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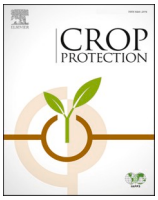

Evaluation of the sensitivity of *Podosphaera xanthii* to several fungicides for management of powdery mildew on squash in Florida

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ABSTRACT

Cucurbit powdery mildew caused by *Podosphaera xanthii* is a major challenge to cucurbit production worldwide. In the United States, the fungus has become resistant to fungicides in FRAC Groups 1, 3, U6, 7, 11, and 13, however the extent of this resistance is not known in the Florida population. Commercially labeled fungicides, thiophanate-methyl (Topsin 70WDG), cyflufenamid (Torino), flutriafol (Rhyme), quinoxyfen (Quintec), and myclobutanil (Rally 40 WSP), were selected to screen for sensitivity against locally obtained isolates under laboratory, greenhouse, and field conditions. Laboratory and greenhouse assessments of disease severity and control were made after a single application of each fungicide, while field trial assessments were made after weekly applications. Quinoxyfen, myclobutanil, cyflufenamid, and flutriafol gave adequate control of powdery mildew, while thiophanate-methyl failed to control *P. xanthii.* No formulation was successful in preventing the establishment and sporulation of *P. xanthii* at the maximum or near-maximum labeled field rate under laboratory, greenhouse, and field conditions. Further research is required to monitor the Florida population for early detection of fungicide resistance, to establish new effective fungicidal concentrations, and to optimize fungicide application regimes.

1. Introduction

The plant family Cucurbitaceae contains agriculturally important crops, including squash, pumpkin, and zucchini in *Cucurbita*; cucumber, cantaloupe, and honeydew in *Cucumis*; and watermelon in *Citrullus*. Cucurbit powdery mildew is a disease caused by two plant pathogens, *Golovinomyces cichoracearum* and *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) and is a recurrent problem in field and greenhouse-grown cucurbits in most regions of the world (Perez-Garcia et al., 2009; Cerkauskas and Ferguson 2014). Both are obligate pathogens, but *P. xanthii* is particularly devastating on Florida cucurbit crops (Maia and Gevens 2009) and predominates over *G. cichoracearum* in the US (Xiang et al., 2020; Maia and Gevens 2009). Florida squash production accounts for 18% of the total US production in 2016 and was valued at $30.1 million (NASS 2018).

Podosphaera xanthii forms visible whitish or silvery powder-like colonies on the upper and lower surfaces of leaves, stems, and petioles. Severe infection leads to defoliation and reduced yield. Yield reductions occur due to reduced fruit production and/or poor fruit quality due to sun scalding and premature or uneven ripening (Lebeda et al., 2010). Severe infections on fruit are rare but can occur (Kousik et al., 2011). Powdery mildew outbreaks in Florida have caused up to 34% yield loss in watermelons (Kousik et al., 2011; Maia and Gevens, 2009), and affect approximately 70% of squash acreage (Nuñez-Palenius et al., 2006).

Controlling powdery mildew requires an integrated pest management (IPM) approach involving cultural practices such as sanitation (removing volunteer plants and weed hosts), using genetically tolerant (resistant) varieties, and the use of fungicides and biorationals to limit losses. Genetically tolerant or resistant varieties are not resistant to all races of powdery mildew (Nuñez-Palenius et al., 2006; Cornell University 2021). Twenty-eight races have been identified worldwide (McCreight 2006) based on their ability to overcome resistance of previously resistant cultivars of melons and other cucurbits (Cohen et al., 2004). In the United States, the designation race 1 was assigned to a strain of *P. xanthii* that was not capable of causing disease on the powdery resistant cultivar, PMR45 (https://powderymildew.ucr.edu/races/) (Jagger 1926; Jagger and Scott 1937) and the strain that subsequently overcame resistance in PMR45 was named race 2 (Jagger et al., 1938). Race 3 was found in Texas in 1976 (Thomas 1978) and race S, originally

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from Japan, was found in California in 2005 (McCreight and Coffey 2011). Other *P. xanthii* races found in the US include race 5 and variants of race 1, 2, 3, 4 and S (McCreight 2006; Coffey et al., 2006; McCreight et al., 2012). Evaluation of commercially available watermelon in South Carolina revealed that within the watermelons evaluated there were no highly resistant varieties (Kousik et al., 2019).

Fungicide resistance management is the hallmark of any good IPM approach, and fungicides with different modes of action must be alternated and label limits for the number of applications (maximum amount of active ingredient) within a season follwed to minimize or slow the development of resistance. However, *P. xanthii* has developed resistance to a number of fungicides, including members of the methyl benzimidazole carbamates (MBC; FRAC Group 1), demethylation inhibitors (DMI; FRAC Group 3) (McGrath and Sexton 2018), quinone outside inhibitors (QoI; FRAC Group 11)(McGrath and Sexton 2018), succinate-dehydrogenase inhibitors (SDHI; FRAC Group 7) (McGrath and Wyenandt 2017; McGrath and Sexton 2018), and quinoline fungicides (FRAC Group 13) (McGrath 2017; Brunelli et al., 2010; Collina et al., 2006; Huang 2013; Maia and Gevens 2009).

Powdery mildew occurs throughout the growing season on cucurbits in Florida and can result in substantial losses if not managed. With the increasing reports of fungicide resistance from cucurbit growing regions around the world and in the United States, monitoring efforts are needed to assess fungicide efficacy in the *P. xanthii* population within Florida. We selected fungicides based upon reports of resistance in other locations to screen for efficacy against locally obtained isolates under laboratory, greenhouse, and field conditions.

2. Materials and methods

2.1. Isolates

Conidia of *Podosphaera xanthii* were collected from naturally infected leaves of summer squash in Collier County from (1) a research field at the UF/IFAS Southwest Florida Research and Education Center (SWFREC) in October 2018 (powdery mildew isolate 1; (PM1)), and (2) a greenhouse in February and March 2019 (PM4 and PM5) and February 2020 (PM7). Isolates from watermelon were collected from two commercial farms in southwest FL in February 2021 (watermelon fruit, PM8, Collier County) and in March 2021 (leaves, PM9, Hendry County). Additional isolates were collected from north and west-central FL in June 2021 from watermelon leaves (PM10, Suwannee County; PM12, Jackson County; PM13, PM14 and PM15 – Levy County). Isolates were maintained on summer squash (c.v. 'Yellow Crookneck', Johnny's Selected Seeds, Winslow, ME, USA) in the greenhouse under domes (Mondi, Canada) and/or in a plant growth chamber (Conviron, Winnipeg, Canada). Conidia were collected directly from infected squash leaves or transferred from watermelon fruit and leaves and maintained on squash for later testing. These sources of inoculum were used for the leaf disc bioassay and greenhouse trials. Field trials relied on inoculation from spores occurring naturally in the surrounding environs.

2.2. Fungicides

Fungicide concentrations and rates of application for the leaf disc bioassay, greenhouse, and field trials are listed in Table 1. Fungicide concentration used in the leaf disc bioassay were based on the maximum labeled rates assuming a spray volume of 935 L per hectare for Quintec (0.44 L/ha), Rhyme (0.51 L/ha) and Topsin (0.56 kg/ha). Rally was calculated at the maximum rate (0.35 kg/ha) in 748 L per hectare. The rates were kept the same for the greenhouse assay except for Topsin which was applied at three times the label rate based on the outcome of the disc assays.

2.3. Leaf disc bioassay

Summer squash (c.v. 'Yellow Crookneck') were started in a plant growth chamber until the appearance of the first true leaf (7–9 days). Six seedlings per treatment were sprayed with fungicide to run-off with an aerosol spray gun (Spra-Tool, Aervoe Industries, NV, USA) and placed in a chemical fume hood for 24 h to dry. Twelve control seedlings were sprayed with ultra-pure water for use as inoculated and uninoculated controls. Cotyledons were detached from treated seedlings and 10 mm discs removed with a #6 cork borer. Leaf discs were placed adaxial side up on 1.0% (w/v) water agar in a 100 mm × 15 mm petri dish. Discs were randomly assigned to account for variability due to spray coverage and 18 discs (technical replicates) were placed on a single Petri plate. Each PM isolate represented a biological replicate of the powdery mildew population in Florida, whether taken from squash or watermelon.

Powdery mildew (10–20 conidia) were transferred onto each disc with the sealed end of a glass pipette under a dissecting microscope. Plates were sealed and maintained at room temperature under 12-h/12-h light-dark cycle. Periodically, plates were examined and condensation removed from lids as needed and resealed. Discs were examined under a dissecting microscope at Day 10 post-inoculation and scored. The leaf disc assay included an uninoculated water control to assess potential contamination with powdery mildew. No powdery mildew growth was seen with the naked eye or under the stereo microscope on these discs. Discs were scored for disease severity on a scale of 0–4. Scores were based on the percentage of leaf disc surface covered in sporulating powdery mildew as follows: 0 = no sporulation; 1 = ≤ 25% of leaf disc surface; 2 = >25% - ≤ 50%; 3 = >50% - ≤ 75% and 4 = sporulating mycelium covering >75% of leaf disc surface (Kristkova et al., 2004; Fernandez-Ortuno et al., 2006; Sedlakova and Lebeda 2008; Lebeda 1984). Powdery mildew isolates PM1, PM4, PM5, and PM7 from squash were tested once (n = 1), watermelon isolates PM8, PM9, PM11, PM12 and PM15 were tested once (n = 1), and the assay was repeated for the watermelon isolates PM10 (n = 3), PM13 (n = 2) and PM14 (n = 2). In total, 11 isolates were tested and the results from these were pooled for the determination of disease severity (DS) and disease control (DC). Disease severity (DS) and disease control (DC) were calculated using the methods of Ishii et al., (2001) with slight variation. Disease severity was calculated as $[(4A + 3B + 2C + D)/4n] \times 100$, where A, B, C, and D were the number of leaf discs corresponding to the scales 4, 3, 2, and 1, respectively, and n was the total number of leaf discs assessed. Disease control was calculated as $\left[\left(Average\ DS_{Water} - Average\ DS_{Fungicide}\right)/\ Average\ DS_{Water}\right] x\ 100$.

2.4. Greenhouse trials

Four trials in May to June 2019 examined the effect of a single application of foliar fungicides for powdery mildew management. The isolate used was PM5, collected from naturally infected greenhouse squash in March 2019. Seedlings of summer squash (c.v. 'Yellow Crookneck') were started (Day 0) in a plant growth chamber until the appearance of the first true leaf then transferred to the greenhouse on Day 7 where they were grown for an additional week. On Day 15, sixteen plants (four plants per replicate) with at least two to three fully expanded leaves were randomly assigned to each of seven treatments. Five of the treatments consisted of commercial formulations of fungicides as detailed in Table 1. The final two treatments were water controls (inoculated and non-inoculated). Plants were sprayed with fungicide to run-off with an aerosol spray gun (Spra-Tool, Aervoe Industries, NV, USA) and placed on a bench to dry. After 24 h, plants were inoculated with a fine mist (approximately 100 ml per 16 plants) of *P. xanthii* conidial suspension prepared by washing squash leaves in ultra-pure water containing 0.02% Tween 20 (Fisher Scientific, USA), diluted to 2×10^4 conidia/ml. Inoculated plants were assigned to a complete randomized block design with four replications and maintained in the greenhouse until disease assessments were made 9 days post-inoculation by counting the number of colonies visible on the adaxial surface of each leaf. Data collected from the oldest four leaves were used in the data analysis, as these would have received fungicide

Table 1
Commercial fungicide formulations used in leaf disc bioassays, greenhouse, and field trials for the control of *Podosphaera xanthii* on summer squash (c.v. 'Yellow Crookneck').

Trade name/ manufacturer	Active ingredient (a.i)	a.i (%)	Type and mode of action	FRAC[a] Code	Leaf Disc Bioassay	GreenhouseTrials	Field Trials	
					a.i. (ppm)	a.i. (ppm)	Product Rate (per ha)	Rate a.i (per ha)
Topsin 70WDG, Bayer CropScience	Thiophanate-methyl	70	MBC[b]; β-tubulin assembly in mitosis	1	420	1260	0.56 kg	0.39 kg
Rally 40WSP, Dow AgroSciences LLC	Myclobutanil	40	DMI[c]; C14-demethylase in sterol biosynthesis	3	152	152	0.35 kg	0.14 kg
Rhyme, FMC Corp.	Flutriafol	22.7	DMI[c]; C14-demethylase in sterol biosynthesis	3	125	125	0.51 L	0.12 L
Quintec, Dow AgroSciences LLC	Quinoxyfen	22.58	Quinolines; disrupts signal transduction	13	.	.	0.29 L	66 mL
Quintec, Dow AgroSciences LLC	Quinoxyfen	22.58	Quinolines; disrupts signal transduction	13	106	106	0.44 L	0.10 L
Torino, Gowan Company LLC	Cyflufenamid	10	Benzamidoxime; unknown moa	U6	27	27	0.25 L	25 mL

[a] Fungicide Resistance Action Committee.
[b] MBC - methyl benzimidazole carbamates.
[c] DMI - demethylation inhibitors.

and been inoculated with PM. Dead or damaged leaves were also recorded. Leaf colony count data was transformed into a scale as follows: 0 = no PM; 1 = 1–25; 2 = 26–50; 3 = 51–75; 4 = 76–100 and 5 > 100 colonies. Disease severity (DS) and disease control (DC) were calculated using the methods of Ishii et al. (2001) with slight variation. Disease severity was calculated as $[(5A+4B+3C+2D+E)/5n] \times 100$, where A, B, C, D and E were the frequency of the corresponding scores 5, 4, 3, 2, and 1, respectively, and n was the total number of leaves assessed. Disease control was calculated as previously described.

2.5. Field trials

Field trials were conducted at the UF/IFAS SWFREC located in Immokalee, FL (26.460852, −81.435593) during Spring 2021 and Fall 2021. The soil is an Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Alaquods). Field preparation, fertility, irrigation, and pest management were conducted based upon UF/IFAS guidelines established in the 2020–2021 Vegetable Production Handbook of Florida (Dittmar et al., 2020). Raised beds were prepared to be 0.81 m wide at the top, spaced on 1.8 m centers, and covered with white on black TIF polyethylene mulch (Berry Global Inc, Sarasota, FL, USA). Field trials were laid out in a complete randomized block design with four replications. Treatments consisted of commercial formulations of fungicides as detailed in Table 1. All treatments were applied using a high clearance sprayer at 3.5 km/h and 275.79 KPa. Each plot consisted of eight plants spaced 0.30 m apart with 3.05 m buffers between plots. A double drop boom equipped with a total of eight nozzles sprayed 841.86 L/ha application volume.

Spring 2021 Trial. 'Yellow Crookneck' (Johnny's Selected Seeds, Winslow, ME, USA) squash seedlings were transplanted on 22 Mar and weekly spray applications began 16 Apr, ended on 7 May, and the trial was terminated on 12 May 2021. The four weekly sprays were Apr 16, 23 and 30, May 7.

Fall 2021 Trial. 'Yellow Crookneck' (Johnny's Selected Seeds, Winslow, ME, USA) squash seedlings were transplanted 13 Sep and received weekly spray application beginning on 30 Sep, with the final application on 28 Oct, and the trial was terminated 12 Nov. The five weekly sprays were Sep 30, Oct 7, 14, 21 and 28.

Disease ratings were taken after the first detection of PM in control plots. Five individual leaves of approximately the same age and position in the canopy were randomly selected and removed from the six middle plants of each plot (avoiding buffer plants at either end). Disease severity of PM was evaluated by visual assessment of the underside of leaves using a modified Horsfall-Barratt Scale (Horsfall and Barratt 1945). Area under the disease progress curve (AUDPC) was calculated from the disease severity ratings.

3. Statistical analysis

3.1. Leaf disc bioassay

Disease severity (DS) and disease control (DC) for sporulating powdery mildew were analyzed using analysis of variance (SAS, Proc GLM). The effects examined were originating crop (watermelon or squash) and isolate on fungicide efficacy. Since the aim of this study was to evaluate the effectiveness of the chosen fungicides on powdery mildew disease control, each sampling of the population (isolate) was treated as a biological replicate of the population irrespective of its fungicidal history. Each disc for a single isolate served as a technical replicate. When an isolate was tested multiple times, the mean of disease severity and control were used in the data analysis. Significance of differences between each fungicide and the control treatment least square means were analyzed using Dunnett's multiple comparison method ($\alpha = 0.05$), which controls for the experiment-wise Type 1 error rate and accounts for the correlation between each comparison. Additionally, treatment means were examined using Tukey's Studentized Range (HSD) test for means separation at $p < 0.05$. All statistical analyses were performed using SAS (SAS v 9.4.; SAS Institute Inc.).

3.2. Greenhouse trials

Mean colony count was modeled as a function of block and treatment (SAS, Proc GLM). Each trial was analyzed separately and the significance of differences among treatments were examined using Tukey's Studentized Range (HSD) test for means separation at $p < 0.05$. For disease control, although the analysis of variance (ANOVA) indicated that trial was not significant ($p = 0.1070$), each trial was analyzed separately to minimize loss of treatment variability that can occurs when data is averaged across multiple trials. The significance of differences among treatments were examined using Tukey's Studentized Range (HSD) test for means separation at $p < 0.05$. All statistical analyses were made using SAS (SAS v 9.4.; SAS Institute Inc.).

3.3. Field trials

Disease ratings were used to calculate area under disease progress curve (AUDPC) for each trial and was subjected to one-way analysis of variance (ANOVA). The comparisons between treatments for trials were

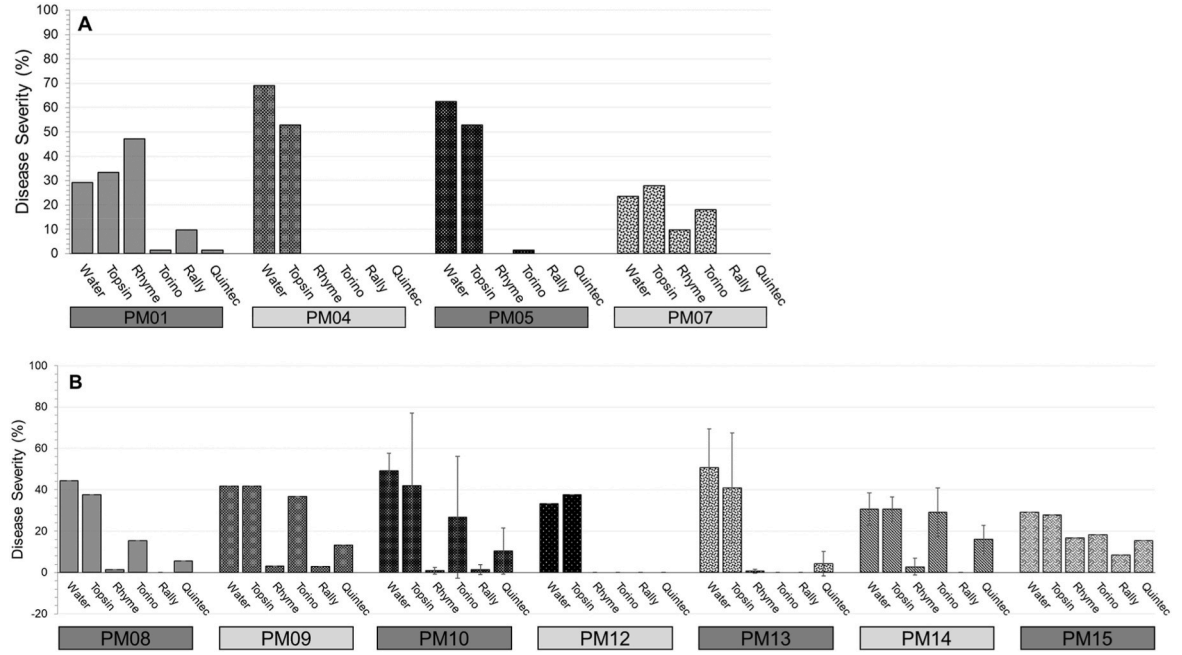

Fig. 1. Disease severity (%) of *Podosphaera xanthii* isolated from (A) squash and (B) watermelon. *P. xanthii* isolates on leaf discs treated with water (untreated control) and five fungicides: 420 ppm thiophanate-methyl (Topsin 70WDG); 125 ppm flutriafol (Rhyme); 27 ppm cyflufenamid (Torino); 152 ppm myclobutanil (Rally 40WSP) and 106 ppm quinoxyfen (Quintec). PM1 though PM7 were isolated from squash and PM8 through PM15 were isolated from watermelon. Bars with error bars represent the standard deviation for isolates where the assay was repeated (PM10: n = 3; PM13 and PM14: n = 2), otherwise n = 1.

made using a post-hoc analysis, with the p-values adjusted for multiple comparisons by the Student–Newman–Keuls test ($\alpha = 0.05$). All the analyses were made using SAS (SAS v 9.4.; SAS Institute Inc.).

4. Results

4.1. Leaf disc bioassay

Disease severity for individual squash and watermelon PM isolates are given in Fig. 1. Mean disease severity in the water control varied from 24 to 69% (Squash isolates: mean 46%, range 24–69%; Watermelon isolates: mean 42%, range 25–64%). Neither the crop from which the isolate was obtained, nor the isolate were statistically significant for disease severity ($p = 0.9705$ and 0.8457, respectively). Disease severity was significantly different between fungicide treatments.

The mean disease control (%) of *P. xanthii* isolates from squash and watermelon is shown in Fig. 2. Dunnett's multiple comparison ($\alpha = 0.05$) indicated that cyflufenamid (Torino), flutriafol (Rhyme), quinoxyfen (Quintec), and myclobutanil (Rally 40WSP) gave significant disease control when compared to the water control. Comparing disease control based on the originating crop for the PM isolate showed slight arithmetic difference, though not statistically significant. Quinoxyfen gave greater than 90% disease control for squash PM isolates but only 74% control in watermelon PM isolates. Cyflufenamid gave significantly lower control on watermelon isolates compared to squash isolates and myclobutanil performed equally well in controlling disease for both squash and watermelon isolates (91.7 ± 16.6% and 94.6 ± 10.5% respectively). There was no statistical difference in disease control of squash isolates when using quinoxyfen, myclobutanil, and cyflufenamid. For the watermelon isolates, there was no statistical difference between disease control for myclobutanil and flutriafol or between quinoxyfen and cyflufenamid (Fig. 2B). Thiophanate-methyl (Topsin 70WDG) consistently underperformed, and was the least effective in controlling disease caused by PM for all isolates tested, and was statistically not different from water (Fig. 2).

4.2. Greenhouse trials

Powdery mildew inoculations consistently resulted in visible infections within 7 days after inoculation with PM on control (water) plants. The overall colony count increased from Trial 1 through Trial 4, reflecting an increase in leaf size at inoculation (Fig. 3A). Thiophanate-methyl applied at triple the concentration used in the leaf disc bioassay failed to prevent development of PM and had similar mean colony counts to the water control. Quinoxyfen consistently resulted in the lowest colony count in all trials and highest disease control in three of the four trials (Fig. 3B). Powdery mildew development on plants treated with flutriafol, cyflufenamid, or myclobutanil was significantly reduced compared to the water.

4.3. Field trials

In Spring (2021), disease was confirmed on plants in control plots on 26 Apr and weekly disease ratings started on 28 Apr and ended 12 May. For Fall 2021, powdery mildew was confirmed on plants in control plots on 11 Oct at very low severity (<1%) and disease ratings were taken weekly starting on 19 Oct and ending 4 Nov. Area under the disease progress curve (AUDPC) for both trials is shown in Table 2.

The Spring 2021 field trial evaluated the minimum and maximum label rates per hectare for quinoxyfen, with no statistical difference in PM control between the two. In both seasons, cyflufenamid, quinoxyfen and myclobutanil performed well in managing PM in the field. In the Spring, cyflufenamid, quinoxyfen and myclobutanil were significantly better at controlling PM than flutriafol. Thiophanate-methyl was not statistically different from cyflufenamid, quinoxyfen, myclobutanil or flutriafol in Spring (2021).

5. Discussion

These studies were initiated based on reports from other cucurbit production regions in the United States of *Podosphaera xanthii* developing resistance to several fungicide active ingredients representing different modes of action. In our studies, isolates were collected from

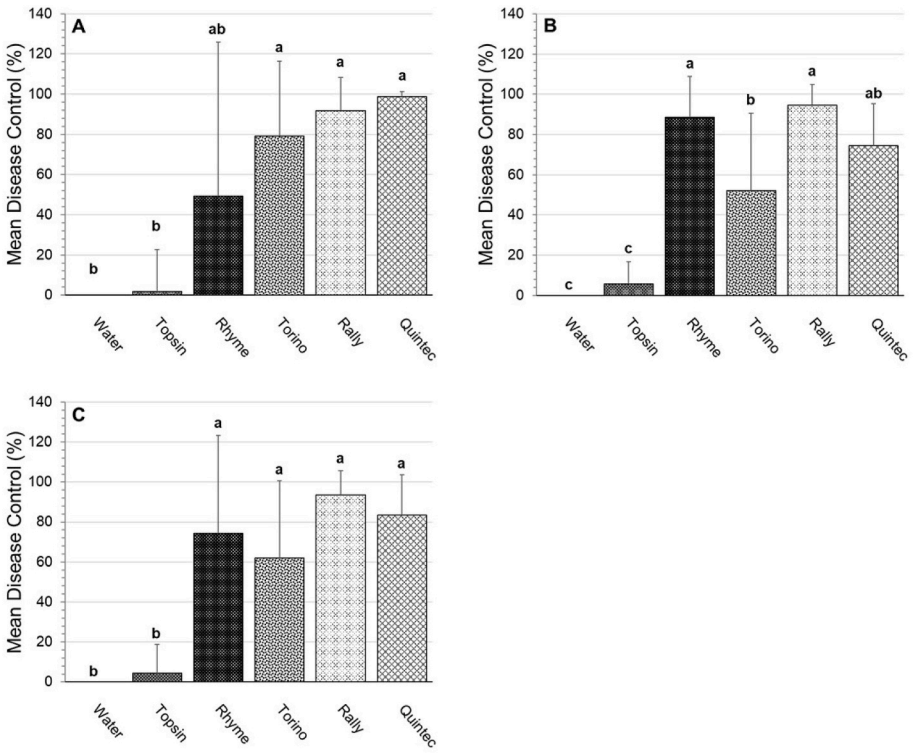

Fig. 2. Mean disease control (%) of *Podosphaera xanthii* isolates from (A) squash (n = 4), (B) watermelon (n = 7), and (C) combined squash and watermelon isolates (n = 11) on leaf discs with untreated control (water) and five fungicides: 420 ppm thiophanate-methyl (Topsin 70WDG); 125 ppm flutriafol (Rhyme); 27 ppm cyflufenamid (Torino); 152 ppm myclobutanil (Rally 40WSP); and 106 ppm quinoxyfen (Quintec). Bars with the same letter are not significantly different (Tukey's Studentized Range (HSD) Test; α = 0.05). Error bars represent the standard deviation.

squash and watermelon at several different locations within Florida and tested against five common commercially available fungicides. The five fungicides tested are protectant fungicides which provide a barrier to infection, either by killing or inhibiting fungal spore germination and pathogen establishment. These fungicides work best when applied to the plant before the entry of the pathogen into the field. It is important to note that the field applications did not follow the label guidelines of rotation in the field experiments, which would not be the standard procedure under commercial field applications.

For each pesticide, the choice of concentration tested was based on the maximum labeled field application rate, except for Topsin 70WDG in the greenhouse trial. The use of the maximum label rate or a discriminatory concentration has been applied where populations of *P. xanthii* exhibit resistance to the maximum rate or where a gradient of resistances to various concentrations of the fungicide has been noted (Bellon-Gomez et al., 2015). *P. xanthii* has shown a gradient of resistance to myclobutanil (Ishii et al., 2021), quinoxyfen (Bellon-Gomez et al., 2015), and cyflufenamid (Pirondi et al., 2014; Pollastro et al., 2022).

In the greenhouse trials, the consistently high number of powdery mildew colonies found on plants treated with thiophanate-methyl (Topsin 70WDG) potentially reflects the existence of an resistant population and was not different to a field trial done by Keinath in 2013 on summer squash (Keinath 2015). *P. xanthii* resistance to thiophanate-methyl is considered qualitative due to mutations in genes encoding fungicide targets. This qualitative resistance was consistent with the failure of increased concentration of thiophanate-methyl (tripling to 1260 ppm in the greenhouse trial) to control the pathogen. Similarly, in the field trials Topsin 70WDG applied weekly did not significantly reduce disease severity as determined by the AUDPC in the Spring. *P. xanthii* resistance to thiophanate-methyl on cucurbit crops has been well documented in field and greenhouse populations in Europe (Bellon-Gomez et al., 2015) and the United States (Matheron and Porchas 2013). Thiophanate-methyl belongs to the methyl benzimidazole carbamates (MBCs; FRAC group 1) and powdery mildew resistance to its pioneer group member benomyl was first reported in cucurbits in 1969 (Schroeder and Provvidenti 1969). The mechanism associated with resistance to this group of compounds are mutations E198 A/G/K and F200Y located in the β-tubulin gene (Hawkins and Fraaije 2016). The E198A mutation and its mechanism in the role of fungicide resistance has been extensively studied in *P. xanthii* (Vela-Corcia et al. 2014, 2018). Beta-tubulin is required for the formation of micro-tubules which play key roles in cell division (mitosis). The results from the disc bioassay and greenhouse studies are not surprising and further substantiate that thiophanate-methyl gives poor control of powdery mildew on cucurbits.

Rally 40WSP (myclobutanil) and Rhyme (flutriafol) belong to FRAC group 3, the demethylation inhibitors (DMIs). These fungicides work by inhibiting sterol biosynthesis; sterols are essential components of cell walls (Joffrion and Cushion 2010). Myclobutanil, the older of the active ingredients, was registered with the Environmental Protection Agency (EPA) in 1952 and the formulation Rally 40WSP as a control agent of cucurbit PM in March 2007; flutriafol was registered in April 2010 and the formulation Rhyme in March 2016. Resistance to DMI fungicides has been associated with mutations in the CYP51 gene, specifically Y136F and overexpression (Ma and Michailides 2005; Hamamoto et al., 2000). Additionally, multiple copies of the CYP51 gene exist in a single cell and increasing numbers of mutated copies appears to correlate with the gradation of resistance seen across field populations in grapevine PM (Jones et al., 2014). Whether this is the same in cucurbit PM has yet to be shown. In the greenhouse trials both DMI fungicides failed to give 100 percent disease control and potentially reflects a mixture of susceptible and resistant conidia present in the PM5 population. In a recent publication on DMI fungicides, preventative treatment with 50 mg/L myclobutanil, one-third the concentration used in this study, against three isolates of *P. xanthii* of varying DMI resistance gave 90% (Chikusei-1, sensitive), 52% (Tsukuba-1, moderate), and 54% (N-E4, highly resistant) control. The resistance seen in N-E4 has been attributed to mutations A372G, I374V, V449L, and G461S in CYP51(Ishii et al., 2021). In the leaf disc bioassay, both DMI fungicides performed equally well in controlling PM. Myclobutanil outperformed flutriafol in suppressing *P. xanthii* growth in the greenhouse and Spring 2021 field trial and could be a reflection of the concentration used, as DMI fungicide

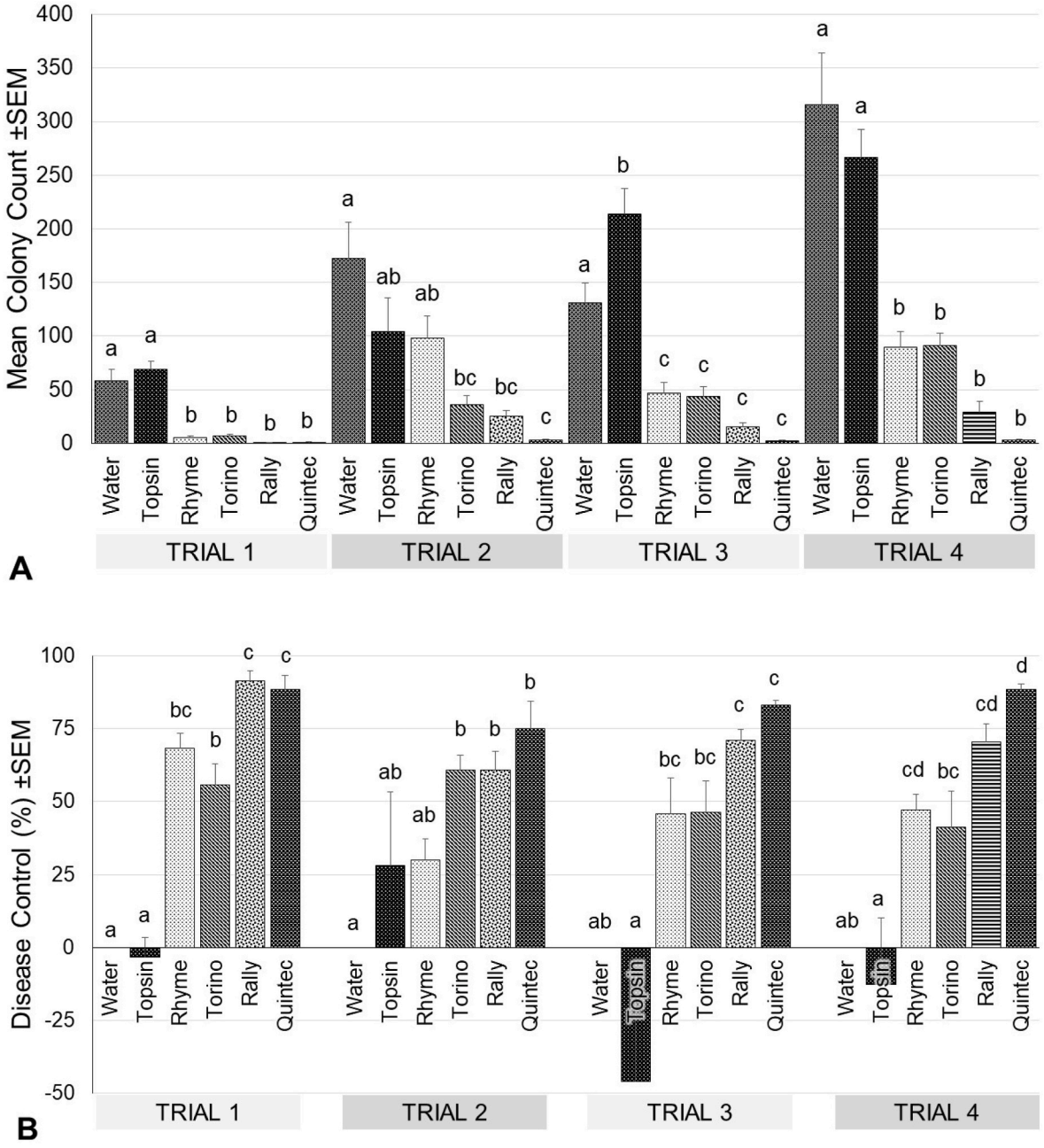

Fig. 3. (A) Mean colony count ± Standard Error of the Mean (SEM) and (B) Disease Control ± SEM (%) on Day 9 for greenhouse summer squash plants (n = 14–16) after a single application of water or one of five fungicides and inoculated with powdery mildew (PM5; *Podosphaera xanthii*) in June 2019. Fungicides were 1260 ppm thiophanate-methyl (Topsin 70WDG), 125 ppm flutriafol (Rhyme), 27 ppm cyflufenamid (Torino), 152 ppm myclobutanil (Rally 40WSP) and 106 ppm quinoxyfen (Quintec). Bars with the same letter within a single trial are not significantly different within the trial according to Tukey's Studentized Range (HSD) Test (α = 0.05).

resistance is quantitative (McGrath 2001), with increasing concentration resulting in increased disease control. The control of PM in the Fall by flutriafol following a Spring season with poor control was, however, surprising. This could be indicative of the introduction of a sensitive population into the field and an indicator of the seasonal fluctuating dynamics of resistance in the PM population. Repeated application of flutriafol at full or reduced doses has been documented to select for highly resistant strains of *Cercospora beticola* (Karaoglanidis et al., 2001), and lowered *Podosphaera leucotricha* control has been noted in apple orchards to both myclobutanil and flutriafol (Cox et al., 2013). For both DMI fungicides, the highest label rate (equivalent ppm of active ingredient) was applied, suggesting that application rates may require reassessment to prevent intermediate strain escape and to delay resistance development.

Quintec (quinoxyfen) belongs to FRAC group 13, the phenoxyquinolines fungicides, and was developed specifically for the control of powdery mildews (Longhurst et al., 1996). The mechanism of action involves disruption of signal transduction, reducing sporulation (Hollomon et al., 1997) and preventing germination and appresoria formation (Wheeler et al., 2000; Lee et al., 2008). Quinoxyfen consistently provided the highest degree of disease control in the greenhouse trial, resulting in the lowest colony counts, and adequate control in the leaf disc bioassay and field trials. In the Spring 2021 field trial, the maximum label rate (0.44 L/ha quinoxyfen) had a lower AUDPC, and not statistically different from the lower rate (0.29L/ha quinoxyfen), but did not prevent disease establishment. Although the risk of fungicide resistance was considered low for quinoxyfen (Hollomon et al., 1997), resistance has been reported in *P. xanthii* in the United States (McGrath 2017). The

Table 2
Area under the disease progress curve (AUDPC) for *P. xanthii* on 'Yellow Crookneck' in field plots treated with thiophanate-methyl (Topsin 70WDG), flutriafol (Rhyme), cyflufenamid (Torino), myclobutanil (Rally 40WSP), and quinoxyfen (Quintec).

Treatment[w]	Product Rate (per ha)	Spring 2021 AUDPC[y]		Fall 2021 AUDPC[y]	
Control		431.67	a	420.00	a
Cyflufenamid; Torino (1–4, 1–5)	0.25 L	33.08	c	59.50	b
Quinoxyfen; Quintec (1–4)	0.29 L	81.03	c	–	–
Quinoxyfen; Quintec (1–4, 1–5)	0.44 L	49.18	c	73.00	b
Myclobutanil; Rally 40 WSP (1–4, 1–5)	0.35 kg	75.78	c	102.25	b
Thiophanate-methyl; Topsin 70 WDG (1–5)	0.56 kg	172.20	bc	–	–
Flutriafol; Rhyme (1–4, 1–5)	0.51 L	269.85	b	108.75	b

[w] Numbers in parentheses represent the number of weekly applications - Spring 2021: Apr 16, 23 and 30, May 7; Fall 2021: Sep 30, Oct 7, 14, 21 and 28.
[y] AUDPCs followed by the same letter are not significantly different according to Student–Newman–Keuls test ($\alpha = 0.05$).

2019 isolate PM5 from squash used in the greenhouse trial had on average three colonies per replicate. In the leaf disc bioassay, six of the seven watermelon isolates had sporulating PM. Considering the number of conidia sprayed in the greenhouse trial vs. the number of conidia placed on a 10 mm leaf disc, 125,000 per plant vs. 10–20 spores per disc, there might be quinoxyfen intermediate or resistant PM populations, yet to be explored. Further testing is needed to confirm this for Florida isolates of *P. xanthii*, especially for watermelon isolates, and to establish new effective concentration and fungicide application regimes.

Torino (cyflufenamid) belongs to FRAC group U6 and was registered for use in the United States in 2012. Greenhouse and leaf disc bioassays in this study used 27 ppm cyflufenamid and showed moderate control of PM. Under field trial conditions, cyflufenamid applied at the maximum label rate was the most effective at reducing disease severity (AUDPC) compared to quinoxyfen, myclobutanil, thiophanate-methyl, and flutriafol in Spring (2021). *P. xanthii* resistance to cyflufenamid was first reported in Japan (Hosokawa 2006), and later in Italy (Pirondi et al., 2014). More recently, resistance was noted in isolates collected in the United States (Long Island, New York) in 2017 (McGrath and Sexton 2018), where growth and sporulation at 50 ppm in leaf disc bioassay was used to define an isolate as resistant. This discriminatory concentration was well above that tested in this study, and evaluation of the Florida *P. xanthii* population using 50 ppm cyflufenamid would prove useful for comparison to the New York findings.

Overall, the Florida PM isolates were well managed with quinoxyfen (Quintec), myclobutanil (Rally 40WSP), cyflufenamid (Torino), and flutriafol (Rhyme), with inadequate control from thiophanate-methyl (Topsin 70WDG). The varying sensitivity of *P. xanthii* to fungicides tested suggests the possibility of a resistant sub-population. Differences in disease control between watermelon and squash isolates, although not statistically different, corroborate some anecdotal reports and a larger data set and further study would be useful in further elucidating these differences. Consistent monitoring of the *P. xanthii* population in Florida is needed for early detection of fungicide resistance, to establish new effective concentration, and to optimize fungicide application regimes.

Authorship contribution statement

Katherine Hendricks: conceptualization, investigation, methodology, data collection and analysis (leaf disc bioassay and greenhouse trials); Writing - original draft, review & editing. Pamela Roberts: conceptualization, investigation, methodology, data collection and analysis (field trials), funding acquisition, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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