Research

Efficacy of Fungicides for *Pseudoperonospora cubensis* Determined Using Bioassays over Multiple Years in the Mid-Atlantic and Northeastern United States

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Abstract

In the United States, fungicides are the primary management option for cucumber growers to protect their crops from *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew. Pathogen resistance to some fungicides can quickly develop with the repeated applications needed to protect yield. In order to determine fungicide efficacy and monitor it over time, bioassays were conducted from 2016 to 2019 in Delaware, Maryland, Pennsylvania, and New York. Potted cucumber plants were either sprayed with fungicides or not treated, placed next to field-grown plants with cucurbit downy mildew for up to 2 days, and then kept in a greenhouse until symptoms developed. Severity of symptoms or number of lesions on leaves was recorded 6 to 14 days after exposure started and used to determine fungicide efficacy. Quadris (azoxystrobin) was ineffective in seven of the nine bioassays, and Revus (mandipropamid) was ineffective in six of seven bioassays. Forum (dimethomorph) and Presidio (fluopicolide) were ineffective in three of eight and four of nine bioassays, respectively. The most effective fungicides were Bravo (chlorothalonil), Zing! (zoxamide + chlorothalonil), and Orondis (oxathiapiprolin), all of which consistently suppressed disease severity more than 90% when compared with the untreated control. Previcur Flex (propamocarb hydrochloride) and Ranman (cyazofamid) were also effective in every bioassay.

Keywords: cucurbit downy mildew, cucumber, fungicide resistance

Cucurbit downy mildew is one of the most significant diseases of an important crop group in the United States. Cucurbit crops including cucumber (*Cucumis sativus* L.), jack-o-lantern pumpkin (*Cucurbita pepo*), giant pumpkin (*Cucurbita maxima*), squash (*Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*), watermelon (*Citrullus lanatus*), and melon (*Cucumis melo*) were grown on over 156,000 ha in the United States with a value of more than $1.6 billion in 2019 (USDA 2020). An annual threat to production is the foliar disease cucurbit downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovez. Epidemics have occurred annually in the Eastern United States since 2004, when a more aggressive population of *P. cubensis* overcame the host resistance of cucumbers and devastated yields (Holmes et al. 2015). Cucumber cultivars with intermediate resistance to this new pathogen population only recently became commercially available (Adams et al. 2020a, b; Everts et al. 2019, Keinath 2019; McGrath et al. 2018). Therefore, fungicides have been the primary tool to manage cucurbit downy mildew and were applied to 83% of cucumber acreage in the United States in 2018 (Cohen et al. 2015; Holmes et al. 2015; USDA 2019; Wyenandt et al. 2017).

Fungicide resistance is a major concern when managing cucurbit downy mildew. The most effective fungicides have single site modes of action, which pose a high risk for resistance development. Additionally, *P. cubensis* has been classified as a pathogen at high risk for developing resistance (Russell 2003). Each registered active ingredient (AI) can be applied multiple times to a cucumber crop during the season to manage cucurbit downy mildew (specified by the product label), thereby increasing selection pressure on *P. cubensis* populations for resistant mutants (Brent and Hollomon 2007). Research conducted by Ojiambo and Holmes (2011) indicated that *P. cubensis* inoculum spreads annually from southern Florida up to the Georgia/South Carolina/

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North Carolina border, where infections in this region likely contribute inoculum to disease outbreaks in the Mid-Atlantic and Great Lakes regions. The pathogen population cycles through numerous crops as it spreads northward and experiences more selection events than just those in a single cropping cycle. Monitoring of fungicide resistance in different regions is vital to avoiding disease control failure (Holmes et al. 2015; Ojiambo et al. 2015). For example, Presidio (AI fluopicolide; Valent USA, Walnut Creek, CA) and Previcur Flex (AI propamocarb hydrochloride; Bayer CropScience, Research Triangle Park, NC) were first labeled for cucurbit downy mildew in 2007 and 2004, respectively, and were among the most efficacious fungicides in the United States, until reduced efficacy was observed starting in 2012 and resistance confirmed in 2018 (Keinath 2016; Langston and Sanders 2013; Ojiambo et al. 2010; Thomas et al. 2018). Fungicides with novel modes of action, such as Orondis Opti and Orondis Ultra (AI oxathiapiprolin + chlorothalonil or mandipropamid; Syngenta Crop Protection, Greensboro, NC) have been released but at a slower rate than the loss of older chemistries (Salas et al. 2019). Rotation among fungicides in different Fungicide Resistance Action Committee (FRAC) groups, which numerically group AIs by modes of action, helps reduce selection pressure on *P. cubensis* to develop resistance. Efficacious chemicals including ethaboxam formulated as Elumin (Valent USA), fluazinam formulated as Omega (Syngenta Crop Protection), and oxathiapiprolin formulated alone without chlorothalonil as Orondis Opti A have not shown apparent signs of resistance within the *P. cubensis* populations (Thomas et al. 2018). Omega, Gavel (AI mancozeb + zoxamide; Gowan Company, Yuma, AZ), Ranman (AI cyazofamid; Summit Agro USA, Durham, NC), and Orondis Opti A were highly effective in reducing cucurbit downy mildew severity in multiple bioassays across Ohio, New York, and South Carolina (Keinath et al. 2019). Comparatively, in Michigan, Elumin, Ranman, Zampro (AI ametoctradin + dimethomorph; BASF Corporation, Research Triangle Park, NC), Gavel, Koverall (AI mancozeb; FMC Corporation, Philadelphia, PA), Bravo Weather Stik (AI chlorothalonil; Syngenta Crop Protection), and Orondis Opti A, Orondis Opti, and Orondis Ultra were all effective (Goldenhar and Hausbeck 2019). Continued monitoring of pathogen populations to determine fungicide sensitivity is important because development of fungicide resistance within *P. cubensis* populations is well documented (Olaya et al. 2009; Thomas et al. 2018). Within Call et al. (2013), it was shown that the incorporation of host-resistant cultivars into cucurbit downy mildew management strategies in cucumber allowed for the use of a protectant (mancozeb) to preserve yield output compared with alternating tank mixes of systemic and protectant fungicides. In New Jersey, moderate and lower efficacy fungicides provided adequate control of cucurbit downy mildew in winter squash (Wyenandt et al. 2017). Disease management efforts can vary based on host, as *P. cubensis* population dynamics and clade-host associations become clearer (Naegele et al. 2016; Quesada-Ocampo et al. 2012; Rahman et al. 2021; Wallace et al. 2020).

Population studies have divided *P. cubensis* into two clades, and although each clade can infect different cucurbit hosts, host preference is apparent, with clade 1 mostly found infecting pumpkins, squash, and watermelons, whereas clade 2 occurs on cucumbers and cantaloupe (Crandall et al. 2018; Wallace et al. 2020). The limited number of U.S. isolates prior to 2004 from cucumber align closely with the clade 1 grouping, and post 2004 align with clade 2 (Kitner et al. 2015; Runge et al. 2011). Pre-epidemic clade 2 samples originated in East Asia, suggesting clade 2 is indigenous to that region (Runge et al. 2011). Clade 2 is now the prominent genotype in the post 1984 and 2004 epidemics infecting cucumbers in Europe and the United States, respectively (Kitner et al. 2015; Runge et al. 2011; Wallace et al. 2020). It has been hypothesized that the 2004 epidemic was caused by a new biotype of *P. cubensis* introduced to the United States that was resistant to mefenoxam and strobilurin fungicides, as well as virulent on the previously resistant cucumbers (Holmes et al. 2015). It is likely this new and aggressive pathotype in 2004 was clade 2 *P. cubensis* (Runge et al. 2011).

Fungicide Bioassays

A bioassay was developed and has been successfully used on different *P. cubensis* populations from multiple states over multiple years to determine the efficacy of fungicides on cucurbit downy mildew and to monitor for reduced sensitivity in the pathogen population, and it can be used to make in-season fungicide recommendations to growers (Keinath 2016; Keinath et al. 2019). Variability in fungicide efficacy has been observed both in time and location using the bioassay (Keinath 2016; Keinath et al. 2019). The speed and ease of the bioassay are key benefits: it can be completed in as little as 4 weeks (from seeding to data collection), it can be repeated multiple times during the growing season, and a large number of fungicides can be included for testing. Bioassays determine fungicide sensitivity at a particular point in time. Efficacy of a fungicide in a bioassay may not match its efficacy on the crop where the bioassay was conducted if the pathogen population fluctuated greatly over the course of the season. The objective of this study was to determine the efficacy of select fungicides on cucurbit downy mildew, monitor for insensitivities to the fungicides, and compare the results across multiple states and years to examine variability of fungicide efficacy across the Mid-Atlantic and Northeast regions.

Nine bioassays were completed across four states (Delaware, Maryland, Pennsylvania, and New York) over 4 years (2016 to 2019) (Table 1). 'Silver Slicer' cucumber was used, which has no resistance to *P. cubensis* but is resistant to powdery mildew caused by *Podosphaera xanthii*. Resistance to *P. xanthii* reduced competition on the leaf surface for infection by *P. cubensis* and helped avoid confusion when rating disease symptoms. Greenhouse-grown cucumber seedlings with two to three true leaves, in 10-cm square pots, were arranged in a randomized complete block design, with four replications. Each replication consisted of single seedlings in pots receiving either fungicide or water in the untreated controls (Table 2). Fungicides were mixed in 200

TABLE 1
Locations and dates of cucumber bioassay experiments used to evaluate the efficacy of fungicides applied preventively for cucurbit downy mildew

Year	State	County	Exposed	Rated
2016	Delaware	Sussex	8/03/16	8/10/16
2016	New York	Suffolk	9/16/16	9/29/16
2017	New York	Suffolk	8/26/17	9/7/17
2017	Pennsylvania	Berks	8/29/17	9/8/17
2017	Pennsylvania	Blair	8/16/17	8/25/17
2017	Maryland	Wicomico	8/1/17	8/11/17
2018	New York	Suffolk	9/15/18	9/25/18
2019	New York	Suffolk	9/13/19	9/23/19
2019	Maryland	Wicomico	8/16/19	8/27/19

ml of water at full label rates. Fungicide applications were made to leaves with a backpack sprayer (New York and Pennsylvania) or a handheld spray bottle (Delaware and Maryland) until runoff. The following day, 12 to 24 h posttreatment, seedlings were exposed to natural inoculum by placing them next to field-grown cucurbit downy mildew-infected cucumber plants, with actively sporulating lesions, in research plots or a commercial field. The growing tip of each seedling was removed to slow the senescence of the treated leaves. There were two water control seedlings for each replication, in case one was damaged, rendering that replicate set of treatments invalid. Seedlings were arranged in replications and left in the field for 24 to 48 h to allow for infection to occur before they were returned to the greenhouse for up to 14 days to allow for symptoms to develop.

Disease severity was rated on a scale of 0 to 100% in New York and Pennsylvania by estimating percent coverage of disease symptoms on the second leaf, whereas lesions were counted on the second leaf in Delaware and Maryland. Relative disease severity (RDS) was calculated as percent severity or number of lesions in fungicide-treated plants divided by average percent severity or average number of lesions in the water control plants of the same replication (Keinath 2016). RDS was used as opposed to disease severity to reduce the impact of variability in disease pressure due to timing or location (Keinath et al. 2019). Fungicides were considered effective when RDS values were below a threshold of 35% (Thomas et al. 2018). RDS values were transformed by adding a constant value of 1 before specifying a "lognormal" distribution in PROC GLIMMIX. Fungicide treatment, date, and state were considered fixed effects, and block nested within state or date was a random effect in PROC GLIMMIX. The "by" statement was used to analyze RDS within state and year. Least square means were separated using Student's *t* test, $P = 0.05$. The water control treatment was excluded from the datasets used in analyses to reduce the inequality of variances.

Quadris (AI azoxystrobin; Syngenta Crop Protection) was ineffective in seven of the nine bioassays, but even when it was effective in 2016 Delaware and 2017 Berks County Pennsylvania, its RDS was close to the 35% cutoff at 34.5 and 31.4%, respectively (Table 3). Resistance and consequent poor efficacy of Quadris and its AI azoxystrobin is well known and widely reported (Ishii et al. 2001; Keinath 2016; Keinath et al. 2019; Miller et al. 2020). Revus (AI mandipropamid; Syngenta Crop Protection) and Forum (AI dimethomorph; BASF Corporation) are both FRAC group 40 fungicides (carboxylic acid amides) and were ineffective in six out of seven and three out of eight bioassays they were included in, respectively (Table 3). Revus was previously reported as ineffective at controlling cucurbit downy mildew in cucumber (Goldenhar and Hausbeck 2019; Keinath 2016; Keinath et al. 2019; Salas et al. 2019). Forum was more effective in our bioassays than in field trials in Michigan and bioassays in Ohio and South Carolina (Goldenhar and Hausbeck 2019; Keinath et al. 2019; Miller et al. 2020). But Forum response in our bioassays was similar to that in bioassays conducted in South Carolina and Ohio, in which lack of efficacy was observed less often with Forum than with Revus (Keinath 2016; Miller et al. 2020). Zampro is a premixture that includes the AI of Forum (dimethomorph) plus ametoctradin. Zampro was effective in two of the three bioassays in which Forum was ineffective, reducing RDS from 37.0 to 13.6% in Maryland in 2017 and 69.9 to 0% in Berks County, Pennsylvania, in 2017, compared with Forum. Zampro was only ineffective in one bioassay out of the eight it was included in and was statistically similar to the most efficacious products in Berks County and Blair County, Pennsylvania, in 2017 and New York in 2018 (Table 3). In Michigan field trials, Zampro was among the most efficacious products on cucurbit downy mildew but was ineffective in Ohio and South Carolina bioassays (Goldenhar and Hausbeck 2019; Keinath et al. 2019).

Presidio was included in all nine bioassays and was ineffective four times: once in Maryland in 2019 and for three consecutive years in New York, in 2017, 2018, and 2019 (Table 3). Failure of Presidio to reduce cucurbit downy mildew severity has also been seen in South Carolina and Michigan (Goldenhar and

TABLE 2
List of fungicides evaluated for control of cucurbit downy mildew across four states

Trade name	Active ingredient	FRAC[v] code	Rate (per hectare)[w]
Bravo Ultrex 82.5WDG	Chlorothalonil 82.5%	M5	1.57 kg
Curzate 50DF	Cymoxanil 60%	27	0.35 kg
Elumin 4SC	Ethaboxam 42.5%	22	0.58 liters
Forum 4.17SC	Dimethomorph 43.5%	40	0.44 liters
Omega 500F	Fluazinam 40%	29	1.75 liters
Orondis Gold 200SC[x]	Oxathiapiprolin 18.7%	49	0.67 liters
Orondis Opti A 0.83OD[y]	Oxathiapiprolin 10.2%	49	0.15 liters
Plenaris 200FS[z]	Oxathiapiprolin 18.7%	49	0.15 liters
Previcur Flex 6SL	Propamocarb 66.5%	28	1.40 liters
Presidio 4SC	Fluopicolide 39.5%	43	0.29 liters
Quadris 2.08F	Azoxystrobin 22.9%	11	1.13 liters
Ranman 400SC	Cyazofamid 34.5%	21	0.20 liters
Revus 2.08SC	Mandipropamid 23.3%	40	0.58 liters
Zampro 525SC	Ametoctradin 26.9% + dimethomorph 20.2%	45 + 40	1.02 liters
Zing! 4.9SC	Zoxamide 6.8% + chlorothalonil 40%	22 + M5	2.63 liters
Zoxamide	Zoxamide (technical grade)	22	400 ppm

[v] Fungicide Resistance Action Committee.
[w] Maximum rate given on fungicide label for use on cucurbit downy mildew.
[x] Orondis formulation used in New York.
[y] Orondis formulation used in Pennsylvania.
[z] Orondis formulation used in Maryland.

Hausbeck 2019; Keinath et al. 2019). Previcur Flex was effective in our bioassays (Table 3), albeit close to the 35% threshold in 2017 in both locations in Pennsylvania and in New York, although it was ineffective in an earlier field trial in Pennsylvania (Gugino and Grove 2016). Variation from year to year in the efficacy of Previcur Flex has been observed in South Carolina, Ohio, and Michigan (Baysal-Gurel et al. 2015; Goldenhar and Hausbeck 2019; Keinath 2016; Keinath et al. 2019; Kenny et al. 2020). Resistance to Presidio and Previcur Flex was found in approximately 65 and 26%, respectively, of 31 isolates collected between 2008 and 2015 in the Eastern United States by Thomas et al. (2018). Seven of the 31 isolates showed multiple resistance to the unrelated fungicides, Presidio (FRAC group 43) and Previcur Flex (FRAC group 28) (Thomas et al. 2018). Even so, a combination of the AIs from Presidio and Previcur Flex (fluopicolide + propamocarb) formulated as Infinito (Bayer CropScience) was effective when used either as a preventive or curative treatment by Salas et al. (2019).

Curzate (AI cymoxanil; Corteva Agriscience, Midland, MI) was included in all nine bioassays and was effective seven times, except 2017 New York, when its RDS was 36.0%, just above the effective RDS value, and 2019 Maryland when its RDS was 41.0% (Table 3). Similar results were seen in Keinath et al. (2019), in which Curzate was ineffective in one of the six bioassays. But reports of Curzate failing to control cucurbit downy mildew are common, with two out of three years in Michigan and half of the bioassays in South Carolina not reducing severity compared with the untreated control (Goldenhar and Hausbeck 2019; Keinath 2016). These failures could at least partly be because residual activity of cymoxanil is about 5 days.

Technical-grade zoxamide was only examined as a standalone treatment in two bioassays and was highly effective in 2018 but ineffective in 2019 in New York (Table 3). In a separate study, zoxamide reduced cucurbit downy mildew severity in New York and South Carolina in 2017 (Keinath et al. 2019). Zoxamide is marketed in premixtures with mancozeb (Gavel) or chlorothalonil (Zing!; Gowan Company). Gavel was not included in our bioassays but was among the most effective fungicides in 50% of the bioassays in New York, South Carolina, and Ohio and 100% of the trials in Michigan (Goldenhar and Hausbeck 2019; Keinath et al. 2019). Koverall and another formulation of the AI mancozeb, Manzate Pro-Stick (AI mancozeb; United Phosphorus, King of Prussia, PA), were not included in this study but are efficacious on cucurbit downy mildew (Goldenhar and Hausbeck 2019; Keinath 2016). Koverall was statistically similar to Gavel in two out of three trials in Michigan, whereas Manzate Pro-Stick was statistically similar to Gavel in five out of six bioassays in Ohio, South Carolina, and New York, suggesting the efficacy of Gavel could be in large part due to the protectant fungicide in the mixture (Goldenhar and Hausbeck 2019; Keinath et al. 2019). Zing! was included in seven of the nine bioassays in our study and was statistically similar to the fungicide with the lowest RDS two times and had the lowest RDS four times (Table 3). Zing! was similar in efficacy to Bravo Ultrex in our bioassays, except in New York in 2016, when Bravo Ultrex RDS was significantly lower. Similar to Gavel, the efficacy of Zing! could be due in large part to the protectant fungicide component. Zing! was also reported as effective in North Carolina and one of the most effective fungicides in a trial in Michigan (Adams et al. 2019; Hausbeck et al. 2017).

Bravo Ultrex was consistently effective and included in eight of the nine bioassays. It was statistically similar to the fungicide with the lowest RDS four times and had the lowest RDS three times (Table 3). The highest RDS of Bravo Ultrex was observed in 2019 in New York, with a value of 7.3%. Bravo Weather Stik, a different formulation of chlorothalonil not used in our bioassays, was effective in both New York and South Carolina in 2015 and 2017 but ineffective in Ohio both years (Keinath et al. 2019). Bravo Weather Stik was statistically similar to the most efficacious fungicides in two of three field trials in Michigan and seven of eight bioassays in South Carolina (Goldenhar and Hausbeck 2019; Keinath 2016). Bravo formulations and

TABLE 3
Relative downy mildew severity values for fungicides tested on cucumbers in bioassays across four states and four years[w]

	2016		2017				2018	2019	
Fungicide	DE	NY	MD	NY	PA[x]	PA[y]	NY	MD	NY
Quadris 2.08F	34.5 a	70.7 a	35.7 ab	85.7 ab	86.2 a	31.4 ab	107.5 a	117.5 a	88.2 ab
Revus 2.08SC	13.5 b	81.5 a	105.9 a	75.8 abc	ND	ND	62.3 a	44.2 bc	122.7 a
Presidio 4SC	0 c	13.0 b	4.1 de	99.6 a	17.2 ab	1.1 c	66.0 a	76.5 ab	36.2 bc
Forum 4.17SC	ND	74.7 a	37.0 abc	22.2 cde	13.4 bc	69.9 a	4.0 b	0.8 d	30.8 bc
Zampro 525SC	16.9 ab	59.4 a	13.6 bcd	20.9 de	7.0 bcd	0 c	1.6 bc	11.0 c	ND
Curzate 50DF	3.1 c	3.2 bc	2.6 de	36.0 bcd	7.6 bcd	28.4 a	1.0 bc	41.0 c	0.6 f
Zoxamide	ND	ND	ND	ND	ND	ND	0 c	ND	44.3 ab
Zing! 4.9SC	0 c	7.5 b	2.6 cde	7.6 ef	0 d	0 c	ND	0 d	ND
Bravo Ultrex 82.5WDG	ND	0.3 c	6.5 cde	4.2 f	5.6 cd	8.3 bc	0.8 bc	0 d	7.3 de
Previcur Flex 6SL	0 c	4.1 b	ND	30.7 cde	26.1 ab	27.9 a	1.9 bc	0 d	4.5 ef
Omega 500F	ND	ND	ND	ND	ND	ND	ND	0 d	24.2 de
Ranman 400SC	0 c	10.3 b	2.3 de	21.9 de	1.1 cd	8.3 bc	0.2 c	0 d	13.3 cd
Elumin 4SC	ND	ND	ND	ND	ND	ND	ND	0 d	ND
Orondis formulations[z]	ND	ND	0 e	ND	0 d	0 c	0.7 bc	0 d	0 f
Fungicide *P* value	<0.0001	<0.0001	0.0013	0.0001	0.0008	0.0015	<0.0001	<0.0001	<0.0001

[w] State means by year within each column followed by the same letter are not significantly different, Student's *t* LSD, *P* = 0.05. ND = no data. States are Delaware (DE), New York (NY), Maryland (MD), and Pennsylvania (PA).
[x] Blair County, PA.
[y] Berks County, PA.
[z] Orondis Gold 200SC in NY, Orondis Opti A 0.83OD in PA, and Plenaris 200FS in MD.

Koverall/Manzate Pro-Stick are broad-spectrum fungicides (FRAC groups M05 and M03, respectively) with a low risk of resistance development and help maximize the effective life of higher risk fungicides when used in tank mixes or as premixtures (FRAC 2020; Hobbelen et al. 2011). These are contact fungicides that are efficacious if applied preventively, with proper spray coverage. When applied alone, repeatedly in a season, control of cucurbit downy mildew typically decreases, and protection of yield becomes inadequate as infections occur on unprotected leaf surfaces, notably the underside of leaves, and are not affected by subsequent applications (Adams et al. 2020a, c; Colucci et al. 2007). Newer targeted chemistries have the advantage over contact fungicides of being able to move into leaves, where they are protected from removal by rain and can redistribute to unprotected leaf surfaces. Therefore, mixtures with newer chemistries that are single-site inhibitors are common, in order to improve disease management and reduce the risk of fungicide resistance (Brent and Hollomon 2007).

Oxathiapiprolin formulated as Orondis Opti A, Orondis Gold 200, or Plenaris 200 was included in six of the nine bioassays and had the lowest RDS in five of the six bioassays (Table 3). The average RDS for the oxathiapiprolin formulations across all bioassays was 0.1%. Orondis Opti A/Zorvec Enicade (Corteva Agriscience) was also among the most efficacious fungicides in multiple studies in Michigan, New York, Ohio, and Delaware (Goldenhar and Hausbeck 2019; Keinath et al. 2019; Miller et al. 2020; Salas et al. 2019). The AI in Orondis fungicides (FRAC group 49) has a medium to high resistance risk (FRAC 2020). In order to manage resistance development, Orondis fungicides are only marketed as premixtures in the United States, and the number of applications per season are limited.

Ranman was included in every bioassay and was consistently efficacious with RDS statistically similar to the most efficacious fungicides in six of the nine bioassays (Table 3). The consistently high efficacy of Ranman was similar to trials in North Carolina and Michigan and bioassays in Ohio, South Carolina, and New York (Adams et al. 2019, 2020b; Goldenhar and Hausbeck 2019; Keinath 2016; Keinath et al. 2019).

Elumin, which was the most recently registered fungicide (2017) included in our study, was a late addition to the bioassay treatment list. It was only included in the 2019 Maryland bioassay and was highly effective at reducing RDS (Table 3). In Michigan, Elumin was statistically similar to the most effective fungicides in two out of three years and significantly more effective than the untreated control the third year (Goldenhar and Hausbeck 2019). Elumin was statistically similar to the most efficacious fungicide in 2019 in Ohio and North Carolina, but not in 2018 in North Carolina when it exhibited a more moderate control of cucurbit downy mildew (Adams et al. 2019, 2020b; Miller et al. 2020). Thirty-five isolates screened by Thomas et al. (2018) were highly sensitive to ethaboxam, the AI in Elumin, although baseline sensitivity varied depending on the geographic location from which the isolates were collected.

Omega was only included twice in our bioassays but was highly effective in Maryland in 2019 and effective in New York in 2019 (Table 3). Omega was similar to the most effective fungicides in New York, Ohio, South Carolina, North Carolina, and Michigan (Adams et al. 2020b; Goldenhar and Hausbeck 2019; Keinath et al. 2019).

AIs from 11 different FRAC groups were evaluated for efficacy in the nine bioassays conducted in four states in our study. Orondis formulations, Zing!, Bravo Ultrex, and Ranman were among the most effective fungicides in the majority of bioassays across all states (Table 4), whereas Quadris, Revus, Presidio, Forum, and Curzate were all ineffective in more than one bioassay (Table 4). The cutoff severity of 35% for determining fungicide efficacy, although significantly lower than the untreated control, was likely not a commercially acceptable level of

TABLE 4
Ranking of fungicides for effectiveness in reducing *Pseudoperonospora cubensis* infection across nine bioassays in four states[w]

	2016		2017				2018	2019	
Fungicide	DE	NY	MD	NY	PA[x]	PA[y]	NY	MD	NY
Quadris 2.08F	E	I	I	I	I	E	I	I	I
Revus 2.08SC	E	I	I	I	ND	ND	I	I	I
Presidio 4SC	*	E	H	I	E	H	I	I	I
Forum 4.17SC	ND	I	I	E	E	I	E	H	E
Curzate 50DF	H	H	H	I	H	E	H	I	H
Zoxamide	ND	ND	ND	ND	ND	ND	*	ND	I
Zampro 525SC	E	I	E	E	H	*	H	E	ND
Previcur Flex 6SL	*	E	ND	E	E	E	H	*	H
Omega 500F	ND	ND	ND	ND	ND	ND	ND	*	E
Ranman 400SC	*	E	H	E	H	H	H	*	E
Bravo Ultrex 82.5WDG	ND	*	H	*	H	H	H	*	E
Zing! 4.9SC	*	E	H	H	*	*	ND	*	ND
Elumin 4SC	ND	ND	ND	ND	ND	ND	ND	*	ND
Orondis formulations[z]	ND	ND	*	ND	*	*	H	*	*

[w] I = ineffective fungicide (relative disease severity [RDS] > 35% [Thomas et al. 2018]); E = effective fungicide (RDS < 35% but significantly higher than fungicide with the lowest RDS); H = highly effective fungicide (statistically similar to the fungicide with the lowest RDS); * = fungicide with lowest RDS in the bioassay; and ND = no data. States are Delaware (DE), New York (NY), Maryland (MD), and Pennsylvania (PA).

[x] Blair County, PA.

[y] Berks County, PA.

[z] Orondis Gold 200SC in NY, Orondis Opti A 0.83OD in PA, and Plenaris 200FS in MD.

control (Thomas et al. 2018). Pavelková et al. (2014) used a cut-off of <10% severity in a leaf disc bioassay to assign *P. cubensis* as sensitive to fungicide treatment, which would correlate more closely to our highly effective fungicides and those with the lowest RDS values in Table 4. The cucurbit hosts with the most acreage vary among the states in our study, with Maryland and Delaware acreage dominated by cucumbers and watermelon and Pennsylvania and New York acreage dominated by squash and pumpkins. Host availability and association with *P. cubensis* clade play important roles in the spread of cucurbit downy mildew and the population dynamics of *P. cubensis* (Rahman et al. 2021; Wallace et al. 2020). The use of cucumbers in the bioassays could have resulted in less inoculum in the Pennsylvania and New York locations and variability in the pathogen populations due to regional fungicide use. Host resistance in a limited number of commercial cucumber cultivars is available and provides growers with an additional tool and more integrated approach to manage cucurbit downy mildew. However, higher seed cost for resistant cultivars, concerns about their yielding ability, and the continued need to apply fungicides for other diseases are potential barriers to adoption. Bioassays provide a fast and easy way to determine fungicide efficacy, and results could be used to guide in-season fungicide recommendations for cucurbit downy mildew, or as the authors use them to monitor trends in efficacy over time and combine with field trial results to make fungicide recommendations in subsequent years.

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