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Confirmation of Sclerotinia homoeocarpa resistance to propiconazole fungicide through field and laboratory evaluation

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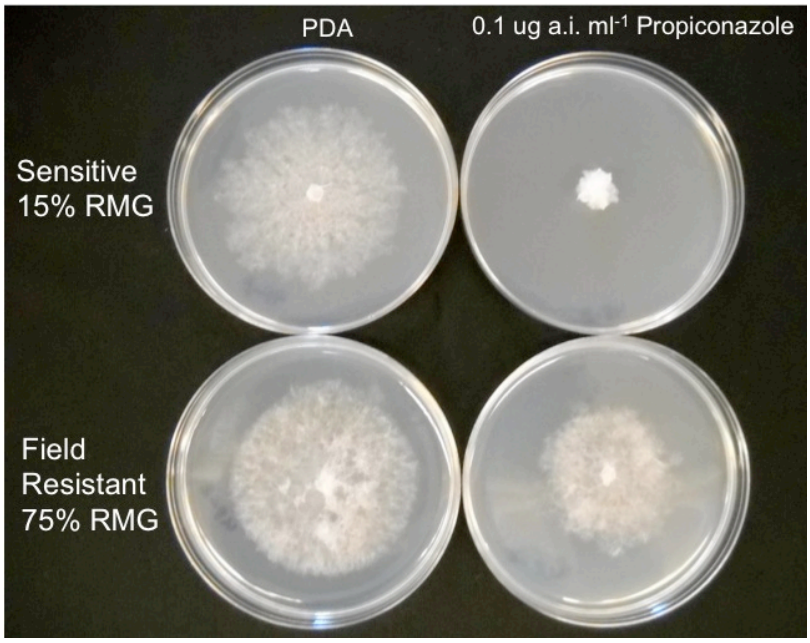
Final Report



Introduction

Dollar spot, caused by the ascomycete fungus *Sclerotinia homoeocarpa* F.T. Bennett, is a major turfgrass disease causing significant damage to turfgrass swards from May to October (9). Cultural practices do not provide adequate *S. homoeocarpa* control and multiple fungicide applications are made each year to maintain acceptable turf quality (9). Systemic fungicides can provide residual control for 14-28 days and are an extremely important tool for dollar spot control. However, frequent fungicide applications on golf courses have led to the selection of *S. homoeocarpa* isolates resistant to benzimidazole and dicarboximide fungicide classes, and with reduced sensitivity to the sterol demethylation inhibitor (DMI) fungicide class (1,2,4). Putman et al. recently confirmed that *S. homoeocarpa* isolates collected from golf courses in New England exhibited decreased sensitivity to DMI fungicides and the association between *in vitro* sensitivity and field efficacy remains unclear (8). Many studies have reported either a reduction in field efficacy or reduced *in vitro* sensitivity to the DMI class (3,4,5,6,8). However, the level of reduced *in vitro* sensitivity that correlates to practical field resistance has yet to be determined. This work sought to determine the association between *in vitro* propiconazole sensitivity and field efficacy at five different locations with differing DMI sensitivity levels.

Objectives

1. Determine propiconazole field efficacy on one baseline population and four DMI-exposed populations.
2. Quantify *in vitro* propiconazole sensitivity of the five populations before, 7 days after treatment and 21 days after propiconazole application.
3. Determine the association between *in vitro* propiconazole sensitivity and reduced field efficacy

Materials and Methods

1. Field Efficacy Testing

Four golf courses and the Joseph Troll Turf Research Facility (JTRF) were chosen and represented a range of ages and five-year DMI spray histories (Table 1). The Joseph Troll Turf Research Facility (JTRF) area examined in this study has not been exposed to DMI fungicides and represented the baseline population. The respective five-year DMI application histories of Hartford Golf Club (HGC), Hickory Ridge Country Club (HRCC), Shuttle Meadow Country Club (SMCC) and Wintonbury Golf Club (WBGC) are listed in Table 1. All five sites consisted of a mixture of annual bluegrass and creeping bentgrass and were mowed approximately 3 times per week at a typical fairway height of approximately 0.5 in.

Experimental plots were set up in a randomized complete block design with four replications. Individual plot size measured 3 ft × 6 ft with a 1-ft buffer strips between each plot for HRCC and JTRF. Buffer strips were not included in the plot design at HGC, SMCC and WBGC due to plot size restrictions. The following treatments were applied at all sites: propiconazole (Banner MAXX 1.3ME, Syngenta Crop Protection, Greensboro, NC) applied at 1.0, 2.0 3.0 and 4.0 oz/1,000 ft^2, chlorothalonil (Daconil Ultrex 82.5WDG, Syngenta Crop Protection, Greensboro, NC) applied at 3.25 oz/1,000 ft^2 and an untreated control. The 1.0 and 2.0 oz/1,000 ft^2 propiconazole rates are the low and high-labeled rates, respectively. The multi-site fungicide chlorothalonil was included because resistance has not been reported to this active

ingredient. Chlorothalonil is a contact fungicide with the suggested curative application interval of 14 days; therefore, some level of disease was expected 14 days after treatment. Applications were made on 21-day intervals for all the treatments at all five locations.

Fungicides were applied at a nozzle pressure of 40 psi using a CO_2 pressurized boom sprayer equipped with two XR Teejet 8004VS nozzles. All fungicides were agitated by hand and applied at the equivalent of 81.5 ml m^{-2}. Disease severity ratings were taken approximately every 7 days by counting individual dollar spot infection centers per plot, beginning on the date of the first fungicide application until 21 days after the last fungicide application was made. Due to differences in disease pressure, field efficacy testing began at differing dates among locations and the number of total applications for each year is listed in Table 2. In order to reduce the volume of data, the results will be presented as relative control percentage (RC %). Data is available upon request from each individual location. The area under the disease progress curve (AUDPC) was calculated for the number of infection centers at each location for both years using the formula $\Sigma[(y_i + y_{i+1})/2](t_{i+1} - t_i)$, where i = 1, 2, 3, …, n-1 and y_i is the amount of disease (number of infection centers) at the time t_i (days) of the i^{th} rating. AUDPC values were converted into RC% percentage with the following formula: [(untreated - fungicide treatment)/untreated] x 100 = RC%. Relative control % data were analyzed to determine the effect of location and treatment for each year separately using analysis of variance in PROC GLM (SAS v. 9.1.3, SAS Institute, Cary, NC). The location x treatment interaction was of most interest and was sliced by treatment. If significant differences existed among locations within treatment, Tukey's HSD test was used for RC% mean separation (P = 0.05).

2. Isolate Collection and In Vitro Propiconazole Sensitivity

The initial sampling was conducted when sufficient dollar spot infection centers (approximately ten infections per plot) were observed in 2009 and 2010. Ten samples were taken from different individual infection centers in each treatment plot by selecting individual leaf blades with bleached hourglass lesions. Sampling of *S. homoeocarpa* isolates were performed three times in 2009 and 2010 as follows: 1) prior to the first fungicide treatment, 2) 7 days after treatment (7-DAT) and 3) approximately 21 days after the final fungicide treatment. The 7-DAT samples were from dollar spot infection centers with active mycelia or recently developed lesions at all locations in 2009 and 2010 except JTRF due to the absence of dollar spot infection centers on all propiconazole treated plots.

Fungal isolation followed the procedures described in Jo et al. (5). *In vitro* fungicide sensitivity assays were conducted after *S. homoeocarpa* isolates had grown in pure culture for 2 to 3 days. Five mm agar plugs were transferred from actively growing mycelia of the pure *S. homoeocarpa* cultures to the center PDA Petri plates amended with the propiconazole discriminatory concentration (0.1 µg a.i. ml^{-1}) and non-amended PDA Petri plates using a sterile 5-mm cork borer and spatula (21). Petri plates were kept for 48 hours at 25°C. Forty-eight hours after transfer, three radial points approximately 120° apart on the circumference of actively growing *S. homoeocarpa* colonies were measured from the edge of the transferred agar plug using digital calipers (Mahr 16EX, Göttingen, Germany). The average radial growth on propiconazole-amended PDA was divided by the average non-amended mycelial radial growth and multiplied by 100 to give the relative mycelial growth percentage (RMG%).

Analysis of variance was conducted on RMG% for the main effects location and treatment using PROC GLM (SAS v. 9.1.3, SAS Institute, Cary, NC). Each sample time was analyzed separately. Mean RMG% of main effects or main effect interactions were separated using Tukey's HSD test (P = 0.05). Histograms describing *S. homoeocarpa* population distribution were constructed for HGC and HRCC to show differences in isolate distribution

3. Association Between In vitro Sensitivity and Field Efficacy

Linear regression analysis was used to determine if there was a relationship between mean RMG% of *S. homoeocarpa* isolates collected during the final sample date in all five locations and mean RC% at the respective locations in 2009 and 2010 using PROC REG (SAS v. 9.1.3, SAS Institute, Cary, NC). Relative control percentage values from each replication were regressed against mean RMG percentage values for each replication. Regression analysis was performed on chlorothalonil (3.25 oz/1,000 ft^2) and all propiconazole treatments (1.0, 2.0, 3.0 and 4.0 oz/1,000 ft^2) separately. Coefficients of determination between the RC% and RMG% values were calculated

Results

2009 and 2010 Field Efficacy

The early summer months of 2009 were unusually cool and dollar spot severity was low in June and July. Conditions became more favorable in August and resulted in higher disease incidence among all locations. The 2010 summer months (June-August) were unusually hot and dry. These conditions resulted in lower dollar spot severity in 2010 than 2009 for all locations (except HRCC).

Analysis of variance determined that relative control percentage (RC%) was significantly different for the main effects location (P = 0.001), treatment (P <0.001) and location x treatment interaction (P <0.001) in 2009. The location x treatment interaction was sliced by treatment and determined significant differences in RC% among location for all propiconazole treatments (1.0, 2.0 3.0 and 4.0 oz/1,000 ft^2), but not chlorothalonil (3.25 oz/1,000 ft^2),) (Table 2). Relative control % was significantly higher at JTRF within the 1.0, 2.0 and 3.0 oz/1,000 ft^2 propiconazole treatments for all locations except SMCC (2.0 and 3.0 oz/1,000 ft^2 propiconazole treatments). The 1.0 and 2.0 oz/1,000 ft^2 propiconazole treatments represent the low and high label rate of propiconazole; therefore, significant reductions in field efficacy among the four exposed locations represent practical DMI field resistance. At the 4.0 oz/1,000 ft^2 propiconazole rate a significant difference in RC% to the baseline site (JTRF) was only observed at HGC (Table 2).

Analysis of variance determined RC% was significantly different for the main effects location (P = 0.002), treatment (P = 0.001) and location x treatment interaction (P = 0.004) in 2010. The location x treatment interaction sliced by treatment determined significant differences in RC% among locations for all propiconazole and chlorothalonil treatments (Table 2). Relative control % observed at JTRF was significantly higher than all other locations, except for SMCC at the 1.0 and 2.0 oz/1,000 ft^2 propiconazole rates (Table 2). Relative control % at HGC and HRCC was significantly lower than JTRF within the 3.0 and 4.0 oz/1,000 ft^2 propiconazole treatments (Table 2). Relative control % within the chlorothalonil treatment was significantly higher at JTRF (91.0%) than at HGC (64.0%), HRCC (49.8%) and WBGC (62.4%), but

not SMCC (71.7%) in Table 2. In 2009 and 2010, a dose response to propiconazole was observed among all rates. As propiconazole rate increased, RC % also increased, however, the baseline location (JTRF) was always numerically higher than the four locations with prior propiconazole exposure.

2009 and 2010 *In Vitro* Propiconazole Sensitivity

Analysis of variance of *in vitro* propiconazole sensitivity determined location was significantly different ($P < 0.001$), but not treatment ($P = 0.309$) or the location x treatment ($P = 0.940$) for the initial sample in 2009. Figure 1 illustrates the different *S. homoeocarpa* population distributions present at the beginning of the study. Mean RMG% of *S. homoeocarpa* isolates collected was lowest at JTRF (18.3%) and significantly higher RMG% means were observed at both HGC (43.8%) and HRCC (45.7%) at the 2009 initial sample. *Sclerotinia homoeocarpa* isolates from HGC and HRCC displayed bimodal distributions in which two subpopulations were present. One subpopulation consisted of isolates ranging from 10 to 40% RMG and the other subpopulation was ranged from 50 to 100% RMG (Fig. 1). SMCC (63.9%) and WBGC (64.1%) *S. homoeocarpa* isolates constituted the highest mean RMG% and displayed unimodal population distributions at the 2009 initial sample time.

Sclerotinia homoeocarpa isolates were collected from HGC, HRCC, SMCC and WBGC 7-DAT in 2009, but not from JTRF since dollar spot infection centers were not observed within the propiconazole-treated plots. Location ($P < 0.001$), treatment ($P = 0.001$), and the interaction ($P < 0.001$), were all significantly different within the 7-DAT sample time. The location x treatment interaction sliced by location determined significant differences in mean RMG% among treatments within HGC and HRCC (Table 3). Mean RMG% of isolates collected from propiconazole treatments 7-DAT at HGC were significantly higher than isolates from the untreated and chlorothalonil treatments except for the 3.0 oz/1,000ft^2 propiconazole treatment, which were statistically similar to chlorothalonil (Table 3). Within HRCC, mean RMG% of *S. homoeocarpa* isolates collected from the 1.0, 2.0 and 3.0 oz/1,000ft^2 propiconazole treatments were significantly higher than isolates collected from the untreated (Table 3). Due to low dollar spot severity, an insufficient number of *S. homoeocarpa* isolates were collected from the chlorothalonil and 3.0 oz/1,000ft^2 propiconazole treatments at HRCC.

Location ($P < 0.001$), treatment ($P < 0.001$), and location x treatment interaction ($P = 0.014$), were all significantly different for the final sample time in 2009. The location x treatment interaction sliced by location determined significant differences in mean RMG% among treatments only for HRCC (Table 3). *Sclerotinia homoeocarpa* isolates collected from all 1.0, 2.0 and 3.0 oz/1,000ft^2 propiconazole treatments exhibited higher mean RMG% than ones from chlorothalonil and untreated treatments in the 2009 final sample (Table 3). In contrast, mean RMG% of isolates collected from HGC, JTRF, SMCC and WBGC were similar among treatments within each respective site.

In 2010 analysis of variance determined location, treatment and the location x treatment interaction was significantly different ($P < 0.001$) for all sample dates (initial, 7-DAT and final). The location x treatment interaction sliced by location determined significant differences in mean RMG% for all sample dates among treatments only for HRCC (Table 3). All propiconazole rates were numerically higher in RMG% than the untreated and chlorothalonil treatments, however, only the 1.0 and 4.0 oz/1,000ft^2 propiconazole treatments were

significantly higher for the 2010 initial sample time (Table 3). All propiconazole treatments from HRCC contained *S. homoeocarpa* isolates with significantly higher mean RMG% than isolates collected from the untreated, but not the chlorothalonil treatment for the 2010 7-DAT sample time (Table 3). All propiconazole treatments from HRCC contained *S. homoeocarpa* isolates with significantly higher mean RMG% than isolates collected from the untreated and chlorothalonil treatment for the 2010 final sample time (Table 3).

Histograms of all sample dates were presented to illustrate changes in the bimodal population distribution of *S. homoeocarpa* isolates collected from HGC and HRCC (Fig. 2) since both populations showed non-normal RMG distributions. The 1.0 oz/1,000 ft^2 rate was the only propiconazole treatment included since all propiconazole rates had a similar effect on mean RMG% (Table 3). The 2009 HGC 7-DAT *S. homoeocarpa* population shifted to a unimodal distribution within the 1.0 oz/1,000 ft^2 propiconazole treatment, whereas the untreated and chlorothalonil treatments remained bimodal in distribution (Fig. 2B).

The HRCC population distribution was similar to HGC for the 1.0 oz/1,000 ft^2 propiconazole treatment throughout all the sample dates; however, both the untreated and chlorothalonil treatments remained bimodal (Fig. 2D-F). *Sclerotinia homoeocarpa* isolates sampled from the chlorothalonil (except the 7-DAT sample time due to low sample number) and untreated treatments at HRCC remained bimodal in population distribution (Fig. 2D-F).

Association between *in vitro* sensitivity and field efficacy

Mean RC% was significantly correlated with mean RMG% on the final sample dates of all five locations for all propiconazole treatments, but not chlorothalonil in 2009 (Fig. 3A-E). Mean RC% decreased as RMG% increased in all propiconazole treatments. In contrast, mean RC% was significantly associated with mean RMG% on the final sample dates for all propiconazole treatments including chlorothalonil in 2010 (Fig. 4F-J). Overall, a higher correlation was detected for most propiconazole rates than for chlorothalonil in both years of this study.

Summary

The operational definition of practical fungicide resistance states: “isolates of pathogens are resistant if their frequencies have increased substantially at sites with poor fungicide performance” (7). The results in this study confirmed (i) reduced propiconazole efficacy existed within the four golf course populations (HGC, HRCC, SMCC, WBGC) with prior DMI fungicide exposure, (ii) the four exposed *S. homoeocarpa* populations were significantly less sensitive to propiconazole *in vitro* than the baseline population (JTRF), and (iii) increased RMG% values on the propiconazole discriminatory concentration 0.1 µg a.i. ml^{-1} were significantly correlated to decreased relative control values. The results presented herein indicate that RMG% values of *S. homoeocarpa* isolates within the range of 50 to 100% on the propiconazole discriminatory concentration 0.1µg a.i. ml^{-1} represent levels that correlate to practical fungicide resistance.

Previous research on *S. homoeocarpa* in turfgrass has correlated reduced *in vitro* DMI sensitivity with reduced DMI efficacy through inoculated field and greenhouse trials; however, the association between native *S. homoeocarpa* populations causing practical field resistance and *in vitro* sensitivity has remained unclear (6,8). The determination of a threshold or level of *in vitro* sensitivity that results in efficacy reductions was yet to be established prior to this work and is critical for making accurate recommendations for DMI fungicides. Moreover, Putman et

al. determined reduced *in vitro* propiconazole sensitivity was present in 18 of the 20 *S. homoeocarpa* populations examined in the New England region; therefore, the ability to determine the level of insensitivity that corresponds to practical fungicide failure is of utmost importance to this region (8).

Our field trials conducted at the HGC, HRCC, SMCC and WBGC revealed reductions in field efficacy for the 1.0 and 2.0 oz/1,000 ft^2 propiconazole treatments (low and high labels rates, respectively) in comparison to JTRF in both years of the study (Table 2). *Sclerotinia homoeocarpa* isolates collected from HGC, HRCC, SMCC and WBGC were significantly more insensitive to propiconazole *in vitro* than ones from JTRF (Fig. 1). The presence of dollar spot infection centers seven days after propiconazole treatment at HGC, HRCC, SMCC and WBGC enabled quantification of the *in vitro* propiconazole sensitivity of *S. homoeocarpa* isolates causing reduced efficacy. Mean RMG values of *S. homoeocarpa* isolates sampled 7-DAT from all propiconazole treatments at HGC, HRCC, SMCC and WBGC were consistently above 50% RMG for both years of the study and provide evidence that reduced field efficacy is caused by isolates exceeding 50% RMG (Table 3). Moreover, the repeated observation of isolates with > 50% RMG over four geographically separated locations adds additional merit to 50% RMG serving as a potential threshold to determine practical field resistance to propiconazole in S. homoeocarpa. In contrast, *S. homoeocarpa* isolates were not collected from JTRF 7-DAT, because dollar spot infection centers were not observed within propiconazole treatments 7-DAT in both years of the study. Furthermore, mean RMG values did not exceed 50% during any sample time in 2009 or 2010 at JTRF which supports the theory that isolates below 50% RMG do not cause practical DMI field resistance. Therefore, these results indicate that *S. homoeocarpa* isolates with RMG values > 50% are capable of causing significant field efficacy reductions or practical DMI field resistance.

Field efficacy results revealed a strong relationship between *in vitro* sensitivity and relative control values (Fig. 3). The baseline population JTRF was adequately controlled by propiconazole during both years of field efficacy testing and was significantly more sensitive *in vitro* than the four locations with prior DMI exposure. Although HGC, HRCC, SMCC and WBGC all displayed mean RMG values > 50%, our results revealed that the level of efficacy reduction varied among locations. Some variation can be attributed to lack of favorable environmental conditions that existed in the 2010 season. For instance, hot and dry conditions reduce guttation fluids (dew formation) in turfgrass and in turn reduce leaf moisture. Leaf moisture created by guttation fluids has been found to play a significant role in the infection process because the sugars and amino acids contained in guttation fluid (dew) provide a nutrition source for *S. homoeocarpa* prior to infection. Taking into account environmental variation, relative control values did show that reduced propiconazole efficacy among HGC, HRCC, SMCC and WBGC was least severe at SMCC in both years (Table 2). The SMCC RMG values from propiconazole treatments were also lower in all sample times except for the 2009 initial (Table 3).

The results from this study have effectively determined the range of *in vitro* sensitivity values of *S. homoeocarpa* populations that cause practical field resistance to propiconazole. Results revealed that *S. homoeocarpa* populations exhibiting RMG values > 50% on the propiconazole discriminatory concentration 0.1 µg a.i. ml^{-1} were capable of causing practical field resistance to propiconazole. This information has been extremely helpful in detecting *S.*

homoeocarpa populations that can cause practical field resistance and has already been implemented within the Fungicide Resistance Assays for New England Dollar Spot Database Development study funded by NERTF. Future studies should focus on developing management strategies for golf superintendents to manage populations of *S. homoeocarpa* that are found to exhibit practical field resistance to DMI fungicides and determination of the molecular mechanisms responsible for practical field resistance to DMI fungicides

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Table 1. Location, age and DMI fungicide application history of field sites.

Golf course	Location	Year established	Average DMI application/year[z]
JTRF	South Deerfield, MA	1995	0.0
SMCC	Kensington, CT	1917	1.5
HRCC	Hadley, MA	1969	1.6
HGC	Hartford, CT	1965	3.4
WBGC	Bloomfield, CT	2003	5.0

[z] Number of DMI applications was averaged over a 5-year period. DMI applications include fenarimol, metconazole, myclobutanil, propiconazole, tebuconazole, triadimefon and triticonazole.

Table 2. Interaction between location and treatment for relative control percentage of dollar spot in 2009 and 2010.

		Relative Control Percentage of Dollar Spot[t]				
Year	Location	PP-1.0[u]	PP-2.0 oz	PP-3.0 oz	PP-4.0 oz	CH-3.25 oz
2009[v]	JTRF	95.4 a[w]	97.5 a	97.6 a	98.2 a	71.0
	SMCC	64.3 bc	87.4 ab	92.7 ab	95.8 a	70.0
	HRCC	75.2 b	80.1 b	83.3 b	88.9 a	75.8
	HGC	52.8 c	68.0 c	64.7 c	76.3 b	75.0
	WBGC	66.6 b	74.2 c	83.6 b	89.9 a	68.4
	Loc x Trt[x]	***[y]	***	***	***	NS
2010[z]	JTRF	95.8 a	96.6 a	97.9 a	97.8 a	91.0 a
	SMCC	73.5 ab	86.9 ab	88.5 a	90.4 a	71.7 ab
	HRCC	39.5 bc	50.5 bc	55.1 b	71.9 b	49.8 b
	HGC	48.8 bc	61.4 bc	18.9 c	59.8 b	64.0 b
	WBGC	54.8 bc	69.4 bc	74.7 ab	83.1 a	62.4 b
	Loc x Trt	***	***	***	***	***

[t] Relative control percentage (RC%) data was collected by counting individual infection centers and calculating area under the disease progress curve values for all rating dates among all treatments. Rating began on the first date of the first fungicide application and concluded 21 days after the final application. RC% was calculated with the following formula: [(untreated-fungicide treated)/untreated] x 100 = RC%.

[u] Treatment abbreviations were as follows: propiconazole = PP and chlorothalonil = CH.

[v] Total fungicide applications in 2009 were as follows: HGC (3), HRCC (2), JTRF (4), SMCC (3) and WBGC (4).

[w] Means in a column followed by the same letter are not significantly different according to Tukey's HSD test ($P < 0.05$).

[x] The location x treatment interaction was sliced by treatment and P values of the respective slices are listed.

[y] *** and NS refer to significance at $P \leq 0.001$ and not significant, respectively.

[z] Total fungicide applications in 2010 were as follows: HGC (3), HRCC (3), JTRF (3), SMCC (3) and WBGC (3).

Table 3. Interaction between location and treatment on mean relative mycelium growth percentage of *Sclerotinia homoeocarpa* isolates collected from five sites, HGC, HRCC, JTRF, SMCC, and WBGC in 2009 and 2010.

	Mean Relative Mycelium Growth Percentage					
	2009			2010		
Treatment	Initial[x]	7-DAT	Final	Initial	7-DAT	Final
	----------JTRF----------					
Untreated	18.3	---[y]	16.7	25.2	---	26.6
CH 3.25 oz	18.8	---	14.1	25.6	---	25.0
PP 1.0 oz	18.6	---	15.8	27.6	---	27.4
PP 2.0 oz	18.5	---	15.7	24.7	---	27.4
PP 3.0 oz	18.6	---	17.5	25.2	---	25.7
PP 4.0 oz	17.1	---	17.0	24.7	---	29.1
P value[z]	0.999	---	0.927	0.893	---	0.827
	----------SMCC----------					
Untreated	63.2	55.5	64.5	57.3	58.0	62.0
CH 3.25 oz	65.4	---	67.2	57.0	59.1	62.9
PP 1.0 oz	63.4	57.4	73.5	58.4	61.2	65.7
PP 2.0 oz	62.0	---	71.7	60.6	60.1	62.9
PP 3.0 oz	65.7	---	70.9	54.2	---	63.3
PP 4.0 oz	63.9	---	70.6	57.3	---	62.5
P value	0.961	0.672	0.081	0.330	0.845	0.803
	----------HRCC----------					
Untreated	50.8	62.4 b	59.8 b	52.2 b	56.7 b	57.5 b
CH 3.25 oz	44.0	---	59.1 b	50.1 b	66.1 ab	66.8 b
PP 1.0 oz	46.2	85.1 a	75.3 a	64.7 ab	75.4 a	78.8 a
PP 2.0 oz	45.5	78.9 a	75.6 a	70.4 a	81.1 a	80.1 a
PP 3.0 oz	47.0	---	76.0 a	74.9 a	84.9 a	83.2 a
PP 4.0 oz	40.6	86.4 a	73.7 ab	64.8 ab	81.5 a	80.5 a
P value	0.324	<0.001	<0.001	<0.001	<0.001	<0.001
	----------HGC----------					
Untreated	46.2	47.0 c	71.2	62.5	60.0	72.4
CH 3.25 oz	47.5	51.7 bc	76.2	64.3	62.0	72.7
PP 1.0 oz	44.2	77.4 a	77.4	61.1	63.3	77.3
PP 2.0 oz	37.8	73.4 a	73.5	65.6	67.3	74.0
PP 3.0 oz	46.9	66.3 ab	74.1	65.7	64.9	73.7
PP 4.0 oz	40.5	72.9 a	78.5	63.6	65.0	73.1
P value	0.183	<0.001	0.265	0.566	0.463	0.427
	----------WBGC----------					
Untreated	61.1	77.4	82.1	91.2	78.9	82.3
CH 3.25 oz	63.2	79.2	83.3	89.1	77.9	81.3
PP 1.0 oz	65.6	76.8	86.3	91.9	81.9	82.5
PP 2.0 oz	65.0	81.0	85.2	87.2	78.9	84.4
PP 3.0 oz	65.6	75.8	84.4	90.8	80.9	82.6
PP 4.0 oz	61.5	---	87.5	90.1	79.9	85.7
P value	0.786	0.767	0.615	0.566	0.898	0.608

[x] Sample time indicates *S. homoeocarpa* isolates were sampled prior to fungicide application at the beginning of the experiment (initial), 7 days after treatment (7-DAT), or approximately 21 days after the final fungicide applications were made (final). Within a sample time, means followed by the same letter are not significantly different according to Tukey's honest significant difference test ($P \leq 0.05$).

[y] Dash marks represent treatments in which *S. homoeocarpa* isolates were not recovered from dollar spot infection centers or dollar spot infection centers were not present at the time of sampling.

[z] The location x treatment interaction was sliced by location and *P* values of the respective slices are listed.

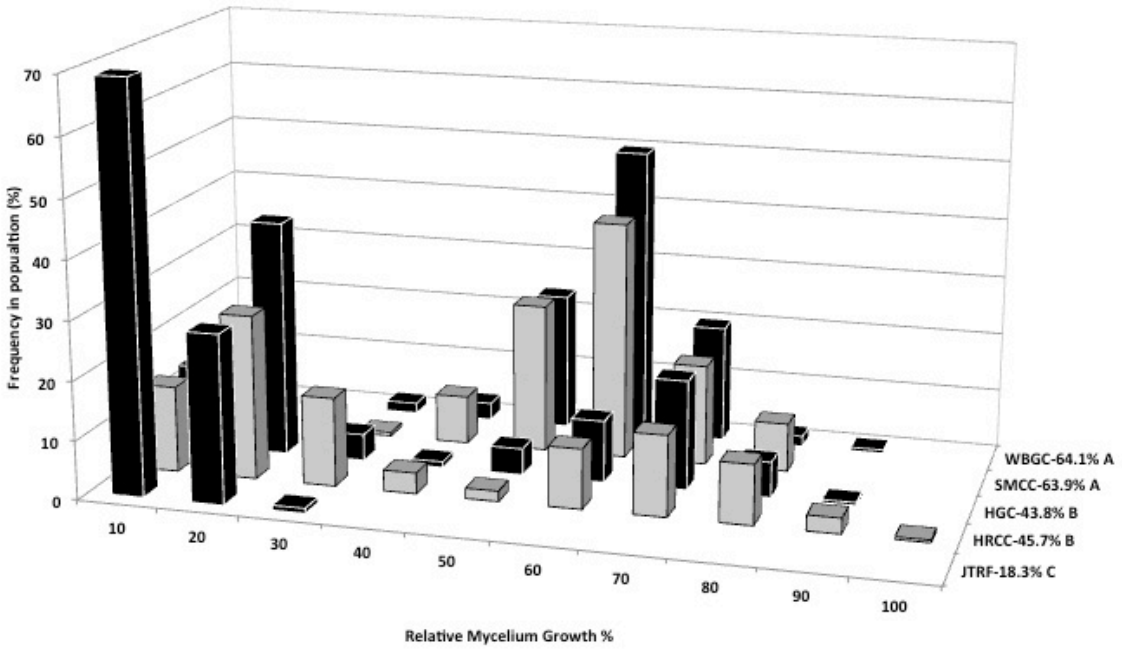

Figure 1. Frequency distributions of relative mycelium growth percentage (RMG%) of *S. homoeocarpa* isolates from 2009 initial sampling (before fungicide application) of Hickory Ridge Country Club (HRCC) and Joseph Troll Turf Research Facility (JTRF) in MA, and Hartford Golf Club (HGC), Shuttle Meadow Country Club (SMCC) and Wintonbury Hills Country Club (WBGC) in CT evaluated on the discriminatory concentration 0.1 µg a.i. ml^{-1} of propiconazole. Analysis of variance determined RMG% was different among location ($P < 0.001$). Mean relative mycelium growth is listed next to each location on the z-axis. Relative mycelium growth means followed by the same letter are not significantly different according to Tukey's honest significant difference test ($P \leq 0.05$).

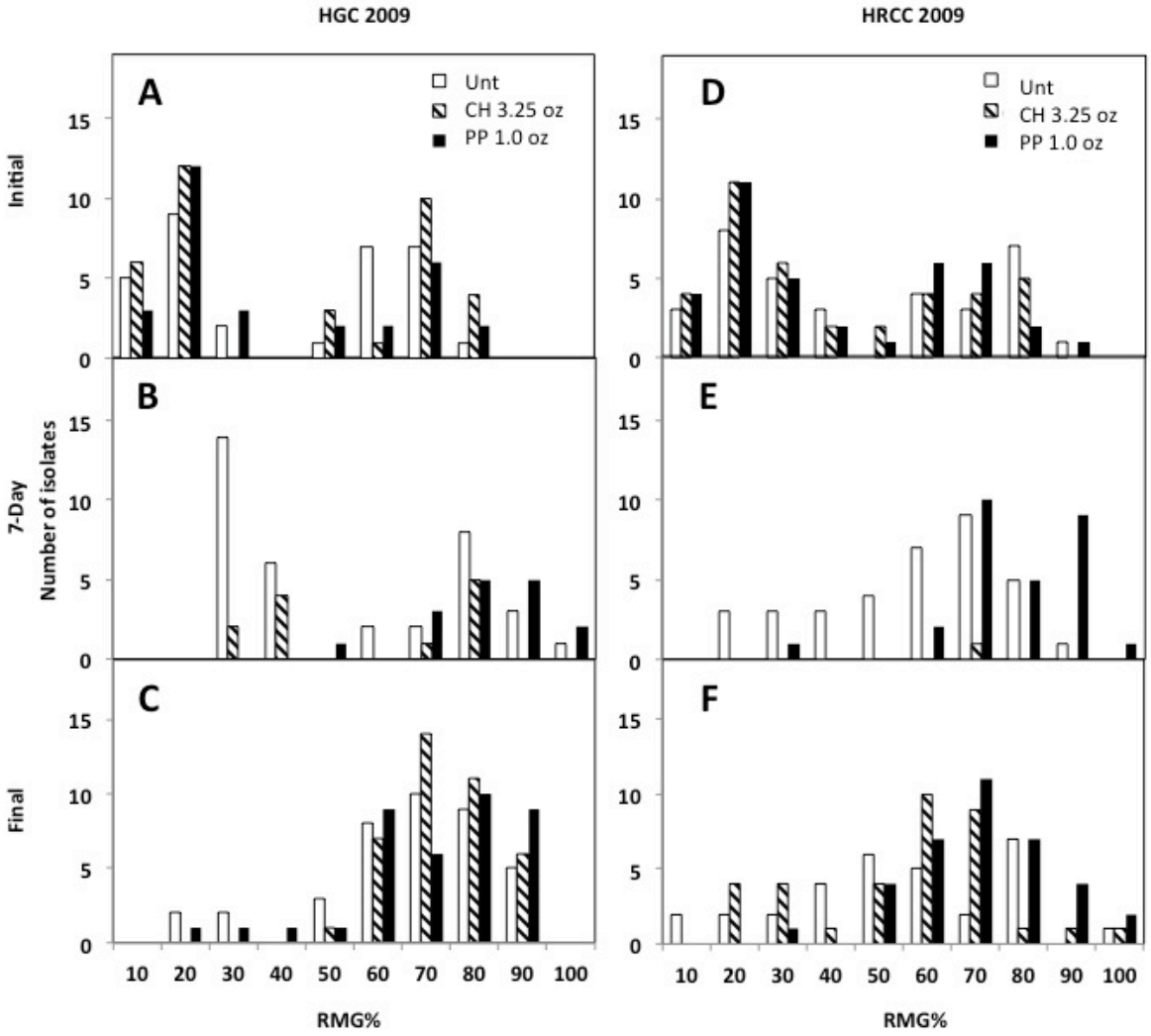

Figure 2. Frequency distribution of relative mycelium growth values (RMG %) of *S. homoeocarpa* isolates on the discriminatory concentration 0.1 µg a.i. ml^{-1} of propiconazole from Hartford Golf Club (HGC) and Hickory Ridge Country Club (HRCC). *Sclerotinia homoeocarpa* isolates were collected during the (A) 2009 initial, (B) 2009 7-DAT, (C) 2009 final sampling times from both locations. Bars represent the number of isolates collected from untreated (white), chlorothalonil 3.25 oz/1,000 ft^2 (hatched) or propiconazole 1.0 oz/1,000 ft^2 (black) treatments.

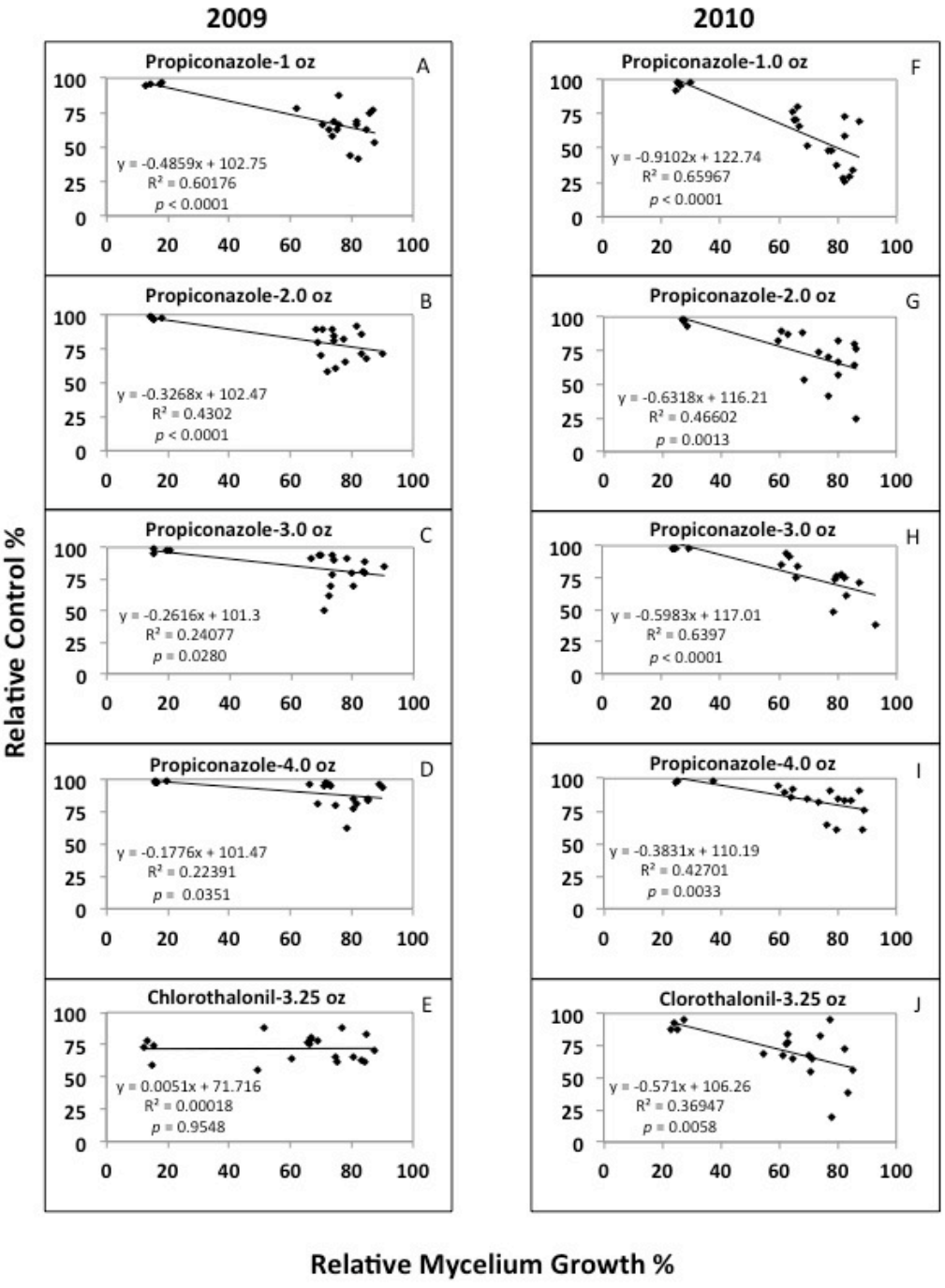

Figure 3. Relationship between mean relative mycelium growth % (RMG%) on the discriminatory concentration 0.1 µg a.i. ml^{-1} of propiconazole and mean relative control (RC%) for 1.0, 2.0, 3.0 and 4.0 oz/1,000 ft^2 propiconazole and chlorothalonil (3.25 oz/1,000 ft^2) treatments at one baseline and four DMI exposed populations on the final sample dates of 2009 and 2010.