Changes in sensitivity of *Clarireedia jacksonii* to the demethylation inhibitor fungicide propiconazole after 30 years of use

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Introduction

Clarireedia jacksonii is the causal agent of dollar spot disease of turfgrass (Fig. 1&2). Turfgrass managers rely heavily on fungicides to manage epidemics of dollar spot, and usually make several applications every year to maintain satisfactory playing conditions (Smiley et al. 2005). Fungicide resistance occurs when frequent use of the same chemical family selects for fungal genotypes which are less sensitive. This phenomenon has been detected in populations of the dollar spot pathogen (Bishop et al. 2008, Golembiewski et al. 1995, Van Den Nieuwelaar and Hsiang 2014).

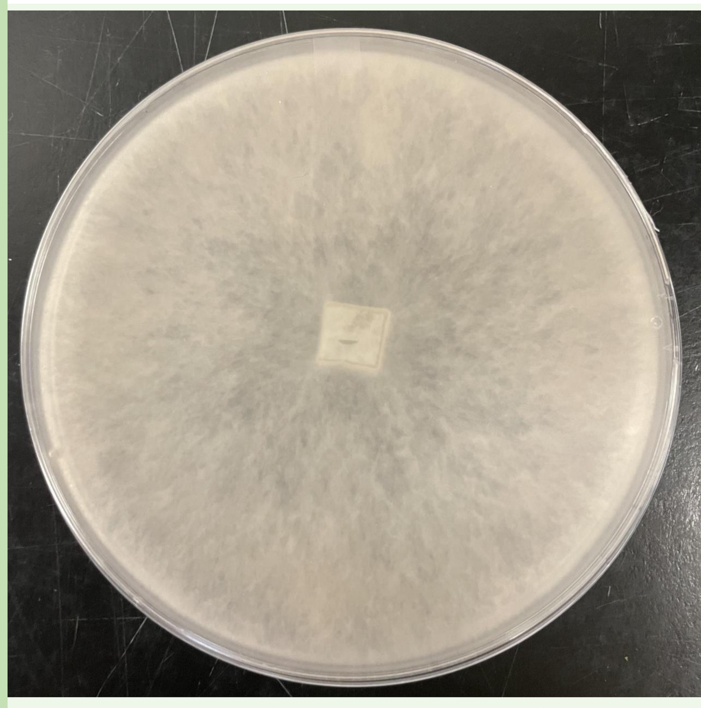


Figure 1: *C. jacksonii* on PDA after 2 days of incubation

Figure 2: Symptoms of dollar spot disease as sunken tan patches on creeping bentgrass (*Agrostis stolonifera*)

The Hsiang lab at the University of Guelph has now monitored resistance of *C. jacksonii* to the DMI fungicide propiconazole on Ontario golf courses over three decades (Fig. 3). They collected dollar spot samples (Fig. 4) from the same golf courses over this time period (Fig. 5) and assessed them for their sensitivity to propiconazole using an amended growth assay (Fig. 6). This current study aims to further assess resistance risks brought on by prolonged DMI fungicide use in Ontario.

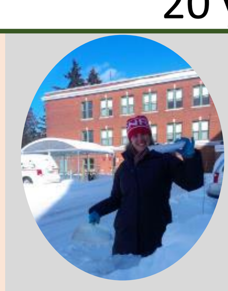
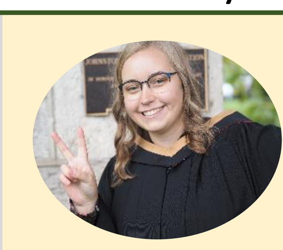
1994 Baseline study	2003 10 years	2013 20 years	2023 30 years
 Wayne Barton	 Angie Liao	 Anne-Miet Deaken	 Andrea Rether
0.007 µg/mL n = 307	**0.026 µg/mL n = 412**	**0.054 µg/mL n = 716**	**0.079 µg/mL n = 981**

Figure 3: Timeline of four studies surveying changes in propiconazole sensitivity in Ontario populations of *C. jacksonii,* showing year of study, each graduate student involved, and the resulting EC_{50} value (Hsiang et al. 1997, Hsiang et al. 2007, Van Den Nieuwelaar and Hsiang 2014).

Objectives: a) collect isolates of *C. jacksonii* from the same golf courses visited in previous studies and measure their sensitivities to propiconazole and b) estimate the number of DMI applications needed for the average EC_{50} value of a population to reach 0.1 µg/mL propiconazole.

Methods

1. Collection: In the summer of 2023, 100 dollar spot samples were collected from 12 golf courses in Ontario (1200 samples). Samples each consisting of a few diseased grass blades were collected in a grid pattern from spots at least 1 m apart, placed in microfuge tube, and transported back to the University of Guelph.

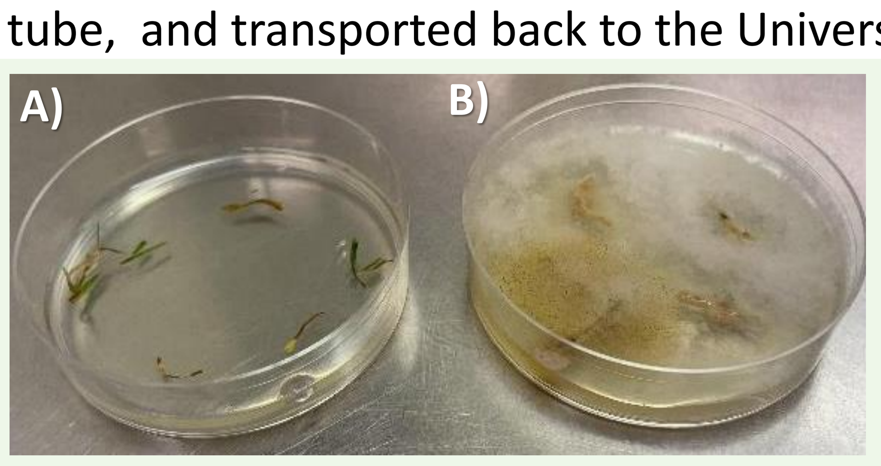


Figure 4: Diseased grass blades placed on antibiotic-amended PDA (A) and growth after 48 hours of incubation (B).

Figure 5: Ontario locations sampled during the 2023 study

2. Isolation: Individual grass blades were placed on potato dextrose agar (PDA) amended with 0.1 g/L each of tetracycline hydrochloride and streptomycin sulfate (BP910-50, Fisher Biotech, NJ, USA) and incubated for 48 hours. Mycelium resembling *C. jacksonii* were placed on non-amended PDA and incubated for 48 hours.

3. Amended agar assay: Media was prepared by pouring molten PDA amended with the fungicide of choice into 10-cm-diameter petri dishes. Solidified agar was cut using a 6-blade stencil and excess agar was removed to create separated strips (Fig. 6) Hyphal agar plugs were cut with a cork borer and placed in the center of an agar strip. Radial growth was marked at 24 and 48 hours (Fig. 7).

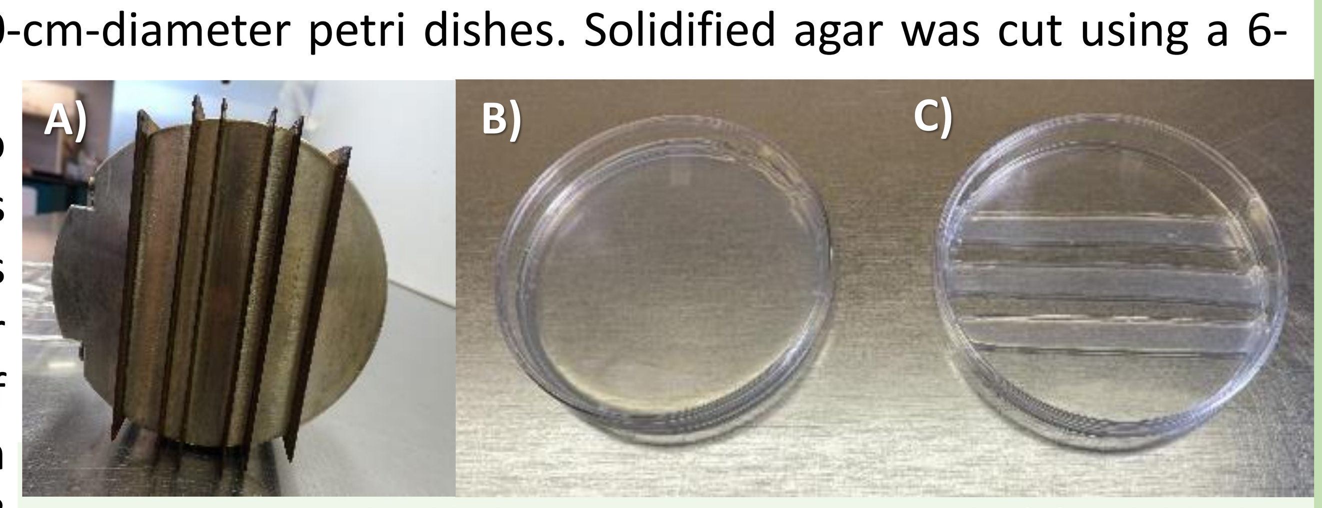
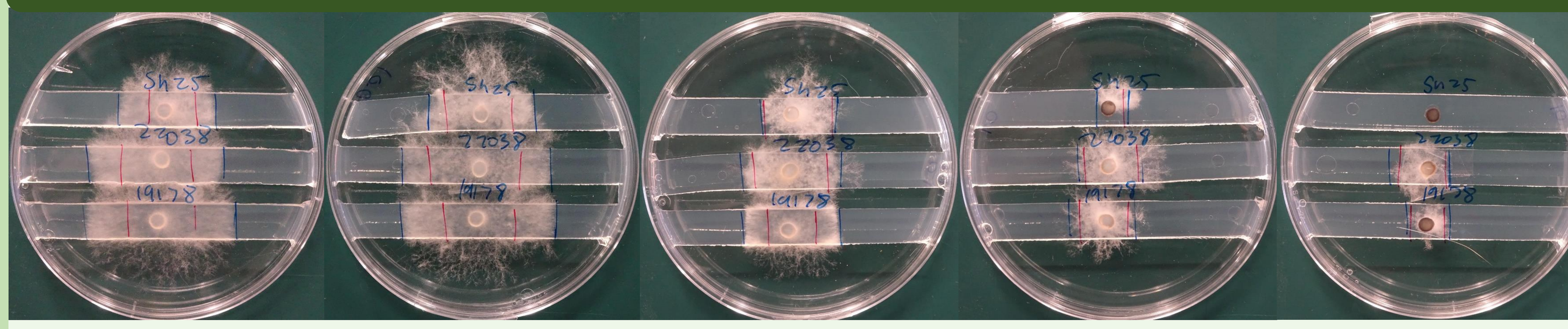


Figure 6: stencil with 6 blades used to cut strips of media (A), solidified media before (B) and after (C) cutting with the 6-blade stencil.

Methods



0 µg/ml | 0.01 µg/ml | 0.1 µg/ml | 1.0 µg/ml | 10 µg/ml

Figure 7: Radial growth of *C. jacksonii* after 48 hours of incubation on agar strips amended with propiconazole. Hyphal plugs of three isolates were placed in the center of each agar strip, and radial growth was marked at 24 and 48 hours. Concentrations of fungicide are indicated below each plate.

4. Statistical Analysis: The growth difference of 48 h – 24 h was used to calculate an EC_{50} value using SAS Proc Probit. Each isolate was assessed once. The geometric mean EC_{50} values from samples obtained from all four studies were analyzed together. If a significant effect was observed in the ANOVA (p<0.05), the geometric mean population $\log_{10}$ EC_{50} values were compared using Fisher's Protected LSD (Least Significant Difference). A regression analysis was performed to assess the relationship between the estimated number of DMI fungicide applications over the last 30 years at each location and the geometric mean EC_{50} values.

Results

Out of 1200 samples, 981 isolates were obtained. Most geometric mean EC_{50} values from sampling sites were increased since the 2013 study, and many surpassed 0.1 µg/mL (Table 1). The geometric mean EC_{50} value for all isolates collected in 2023 was 0.079 µg/mL, which is higher than the 2013 study (Fig. 8). The number of applications estimated to reach an average EC_{50} value of 0.1 µg/mL was determined to be 86 (Fig. 9).

Table 1: Geometric mean EC_{50} values (µg/mL propiconazole) and the estimated number of DMI applications made since 1994 for all *C. jacksonii* sampling sites, with location in Ontario. Means from the 1994, 2003, 2013, and 2023 studies followed by a letter in common are not significantly different.

Location	Geometric mean EC_{50} (µg/mL)				Estimated number of DMI applications made since 1994
	1994	2003	2013	2023	
Guelph 1	0.005 q	0.008 p	0.042 ijkl	0.098 de	62
St. Catharines	0.006 pq	0.020 n	0.038 klm	0.161 c	74
London	0.008 p	0.012 o		0.066 fgh	53
Windsor	0.022 n[a]	0.047 ijk	0.034 lm	0.139 c	107
Toronto	0.008 p	0.027 mn	0.039 jkl	0.053 ghij	97
Point Pelee	0.009 p	0.048 hijk	0.118 cd	0.252 b	88
Oshawa		0.007 pq		0.067 fg	57
Mississauga		0.023 n	0.041ijkl	0.067 fg	93.1
St. Thomas			0.088 def	0.373 a	102.5
Burlington			0.077 ef	0.006 pq	93.5
Guelph 2			0.020 n	0.050 ghijk	35.3
Halton Hills			0.055 ghi	0.084 ef	57

[a]suspected of having used DMI fungicides illegally, not included in mean for 1994 | <0.01 µg/mL | > 0.1 µg/mL

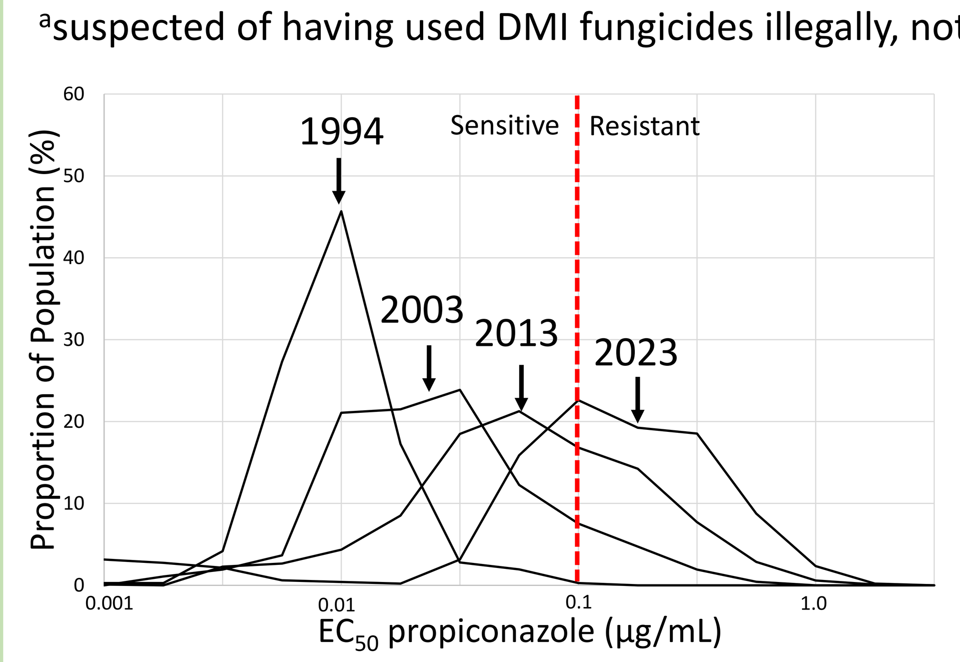


Figure 8: Distribution of isolates of *C. jacksonii* from sites in southern Ontario for each sampling year

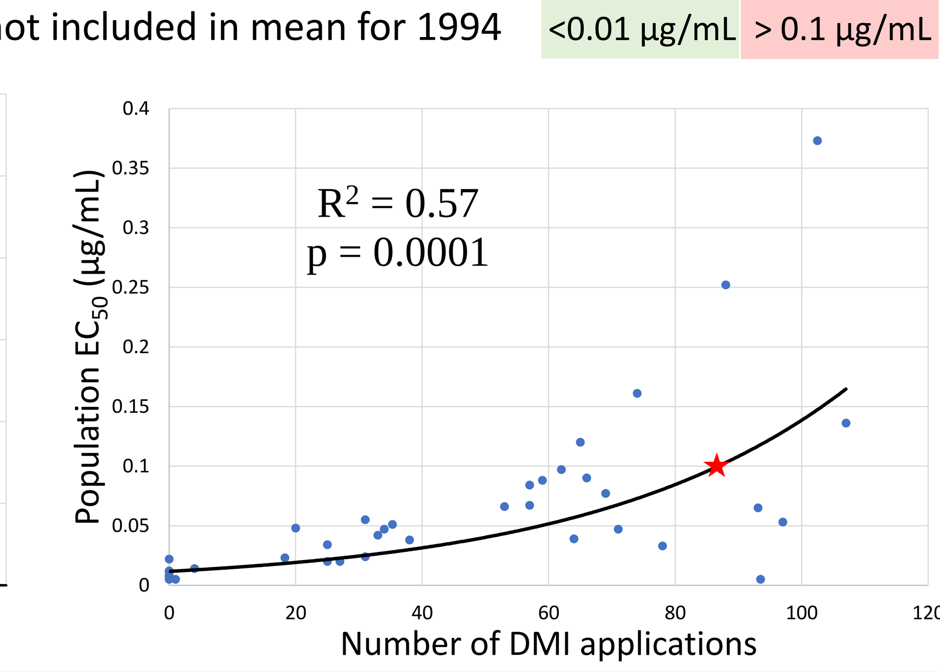


Figure 9: Relationship between the estimated number of DMI applications and geometric mean EC_{50} value

Conclusion

Population shifts above 0.1 µg/mL have been observed in Ontario in both 2013 and 2023. Turfgrass managers from some sampling sites with a geometric mean EC_{50} > 0.1 µg/mL are reporting fewer days of control than indicated by the product label for some DMI fungicides. The onset of DMI resistance makes managing dollar spot populations difficult as a population showing resistance to one DMI has reduced sensitivity to other DMIs as the number of new fungicidal active ingredients for turfgrass every year is becoming fewer, and some actives have reduced use due to government policy, so switching active ingredients to control a resistant population is becoming more difficult. These results highlight the importance of avoiding the onset of resistance to active ingredients currently available for use on turfgrass in Canada.

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References:
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