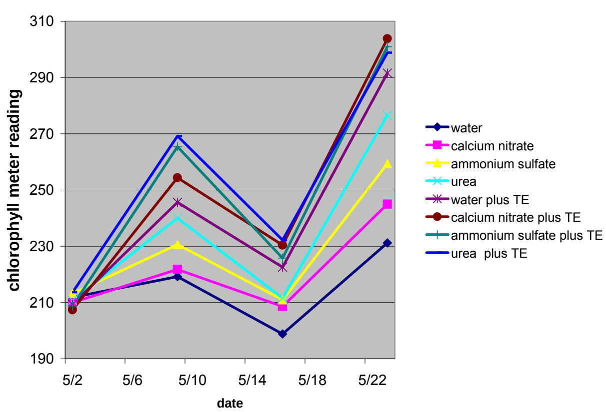


Figure 9. Color Ratings for Trial 1 (L) and Trial 2 (R) Conducted on Green 7

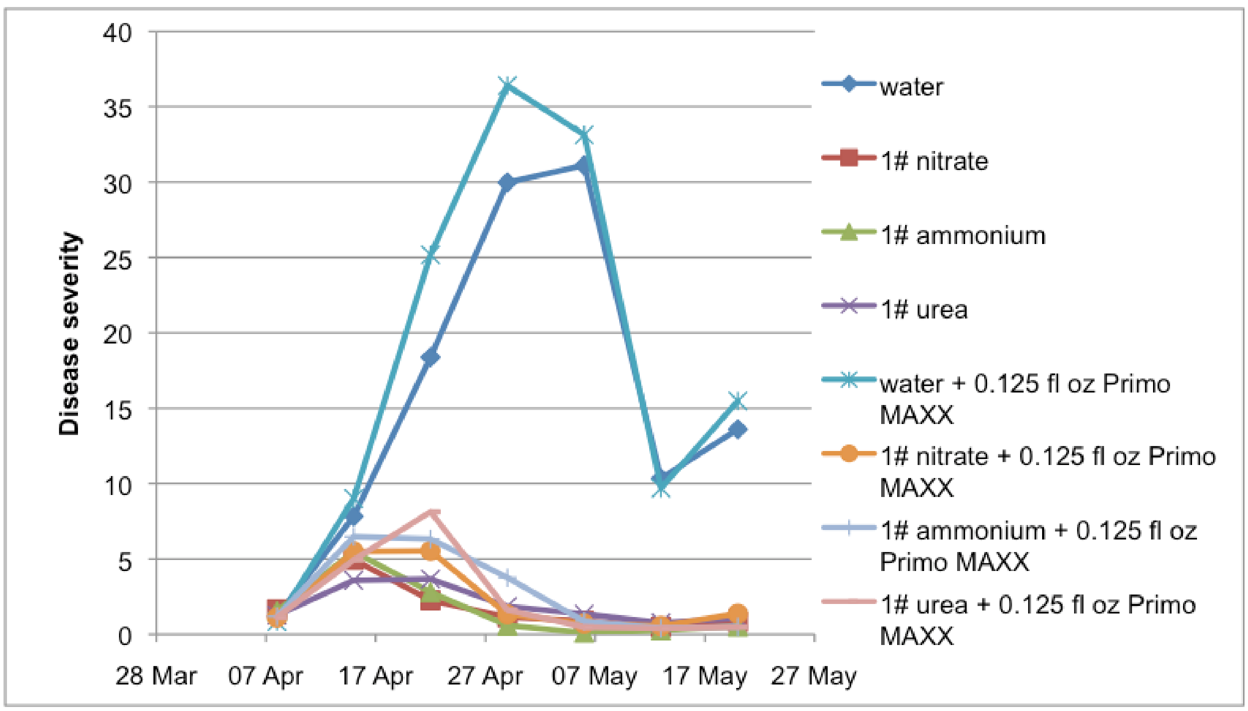
Direct effects on disease development were determined by photographic analysis. Diseased areas in images were calculated as % pixels in 800 x 800 images and converted to a disease severity rating.

Generally, application of nitrogen, regardless of source or in combination with Primo MAXX, resulted in significantly less disease than those treated with water or Primo MAXX only. (Table 5, Figure 10). Results are shown only for Trial 1, but results from Trial 2 were similar. Plots treated with Primo MAXX had significantly more disease than water-only treated plots on two of four rating dates.

Table 5. Nitrogen and Trinexapac-ethyl Effects on Brown Ring Patch Severity

	Disease severity rating*						
Treatment and rate per 1,000 sq ft	08 Apr	15 Apr	22 Apr	29 Apr	06 May	13 May	20 May
water	1.21	7.82	18.38 b	29.97 b	31.11 a	10.34 a	13.60 a
1 lb calcium nitrate	1.66	4.94	2.25 c	1.14 c	0.89 b	0.50 b	0.83 b
1 lb ammonium sulfate	1.53	5.47	2.80 c	0.59 c	0.11 b	0.26 b	0.52 b
1 lb urea	1.26	3.58	3.67 c	1.85 c	1.34 b	0.76 b	1.07 b
0.125 fl oz Primo MAXX	0.85	9.01	25.17 a	36.40 a	33.14 a	9.69 a	15.48 a
0.125 fl oz Primo MAXX plus 1 lb calcium nitrate	1.11	5.49	5.53 c	1.32 c	0.71 b	0.55 b	1.37 b
0.125 fl oz Primo MAXX plus 1 lb ammonium sulfate	1.19	6.48	6.33 c	3.76 c	0.86 b	0.40 b	0.50 b
0.125 fl oz Primo MAXX plus 1 lb urea	1.12	4.94	8.13 c	1.61 c	0.47 b	0.40 b	0.47 b
ANOVA *P*	0.94	0.12	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* The mean of four replicated plots using Sigma Scan to determine % pixles affected by disease; means followed by the same letters are equal according to Fisher's Protected LSD (α=0.05).



Effects of Fungicide, Nitrogen and Trinexapac-ethyl Applications

In both trials, Heritage TL applied at 1 fl oz with 0.5 lb calcium nitrate or with calcium nitrate and 0.125 fl oz Primo MAXX resulted in less disease than the check or Heritage applied alone. (Table 6, Figure 11). Previous studies had suggested that low rate applications of Heritage were not completely effective, so the inability of one application to provide control was not unexpected. Disease pressure decreased by 23 May, but overall, Heriage applied with nitrogen or nitrogen plus Primo MAXX resulted in less diease fro the 4-week evaluation period.

Table 6. Effects of Fungicide, Nitrogen and Trinexapac-ethyl Applications

	Disease severity (0 to 10)*									
	Green 2					Green 6				
Treatment and rate per 1,000 sq ft	25 Apr	02 May	09 May	16 May	23 May	25 Apr	02 May	09 May	16 May	23 May
water	5.0	7.0 a	5.5 a	3.8 a	2.3 ab	4.3	6.8	6.8 a	5.0 a	3.0 a
Heritage TL 1 fl oz	5.8	7.3 a	4.0 ab	4.3 a	4.0 a	2.8	6.0	5.5 a	4.8 a	4.0 a
Heritage TL 1 fl oz plus $CaNO_3$ 0.5 lb	4.3	5.5 b	2.8 bc	3.0 ab	2.8 ab	2.5	5.3	2.0 b	1.8 b	2.3 a
Heritage TL 1 fl oz plus CaNO3 0.5 lb plus Primo MAXX 0.125 fl oz	4.8	5.3 b	1.8 c	2.0 b	1.5 b	3.0	6.0	3.3 b	2.0 b	2.3 a

* Plots rated on a 1 to 10 scale for disease severity, values reflect the mean of 4 replicated plots. Means followed by the same letters are equal according to Fisher's Protected LSD (α=0.05).

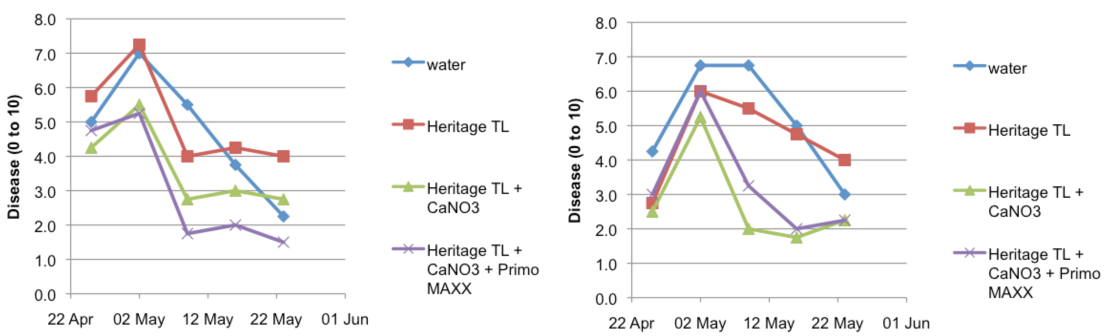


Figure 11. Effects of Fungicide, Nitrogen and Trinexapac-ethyl Applications

Population and Molecular Analyses of *Waitea circinata* var. *circinata*

Multiple isolates were collected from experiments conducted at Torrey Pines Golf Course and stored for analysis. Additional Waitea-like fungi from creeping bentgrass (Idaho) and kikuyugrass were collected in 2008 through the disease diagnosis lab.

No molecular analyses using AFLP or to characterization of fungicide resistance were completed in 2008, but the work will continue throughout 2009.

E. Current Research Plans and Changes to Proposed Work

Results from 2008 indicate a clear response of brown ring patch to certain fungicides and nitrogen fertility. Due to the availability of greens space in 2008, there was a greater focus on field experiments than laboratory work. Although some field experiments should be replicated in 2009, including some follow up work on the effect of soil surfactants and some fungicides, the focus of 2009 work should be in the analysis of *Waitea circinata* var. *circinata* population structure and the molecular basis of fungicide resistance.

F. Literature Cited

- Burpee, L. L. and Martin, S. B. 1992. Biology of *Rhizoctonia* species associated with turfgrass. Plant Disease 76:112-117.
- Chen, C. M., Douhan, G. W. and Wong, F. P. First Report of *Waitea circinata* var. *circinata* Causing Brown Ring Patch on *Poa trivialis* in California. Plant Disease 91:1687
- Couch, H. B. 1995. Diseases of Turfgrass, 3rd ed. Krieger Publishing, Malabar, Florida.
- Cubeta, M. A and Vilgalys, R. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology 87:480-484.
- de la Cerda, K. A., Douhan, G. W., and Wong, F. P. 2006. Discovery and Characterization of *Waitea circinata* var. *circinata* affecting Annual Bluegrass from the western United States. Plant Disease 91:791-797.
- King, R. W., Blundell, C., Evans, L. T., Mander, L. N., and Wood, J. T. 1997. Modified gibberellins retard growth of cool-season turfgrasses. Crop Science. 37:1878-1883.
- Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Annu. Rev. Phytopathol. 34:457-477.
- Milgroom, M. G. and Peever, T. L. 2003. Population biology of plant pathogens. Plant Disease 87: 608-617
- Rios, J. C., de la Cerda, K. A., and Wong, F. P. 2006. In vitro sensitivity of *Waitea circinata* var *circinata* to benzimidazole, carboxin, dicarboximide, QoI and SI-fungicides. Phytopathology 96:S97
- Toda, T., Mushika, T., Hayakawa, T., Tanaka, A., Tani, T., and Hyakumachi, M. 2005. Brown ring patch: A new disease on bentgrass caused by *Waitea circinata* var. *circinata*. Plant Disease 89:536-542.
- Wong, F. P. and Kaminski, J. E. 2007. A new *Rhizoctonia* disease of bluegrass putting greens. Golf Course Management 75(9):98-103.

Appendix A: Materials Under Trial

Material Used	Active Ingredient	Manufacturer
Banner MAXX	propiconazole	Syngenta
Chipco 26GT	iprodione	Bayer
Concert 4.3SE	chlorothalonil + propiconazole	Syngenta
CX-09 45WP	NA	Cleary Chemical
Daconil Ultrex	chlorothalonil	Syngenta
Disarm	fluoxastrobin	Arysta
Emerald	boscalid	BASF
Endorse	polyoxin-D	Cleary
Headway	azoxystrobin + propiconazole	Syngenta
Heritage	azoxystrobin	Syngenta
Heritage TL	azoxystrobin	Syngenta
Humabalance	Bacillus app.	3Tier
Insignia	pyraclostrobin	BASF
Instrata	chlorothalonil + fludioxonil + propiconazole	Syngenta
Medallion	fludioxonil	Syngenta
PBI Exp 1	NA	PBI Gordon
PBI Exp 2	NA	PBI Gordon
Primo MAXX	trinexapac-ethyl	Syngenta
ProStar	flutolanil	Bayer
Renown	azoxystrobin + chlorothalonil	Syngenta
Revolution	modified akyl polyol	Aquatrols
Rhapsody	Bacillus spp.	Agraquest
Tartan	bayleton + trifoxystrobin	Bayer
Tourney	metconazole	Valent
Trinity	triticonazole	BASF
Triton Flo	triticonazole	Bayer