Resistance to Fluopicolide and Propamocarb and Baseline Sensitivity to Ethaboxam Among Isolates of *Pseudoperonospora cubensis* From the Eastern United States

A. Thomas and K. N. Neufeld, Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695; K. W. Seebold, Valent USA LLC, Lexington, KY 40511; C. A. Braun, Bayer CropScience, 40789 Monheim am Rhein, Germany; M. R. Schwarz, Bayer CropScience, Research Triangle Park, NC 27709; and P. S. Ojiambo,[†] Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695

Abstract

Chemical control is currently the most effective method for controlling cucurbit downy mildew (CDM) caused by *Pseudoperonospora cubensis*. Most commercial cucurbit cultivars, with the exception of a few new cucumber cultivars, lack adequate disease resistance. Fluopicolide and propamocarb were among the most effective fungicides against CDM in the United States between 2006 and 2009. Since then, reduced efficacy of these two fungicides under field conditions was reported starting around 2013 but occurrence of resistance to fluopicolide and propamocarb in field isolates of *P. cubensis* had not been established. Thirty-one isolates collected from cucurbits in the eastern United States were tested for their sensitivity to fluopicolide and propamocarb using a leaf disc assay. This same set of isolates and four additional isolates (i.e., 35 isolates) were also used to establish the baseline sensitivity of *P. cubensis* to ethaboxam, an ethylamino-thiazole-carboxamide fungicide, which was recently granted registration to control CDM in the United States. About 65% of the isolates tested were resistant to fluopicolide

with at least one resistant isolate being present in samples collected from 12 of the 13 states in the eastern United States. About 74% of the isolates tested were sensitive to propamocarb with at least one resistant isolate being among samples collected from 8 of the 12 states in the study. The frequency of resistance to fluopicolide and propamocarb was high among isolates collected from cucumber, while the frequency was low among isolates collected from other cucurbit host types. All isolates tested were found to be sensitive to ethaboxam and EC₅₀ values ranged from 0.18 to 3.08 mg a.i./liter with a median of 1.55 mg a.i./liter. The ratio of EC₅₀ values for the least sensitive and the most sensitive isolate was 17.1, indicating that *P. cubensis* isolates were highly sensitive to ethaboxam. The most sensitive isolates to ethaboxam were collected from New York, North Carolina, and Ohio, while the least sensitive isolates were collected from Georgia, Michigan, and New Jersey. These results show that ethaboxam could be a viable addition to fungicide programs used to control CDM in the United States.

Cucurbit downy mildew (CDM), caused by the oomycete *Pseudoperonospora cubensis*, is considered the most economically important disease in cucurbit production worldwide (Lebeda and Cohen 2011; Ojiambo et al. 2015). The pathogen infects wild cucurbits and commercially cultivated cucurbit crops such as cantaloupe, cucumber, squash, pumpkin, and watermelon. Up to 60 species within the Cucurbitaceae family have been reported as hosts of *P. cubensis* (Lebeda and Urban 2007). In the United States, cucurbits are grown primarily in the eastern part of the country and California on a total area of 155,315 ha and the produce was valued at \$1.4 billion in 2015 (USDA-NASS 2016). In the continental United States, *P. cubensis* is widely thought to overwinter in southern Florida and along the Gulf of Mexico where mild winters are conducive to year round production of cucurbits. Sporangia of *P. cubensis* transported aerially from overwintering sources are responsible for initiation of epidemics in disease-free fields (Ojiambo and Holmes 2011). Disease development is favored by warm and humid weather conditions that typically prevail during the growing season in the eastern United States.

During the past two decades, a resurgence of CDM has been reported globally including the United States, resulting in severe disease epidemics (Cohen et al. 2015; Holmes et al. 2015; Kitner et al. 2015). In the United States, the *dm-1* gene from the plant introduction accession 197087 deployed in the 1960s (Barnes and Epps 1954) conferred resistance in cucumber and CDM was of minor concern, requiring minimal use of fungicides. However, this resistance was overcome in 2004 when CDM resurged in the United States, resulting in significant

losses (Holmes et al. 2006). Since the resurgence of CDM around the world, use of resistant cultivars alone for disease control has been largely ineffective due to the high pathogenic variability in *P. cubensis* (Lebeda and Cohen 2011; Thomas et al. 2017c). Since the pathogen is considered to have a high evolutionary potential (Cohen et al. 2015), available cultivars should be used in combination with other disease control methods, such as fungicides, to reduce the risk of *P. cubensis* overcoming host resistance. In addition, the high evolutionary potential of *P. cubensis* is associated with an increased risk of development of fungicide resistance, which in turn may result in fewer effective products being available for inclusion in fungicide spray programs. Consequently, growers will intensively use the few remaining effective fungicides, which further increases the risk of fungicide resistance development in *P. cubensis* (Zhu et al. 2008). Thus, a continuous introduction of products with new modes of action is required to maintain adequate disease control (Russell 2004).

Fungicide resistance management guidelines proposed by the Fungicide Resistance Action Committee (FRAC) should be followed to maintain product efficacy over the years. Fungicides in at least 11 chemical groups with different modes of action have been evaluated to control CDM in the United States (Cohen et al. 2015; Gisi and Sierotzki 2008; Ojiambo et al. 2010). Copper salts, mancozeb, and chlorothalonil are some of the most important multisite fungicides (FRAC codes M1, M2, and M3). Cyazofamid (quinone inside inhibitors, FRAC code 21), cymoxanil (cyanoacetamide-oxime, FRAC code 27), dimethomorph (carboxylic acid amides, FRAC code 40), famoxadone (quinone outside inhibitors, FRAC code 11), fluopicolide (benzamides, FRAC code 43), mefenoxam (phenylamides, FRAC code 4), and propamocarb (carbamates, FRAC code 28) are some of the single-site inhibitors that have been used to control CDM. Fungicides belonging to FRAC codes 4 and 11 are no longer effective for CDM control due to development of resistance in *P. cubensis*. Recently, an oxysterol binding protein inhibitor (oxathiapiprolin, FRAC code 49) (Sweigard et al. 2014) was registered as a novel compound to control CDM in the United States.

[†]Corresponding author: P. S. Ojiambo, E-mail: pojiambo@ncsu.edu

Funding: Bayer CropScience; Valent USA LLC.

Accepted for publication 28 February 2018.

A synthesis of fungicide field trials undertaken to determine the efficacy of different chemical groups in controlling CDM from 2000 to 2008 showed that fluopicolide (Presidio, Valent USA, LLC, Walnut Creek, CA) and propamocarb (Previcur Flex, Bayer CropScience, Research Triangle Park, NC) were the most effective fungicides during that period (Ojiambo et al. 2010). In the United States, Presidio (fluopicolide) was labeled for CDM in 2007, while Previcur Flex (propamocarb) was registered for use in 2004. The efficacy of fluopicolide and propamocarb made these two single-site inhibitors a good choice of fungicides for growers to include in their spray programs to control CDM. However, as with many single-site fungicides, reduced efficacy of fluopicolide developed shortly thereafter and was first reported in 2012 in field trials conducted in Georgia, Michigan, and North Carolina from 2012 to 2014 (Adams and Quesada-Ocampo 2014; Adams et al. 2015; Hausbeck and Linderman 2014; Langston and Sanders 2013). Similar observations of reduced efficacy of propamocarb were reported in Pennsylvania (Gugino and Grove 2016) and South Carolina (Keinath 2016) in 2015. However, no studies have been conducted to determine the prevalence of resistance of *P. cubensis* to fluopicolide or propamocarb in the United States. Monitoring development of fungicide resistance within pathogen populations is critical for effective disease management.

Identification of new, effective fungicide compounds can provide practical alternatives to fungicides that are less effective in controlling CDM. In 1998, ethaboxam (thiazole carboxamide, FRAC code 22) was introduced as a novel thiazole carboxamide to control a range of vegetable diseases caused by oomycetes including *P. cubensis* in

Korea (Kim et al. 1999). Ethaboxam interferes with the β -tubulin assembly during cell division and mitosis and has good protective, curative, systemic, and persistent activity and resulted in effective control of CDM when applied at 125 mg a.i./liter (Kim 1997). In the United States, ethaboxam is currently registered as a seed treatment (Intego Solo; Valent USA Corp., Walnut Creek, CA) for the control of *Phytophthora* and *Pythium* spp. In 2017, the Environmental Protection Agency granted a section 3 registration for use of ethaboxam throughout the United States to control CDM. Currently, there are no reports of insensitivity of *P. cubensis* to ethaboxam and it is important to establish the baseline sensitivity of *P. cubensis* to ethaboxam. Establishing the baseline sensitivity is useful in the detection of any future shifts toward insensitivity in pathogen populations by comparing detected insensitivity levels to baseline sensitivity in pathogen populations not exposed to ethaboxam. This study was therefore undertaken with the following objectives: i) determine the occurrence of resistance to fluopicolide and propamocarb in isolates of *P. cubensis* collected primarily in the eastern United States, and ii) establish the baseline sensitivity and the minimum inhibitory dose to ethaboxam in isolates of *P. cubensis* collected in the eastern United States. Previous studies (Thomas et al. 2017b, c) reported an association between specific cucurbit host types and either the mating type or pathotype of *P. cubensis*. Thus, to explore the potential for using these two phenotypes of the pathogen to screen isolates for fungicide resistance, we further examined if resistance to fluopicolide or propamocarb was associated with the mating type or pathotype of *P. cubensis*.

Materials and Methods

Isolate collection and maintenance. A total of 36 isolates of *P. cubensis* collected from 2007 to 2015 from cucumber, cantaloupe, acorn and butternut squash, and pumpkin were used in this study (Table 1). Thirty-five of these isolates were collected from 13 states in the eastern United States (Alabama, Florida, Georgia, Illinois, Maryland, Michigan, New Jersey, New York, North Carolina, Ohio, South Carolina, Tennessee, and West Virginia) from 2007 to 2015, while one isolate was collected from California in 2008. These states are part of the Cucurbit Downy Mildew ipmPIPE network (<http://cdm.ipmPIPE.org>) that facilitates monitoring of CDM outbreaks and is a source for information on fungicide spray recommendations for CDM within the eastern United States. These isolates were primarily collected from nontreated plants within the disease monitoring network. Some isolates were also collected from plants in research plots and commercial fields that were either treated or not treated with fungicides. All isolates collected in 2008 were previously stored at -80°C and maintained by periodic reinoculation on their respective host types. Isolates were obtained from leaves with discrete lesions collected from 2012 to 2015 and subsequently maintained on their respective host plants as single-lesion isolates. Leaf samples without discrete lesions were further processed to generate single-lesion isolates as described by Thomas et al. (2017b). All isolates were maintained in the laboratory using a detached leaf assay by propagating them on their respective host types from which they were originally isolated. Briefly, detached leaves of 4-week-old plants were placed on moist paper towels in clear acrylic boxes and the abaxial side was spray-inoculated with a sporangial suspension at a concentration of 2×10^4 sporangia/ml. Inoculated leaves were incubated in a growth chamber at $21/18^{\circ}\text{C}$ under 12/12 h light/dark cycle and then reinoculated to produce adequate amounts of sporangia for use in subsequent assays. Maintenance and propagation of isolates was repeated as necessary until isolates were ready for use in the fungicide assay experiments.

Baseline sensitivity and discriminatory concentrations for fluopicolide and propamocarb. Baseline sensitivity of *P. cubensis* to fluopicolide and propamocarb was determined using reference isolates 05A1 and 05A2. These isolates were originally collected in 2005 from cucumber in Oceana County, MI. Fungicide concentrations to be tested were determined based on the recommended labeled spray rate and volume per ha. The recommended spray volume for fluopicolide and propamocarb was determined to be 400 liters/ha. Stock suspensions of fluopicolide (Presidio, 39.5% active ingredient) were prepared at 50 mg a.i./liter in sterile water and serial dilutions

Table 1. Source and description of *Pseudoperonospora cubensis* isolates used to determine their sensitivity to fluopicolide and propamocarb and establish the baseline sensitivity to ethaboxam

Isolate	State	County	Host	Year of collection
2013A15	Alabama	Macon	Cucumber	2013
2013A13	Alabama	Baldwin	Cucumber	2013
2013D6	Alabama	Escambia	Butternut squash	2013
08A1	California	Salinas	Cucumber	2008
2013D2	Florida	Hillsborough	Butternut squash	2013
2013A2	Florida	Collier	Cucumber	2013
08F1	Georgia	Tift	Acorn squash	2008
2013A3	Georgia	Worth	Cucumber	2013
2013A64	Georgia	Tift	Cucumber	2013
2013A74	Georgia	Tift	Cucumber	2013
2014A50	Illinois	Douglas	Cucumber	2014
2014A8	Maryland	Wicomico	Cucumber	2014
2013A23	Michigan	Monroe	Cucumber	2013
2013A77	Michigan	Ingham	Cucumber	2013
2013A78	Michigan	Ingham	Cucumber	2013
2014A12	Michigan	Ingham	Cucumber	2014
2014A13	Michigan	Calhoun	Cucumber	2014
2014A14	Michigan	Calhoun	Cucumber	2014
2013A18	New Jersey	Salem	Cucumber	2013
2013B17	New York	Ontario	Cantaloupe	2013
2014A15	New York	Suffolk	Cucumber	2014
08C1	New York	Suffolk	Pumpkin	2008
2013D4	North Carolina	Johnston	Butternut squash	2013
07B1	North Carolina	Sampson	Cantaloupe	2007
2012A11	North Carolina	Johnston	Cucumber	2012
2014A21	North Carolina	Johnston	Cucumber	2014
2013C3	North Carolina	Johnston	Pumpkin	2013
2013B4	Ohio	Wayne	Cantaloupe	2013
2013A19	Ohio	Sandusky	Cucumber	2013
2013A20	Ohio	Medina	Cucumber	2013
2013F2	South Carolina	Charleston	Acorn squash	2013
2012D3	South Carolina	Charleston	Butternut squash	2012
2013A10	South Carolina	Charleston	Cucumber	2013
2015C2	South Carolina	Chester	Pumpkin	2015
2014A19	Tennessee	Franklin	Cucumber	2014
2013A44	West Virginia	Mercer	Cucumber	2013

were made from the stock solution to obtain the desired concentrations of 1, 5, 10, 20, and 30 mg a.i./liter. Similarly, stock solutions of propamocarb hydrochloride (Previcur Flex, 66.5% active ingredient) were prepared at 7,500 mg a.i./liter in sterile water and serial dilutions of the stock solution were then prepared to obtain the desired concentrations of 25, 75, 250, 750, and 2,500 mg a.i./liter.

A leaf disc assay (Lebeda and Urban 2007) was used to assess the sensitivity of the above reference isolates of *P. cubensis* to fluopicolide and propamocarb. Briefly, leaf discs (15 mm in diameter) were cut from the first, second, or third leaves of 3- to 4-week-old plants and placed into wells (one disc per well) of a 24-well plate filled with 1 ml of 0.5% water agar, with the adaxial side on the water agar. Each fungicide concentration was replicated four times using leaf discs from the same leaf. The assay was repeated once and a new leaf was used for each run of the assay. The cucurbit host from which each reference isolate was originally obtained was used in the leaf disc assay. Each leaf disc was treated by pipetting 10 μ l of the respective fungicide in about 1- μ l droplets across the surface of the leaf disc. These droplets were then spread evenly using a sterile paint brush dipped in the respective fungicide and dabbed to remove excess fluid. After 24 h, fungicide treated leaf discs were spray-inoculated with a sporangial suspension at a concentration of 1×10^4 sporangia/ml. A nontreated control (sprayed with sterile distilled water only) was maintained for each plate during all experiments. Treated and nontreated control leaf discs were then incubated in a growth chamber at 21/18°C under 12/12 h light/dark cycle for 5 days.

Disease severity on each fungicide-treated and nontreated leaf disc was visually assessed 5 days postinoculation (DPI) to estimate the percent leaf disc area infected. Disease severity data were analyzed using PROC GLIMMIX (SAS version 9.4; Cary, NC) to determine differences among isolates and fungicide concentrations. The lowest concentration above which no disease was observed on treated leaf discs was identified as the discriminatory concentration for either fluopicolide or propamocarb. In addition, for each isolate, mean relative severity (RS), i.e., disease severity on treated leaf disc relative to severity on nontreated control, was linearly regressed to the $\log_{10}$ of the fungicide concentration using PROC REG in SAS. The effective concentration (i.e., EC_{50}) for each isolate was subsequently calculated as the point on the regression line at which the RS = 50%.

Sensitivity of isolates to fluopicolide and propamocarb. The sensitivity of 31 isolates of *P. cubensis* (Table 2) to fluopicolide and propamocarb was determined using the leaf disc assay described above. Of these isolates, 22 were from cucumber, while nine were from other cucurbit host types. The cucurbit host type from which each isolate was originally obtained was used in the assay to determine the sensitivity of each respective isolate. Each leaf disc was treated with one of the fungicides at the discriminatory concentration identified for that fungicide. Plates with treated leaf discs were left on a laboratory bench at room temperature for 24 h, after which leaf discs were spray-inoculated with 1×10^4 sporangia/ml. Treated and nontreated control leaf discs were then incubated for 5 days at 21/18°C as described above. Each fungicide concentration was replicated four times and the assay was repeated once.

Disease severity on leaf discs was visually assessed as the percent leaf disc area affected. The relative severity, RS, was calculated for each of the five serial dilutions per fungicide as defined above. In addition, isolates were identified as either resistant or sensitive to fluopicolide or propamocarb based on the RS. Isolates with a RS $\leq 35\%$ were identified as sensitive, while isolates with RS $>35\%$ were identified as resistant (Lebeda and Urban 2007) to either fluopicolide or propamocarb.

Baseline sensitivity to ethaboxam. The baseline sensitivity of *P. cubensis* to ethaboxam was determined using a total of 35 isolates (Table 1). Stock solutions of ethaboxam (ethaboxam technical grade, 98.9% a.i.) were prepared at 100 mg a.i./liter in a 1% acetone-distilled water mixture. Serial dilutions of stock solutions were then made with the 1% acetone-distilled water mixture to the desired concentrations of 1, 3, 5, 10, and 15 mg a.i./liter. Sensitivity of isolates to ethaboxam was determined using the leaf disc assay as described above using leaf discs from the second true leaves of cucurbit host types from which the

isolates were originally obtained. Leaf discs were inoculated using 1×10^4 sporangia/ml and treated and nontreated control leaf discs were incubated for 5 days at 21/18°C. Each fungicide concentration was replicated four times and the assay repeated once.

Disease severity on fungicide-treated and nontreated leaf discs was assessed visually to estimate the percent leaf area infected 5 days after inoculation. Disease severity data were analyzed using PROC GLIMMIX in SAS to determine differences among isolates and fungicide concentrations. The lowest concentration above which no disease was observed on treated leaf discs was identified as the minimum inhibitory concentration (MIC) for ethaboxam. Linear regression of RS on $\log_{10}$ of fungicide concentration was used to estimate the EC_{50} values as described above.

Mating and pathotype determination. Mating type was determined for a subset of isolates (Table 2) by coinoculating an isolate with a known A1 and A2 tester strain (Thomas et al. 2017b). Equal volumes (1:1) of sporangial suspension (2×10^4 spores/ml) of the tester strain and each isolate were mixed together and used to inoculate first true leaves of 3-week-old cantaloupe or cucumber plants. Detached leaves were placed, adaxial side down, on moist paper towels in clear acrylic boxes and the abaxial side of each leaf was spot inoculated with 10 μ l of the sporangial mixture. Inoculated leaves were incubated in a growth chamber at 21°C at 50 to 60% relative humidity under 12/12 h light/dark cycle. About 10 DPI, discs measuring 11 mm diameter were randomly cut from infected leaves and clarified for 24 h in ethyl alcohol-acetic acid solution (3:1 v/v).

Table 2. Fluopicolide and propamocarb sensitivity phenotype and characteristics for some selected isolates of *Pseudoperonospora cubensis* collected in the eastern United States

Isolate	Fluopicolide		Propamocarb		Mating type	Pathotype ^c
	RS ^a	Sensitivity ^b	RS ^a	Sensitivity ^b		
2013A2	1.00	Resistant	0.45	Resistant	A1	— ^d
2012A11	0.96	Resistant	0.67	Resistant	A1	3
2013A3	0.94	Resistant	0.98	Resistant	A1	1
2014A50	0.91	Resistant	0.98	Resistant	—	—
2013A77	0.88	Resistant	0.91	Resistant	—	—
2014A8	0.57	Resistant	0.69	Resistant	—	—
08A1	0.41	Resistant	0.59	Resistant	A1	3
2013A19	1.00	Resistant	0.13	Sensitive	A1	3
2013A64	1.00	Resistant	0.12	Sensitive	—	—
2014A13	0.89	Resistant	0.07	Sensitive	—	—
2014A19	0.89	Resistant	0.01	Sensitive	—	—
2014A14	0.87	Resistant	0.06	Sensitive	—	—
2013A18	0.84	Resistant	0.12	Sensitive	A1	3
2013A15	0.84	Resistant	0.06	Sensitive	A1	3
2014A12	0.76	Resistant	0.14	Sensitive	—	—
2014A15	0.75	Resistant	0.14	Sensitive	—	—
2013C3	0.65	Resistant	0.03	Sensitive	A1	1
2013A44	0.57	Resistant	0.13	Sensitive	—	—
08F1	0.39	Resistant	0.09	Sensitive	A2	6
2013A20	0.38	Resistant	0.34	Sensitive	A1	3
2015C2	0.14	Sensitive	0.18	Sensitive	—	—
2014A21	0.12	Sensitive	0.12	Sensitive	—	—
08C1	0.10	Sensitive	0.05	Sensitive	A1	1
2012D3	0.08	Sensitive	0.02	Sensitive	A2	5
2013A13	0.01	Sensitive	0.96	Resistant	—	—
2013A78	0.00	Sensitive	0.24	Sensitive	—	—
2013D2	0.00	Sensitive	0.04	Sensitive	A2	—
2013A23	0.00	Sensitive	0.04	Sensitive	—	—
2013B17	0.00	Sensitive	0.04	Sensitive	A1	1
2013D4	0.00	Sensitive	0.00	Sensitive	A2	5
2013F2	0.00	Sensitive	0.00	Sensitive	A2	5

^a Mean relative disease severity.

^b Sensitive isolates have RS ≤ 0.35 , while resistant isolates have RS > 0.35 (Urban and Lebeda 2007).

^c Pathotype designation is based on the compatibility pattern of the isolate with a differential host set (Thomas et al. 2017c).

^d Mating type or pathotype of the isolate was not determined.

Clarified leaf discs were examined for the presence of oospores using a compound microscope (100× magnification). An unknown isolate was considered to be an A1 or A2 if oospores were produced when coinoculated with an A2 or A1 tester strain, respectively.

Pathotypes of a subset of isolates collected in this study (Table 2) were determined using a differential host set (Lebeda and Widrechner 2003) as described by Thomas et al. (2017b). Briefly, first and second true leaves from 4-week-old host differential plants were placed adaxial side down on moist paper towels in clear acrylic boxes and the abaxial sides were spray-inoculated with 5×10^3 sporangia/ml. Inoculated leaves were then incubated in a growth chamber at 21/18°C under 12/12 h light/dark cycle. Disease severity (%) on each leaf was assessed 5 to 7 days after inoculation as the percent leaf area infected. Two leaf discs measuring 9 mm in diameter were cut from each leaf at 7 DPI and shaken well in microcentrifuge tubes with 500 μ l of 50% ethanol to dislodge the sporangia. Sporangial counts

were then determined using a hemocytometer and sporangia production expressed as spores per cm^2 leaf area. The entire assay was repeated once. A concentration $\geq 5 \times 10^3$ sporangia/ cm^2 and a disease severity $\geq 50\%$ was considered a compatible (+) reaction and an absence of sporulation or very low sporulation ($\leq 3 \times 10^3$ sporangia/ cm^2) and $<20\%$ disease severity was considered an incompatible (–) reaction (Cohen et al. 2003, 2015; Thomas et al. 2017b). Pathotype designation was based on the pattern of compatible and incompatible reactions of each isolate with the differential host set (Cohen et al. 2015; Thomas et al. 1987).

Data analysis. To determine whether disease severity data recorded from the two experimental runs could be combined prior to analysis, the homogeneity of variance assumption for equality of variance among runs was tested using GLIMMIX procedure in SAS. The homogeneity test for equality of variance among runs was not significant ($P > 0.05$) for all the assays and data from experimental runs were combined for subsequent analysis described above. Association between original host of isolation and sensitivity of isolates to either fluopicolide or propamocarb was examined using contingency tables. Statistical differences in the distribution of resistant and sensitive isolates among cucurbit host types were examined based on Fisher's exact test using PROC FREQ in SAS. The distribution of EC_{50} values for ethaboxam was analyzed using the UNIVARIATE procedure in SAS.

Results

Baseline sensitivity and discriminatory concentration for fluopicolide and propamocarb. Relative severity (RS) was not significantly affected by isolate ($P = 0.0801$) but was significantly affected by fungicide concentration ($P < 0.0001$) when reference isolates were tested against fluopicolide. Specifically, RS ranged from 0 (at 30 mg a.i./liter) to 1 (for the nontreated control). In addition, RS at 10 mg a.i./liter (RS = 0.01) and 20 mg a.i./liter (RS = 0.02) was significantly ($P < 0.05$) higher than that at the nontreated control and 1 mg a.i./liter (RS = 0.93) but was substantially lower than at 30 mg a.i./liter. Thus, 10 mg a.i./liter was identified as the discriminatory concentration to evaluate sensitivity of test isolates to fluopicolide. RS values at 10 mg a.i./liter were 0.01 and 0.02 for reference isolate 05A1 and 05A2, respectively.

Similarly, RS was not significantly affected by isolate ($P = 0.4192$) but was significantly affected by propamocarb concentration ($P < 0.0001$). Values for RS ranged from 0 (at 2,500 mg a.i./liter) to 1 (nontreated control). In addition, RS at 750 mg a.i./liter was significantly ($P < 0.05$) lower than that at all other concentrations except 2,500 mg a.i./liter. Thus, 750 mg a.i./liter was selected as the discriminatory dose for subsequent testing of sensitivity of *P. cubensis* isolates to propamocarb. RS at 750 mg/liter for the reference isolates were 0.06 for 05A1 and 0.09 for 05A2. EC_{50} values for fluopicolide were 2.65 and 3.57 mg a.i./liter for 05A1 and 05A2, respectively. The EC_{50} values for propamocarb were comparatively lower and were 0.11 mg a.i./liter for 05A1 and 0.07 mg a.i./liter for 05A2.

Sensitivity to fluopicolide and propamocarb. Relative severity was significantly ($P < 0.0001$) affected by isolate in leaf disc assays for fluopicolide sensitivity. Some of the isolates that had the highest values (RS > 0.98) when tested against fluopicolide included 2012A11, 2013A3, 2013A19, and 2013A64, while isolates with the lowest values (RS = 0) included 2013B17, 2013D2, and 2013F2 (Fig. 1). Of the 31 isolates tested, 20 (65% of total) had an RS > 0.35 and were classified as resistant to fluopicolide, while 11 (35% of total) had an RS ≤ 0.35 and were classified as sensitive to fluopicolide (Table 2). Isolates classified as resistant to fluopicolide originated from field samples collected from Alabama, Georgia, Florida, Illinois, Michigan, New York, North Carolina, Ohio, Tennessee, and West Virginia. The range of RS for these resistant isolates was 0.38 to 1.0 with a mean of 0.80 (Table 2). In contrast, RS values for the sensitive isolates ranged from 0 to 0.27 with a mean of 0.07.

Relative severity was also significantly ($P < 0.0001$) affected by isolate in the leaf disc assay for sensitivity to propamocarb. Isolates 2013A3, 2013A13, 2013A77, and 2014A8 had the highest values (RS > 0.90), while 2013D4, 2013F2, and 2014A12 were among the isolates with the lowest RS values (Fig. 2). Of the 31 isolates

Fig. 1. Mean relative disease severity of *Pseudoperonospora cubensis* isolates on detached leaf discs of cucumber treated with fluopicolide at the discriminatory concentration of 10 mg a.i./liter. Disease severity was assessed 5 days following inoculation of fungicide treated leaf discs with sporangia of respective isolates. The dashed horizontal line is the relative degree of infection above which an isolate was classified as being resistant to fluopicolide.

Fig. 2. Mean relative degree of infection of *Pseudoperonospora cubensis* isolates on detached leaf discs of cucumber treated with propamocarb at the discriminatory dose of 750 mg a.i./liter. Degree of infection was assessed 5 days following inoculation of fungicide treated leaf discs with sporangia of respective isolates. The dashed horizontal line is the relative degree of infection above which an isolate was classified as being resistant to propamocarb.

tested, eight (26% of total) had an RS > 0.35 and were classified as resistant, while 25 (74% of total) had an RS ≤ 0.35 and were classified as sensitive to propamocarb (Table 2). Propamocarb-resistant isolates were collected from infected samples from Alabama, California, Georgia, Florida, Illinois, Maryland, Michigan, and North Carolina. RS values for resistant isolates ranged from 0.45 to 0.98 with a mean of 0.78, while that of sensitive isolates was 0 to 0.34 with a mean of 0.09 (Table 2).

Association of fluopicolide and propamocarb resistance with cucurbit host type. The distribution of isolates that were found to be resistant or sensitive to fluopicolide differed significantly ($\chi^2 = 9.91$; $P = 0.0033$) between cucumber and other cucurbit host types from which isolates were obtained (Fig. 3). About 82% of isolates originating from cucumber were resistant and only 18% were sensitive to fluopicolide. However, only 22% of isolates from other cucurbit hosts were resistant with 78% of the isolates being sensitive to fluopicolide (Fig. 3). Although isolates collected from other cucurbit host types were all sensitive to propamocarb, while 35% of isolates collected from cucumber were resistant, the distribution of propamocarb-resistant or propamocarb-sensitive isolates was not significantly ($\chi^2 = 4.41$; $P = 0.0677$) different among cucurbit host types (Fig. 3).

Baseline sensitivity to ethaboxam. All isolates of *P. cubensis* tested were sensitive to ethaboxam, even though the MIC differed among isolates (Table 3). Of the 35 isolates tested, 11 (31% of total) had an MIC of 5 mg a.i./liter, while 20 isolates (57% of total) had an MIC of 10 mg a.i./liter. Four isolates (11% of total), 07B1, 2013D4, 2013F2, and 2013A13 had an MIC of 15 mg a.i./liter (Table 3). The three isolates that had the highest MIC of 15 mg a.i./liter were collected from field samples from Johnston (isolate 07B1) and Sampson (isolate 2013D4) counties in North Carolina and Charleston, South Carolina (isolate 2013F2).

The mean ethaboxam EC₅₀ values for the test isolates ranged from 0.18 mg a.i./liter for 2013B4 collected from field samples from Ohio to 3.08 mg a.i./liter for 2013A3 collected from field sample in Georgia (Table 3). The mean EC₅₀ value across all the 34 isolates tested in the study was 1.35 mg/liter. Ethaboxam EC₅₀ values were not normally distributed ($P < 0.0158$) and the observed distribution (Fig. 4) had a mean and median value of 1.38 and 1.55 mg a.i./liter, respectively. Ten isolates (28% of total) had EC₅₀ values ranging from 0 to 1 mg a.i./liter, 18 isolates (51% of total) had EC₅₀ values ranging from 1 to 2 mg a.i./liter, and seven isolates (21%) had EC₅₀ values ranging from 2 to 3 mg a.i./liter. The mean EC₅₀ value for isolates with the lowest MIC of 5 mg a.i./liter was 1.23 mg a.i./liter. The latter mean EC₅₀ value was comparatively lower than that of isolates with an MIC of 10 and 15 mg a.i./liter whose mean EC₅₀ values were 1.41

and 1.38 mg a.i./liter, respectively. Ratios between the EC₅₀ for least and most sensitive isolate (i.e., diversity factor, DvF) were low and were 12.6, 14.0, and 5.5 for the group with lowest, intermediate, and highest MICs with a DvF of 17.1 across all isolates (Table 3). Among states, isolates with the lowest EC₅₀ values were from New York and Ohio, while those with the highest EC₅₀ values were isolated from field samples from Georgia and New Jersey (Fig. 5).

Mating type and pathotype determination. A1 and A2 mating types were recovered among the 16 isolates characterized for mating type. Only the A1 mating type was found among isolates that were resistant to fluopicolide, while both A1 and A2 mating types were found among isolates that were sensitive to fluopicolide. Similarly, only the A1 mating type was found among propamocarb-resistant isolates, while both A1 and A2 mating types were found among sensitive isolates. Isolates characterized in this study belonged to pathotypes 1, 3, 5, and 6. Isolates that were resistant to fluopicolide were pathotypes 1 and 3, while those that were sensitive were pathotypes 1, 5, and 6. Similarly, pathotypes 1 and 3 were found among propamocarb-resistant isolates, while pathotypes 1, 3, 5, and 6 were found among sensitive isolates.

Discussion

Chemical control is currently the most effective method for CDM since most commercial cucurbit varieties, with the exception of a few new cucumber cultivars, lack adequate disease resistance. Fungicides with a single-site mode of action are highly effective but the high

Table 3. Ethaboxam minimum inhibitory concentration (MIC) and effective concentration (EC₅₀) values for isolates of *Pseudoperonospora cubensis* collected in the eastern United States

Isolate	MIC (mg/liter)	EC ₅₀ (mg/liter)	Range EC ₅₀ (mg/liter)	Resistance diversity factor ^a
2013B4	5	0.18	0.03–0.25	
2014A21	5	0.24	0.18–0.56	
08F1	5	0.33	0.26–1.23	
2014A8	5	1.12	0.72–1.26	
2014A50	5	1.26	0.91–1.47	
2014A12	5	1.55	0.97–1.83	
2014A19	5	1.59	1.21–1.73	
2013A44	5	1.62	1.52–1.69	
2014A14	5	1.67	1.46–1.75	
2014A13	5	1.70	1.57–1.74	
2013A10	5	2.26	2.17–2.38	12.6
2013B17	10	0.22	0.03–0.31	
08C1	10	0.23	0.60–0.34	
2013C3	10	0.25	0.03–0.32	
2012D3	10	0.27	0.24–0.34	
2013A15	10	0.32	0.28–0.81	
2013A64	10	0.45	0.23–1.76	
2013D2	10	1.18	0.81–1.20	
2013A2	10	1.19	0.80–1.21	
2014A15	10	1.32	0.87–1.56	
2012A11	10	1.37	1.27–1.40	
2013A19	10	1.52	1.28–1.63	
2013D6	10	1.52	1.20–1.54	
08A1	10	1.55	0.59–1.69	
2013A77	10	1.61	1.58–1.63	
2013A78	10	2.07	1.90–2.11	
2013A20	10	2.25	1.71–2.47	
2013A23	10	2.31	2.05–2.41	
2013A18	10	2.69	2.42–2.73	
2013A74	10	2.87	2.74–2.89	
2013A3	10	3.08	2.58–3.20	14.0
07B1	15	0.35	0.17–0.42	
2013A13	15	1.44	1.07–1.59	
2013F2	15	1.81	1.69–1.90	
2013D4	15	1.93	1.64–1.97	5.5

^a Ratio between EC₅₀ value of the least sensitive and most sensitive isolate within each group of minimum inhibitory concentration.

Fig. 3. Frequency distribution of resistant and sensitive isolates of *Pseudoperonospora cubensis* based on isolates collected from cucumber and other cucurbit host types. The total number of isolates (*n*) from cucumber and other cucurbits, namely cantaloupe, pumpkin, and squash (i.e., other) that were assayed for fungicide sensitivity is shown in parentheses within each bar.

potential of *P. cubensis* to develop resistance implies that such fungicides could become less effective due to buildup of resistant isolates within pathogen populations. In this study, we examined shifts in sensitivity of *P. cubensis* to fluopicolide and propamocarb among a sample of isolates collected in the eastern United States. Variation in baseline sensitivity of *P. cubensis* to ethaboxam, a newly introduced fungicide against oomycetes, was also examined and is reported here for the first time, to facilitate detection of any future shifts toward insensitivity in pathogen populations in the United States. Although the sample size of isolates evaluated in this study was limited, with three states being represented by one isolate, a high proportion of isolates of *P. cubensis* examined was resistant to fluopicolide, while most of these isolates were sensitive to propamocarb. Multiple fungicide resistance was also observed among some isolates that exhibited a high degree of insensitivity to both fluopicolide and propamocarb. All isolates examined were sensitive to ethaboxam as exhibited by the relatively low ratio of the EC₅₀ values of the most sensitive to least sensitive isolate. This study represents the first documentation of the extent of fluopicolide and propamocarb sensitivity and the baseline sensitivity to ethaboxam among *P. cubensis* isolates across the eastern United States.

Isolates resistant to fluopicolide were widespread across the eastern United States and present in Alabama, Georgia, Florida, Illinois,

Fig. 4. Frequency distribution of EC₅₀ values (concentrations that inhibit growth by 50%) for the growth of *Pseudoperonospora cubensis* isolates tested on 1, 3, 5, and 10 mg a.i./liter of ethaboxam.

Fig. 5. Range of EC₅₀ values (concentrations that inhibit growth by 50%) across 13 states from which isolates of *Pseudoperonospora cubensis* were collected in the eastern United States.

Michigan, New York, North Carolina, Ohio, Tennessee, and West Virginia. These resistant isolates were collected in 2008, 2012, 2013, and 2014. This high proportion of fluopicolide-resistant isolates in the United States was not unexpected as it explains reports of reduced efficacy of fluopicolide from field trials conducted in Georgia (Langston and Sanders 2013), Michigan (Hausbeck and Linderman 2014), and North Carolina (Adams and Quesada-Ocampo 2014; Adams et al. 2015). Additional evidence that a shift toward increased resistance to fluopicolide has occurred is that reduced efficacy was initially reported from field observations. Growers are risk averse by nature and when a fungicide becomes less effective, more applications will be needed to achieve effective disease control. Growers are also likely to discontinue the use of less effective products and to rely more heavily on those that are effective. Successive use of a single fungicide over time can select for resistant isolates in a given pathogen population. For example, use of flumorph over an extended period of time was shown to rapidly select for resistance in *P. cubensis* under laboratory conditions (Zhu et al. 2008). More recently, reduced efficacy of fluopicolide and propamocarb has been reported in Pennsylvania (Gugino and Grove 2016, 2017) and South Carolina (Keinath 2016). About 39% of the isolates examined in this study (e.g., 2013A2, 2013A64, 2012A11) had an RS > 0.80 when tested against fluopicolide and these isolates could be classified as highly resistant. The highly resistant isolates identified in this study were geographically widespread, having originated from all the states included in this study except Maryland, New York, and West Virginia. This latter observation could also further explain reports of reduced efficacy of fluopicolide across several states in the eastern United States. Based on these results, we recommend fluopicolide not to be used for a few years where efficacy is no longer acceptable. Additional studies could be conducted thereafter to determine whether the pathogen population has reverted back to one that is largely sensitive to fluopicolide to again warrant its use for CDM control.

About 26% of the isolates tested in this study were resistant to propamocarb and these specific isolates were collected in 2008, 2012, 2013, and 2014. This level of resistance was unexpected for two reasons. First, reports of reduced efficacy of propamocarb in the United States have been reported only in recent years in North Carolina (Adams et al. 2015), Pennsylvania (Gugino and Grove 2016), and South Carolina (Keinath 2016). However, this study shows that *P. cubensis* isolates resistant to propamocarb were also present in Alabama, Florida, Georgia, Maryland, Michigan, and Illinois in the United States. Secondly, propamocarb resistance in *P. cubensis* has been rare globally and has previously been reported only in Israel (Cohen and Samoucha 1984) and the Czech Republic (Pavelková et al. 2014; Urban and Lebeda 2007). Shifts toward increased insensitivity to propamocarb in *P. cubensis* have been monitored over the years in the Czech Republic with insensitivity to propamocarb being highly unpredictable (Urban and Lebeda 2007). It has been suggested that mutation, migration, and gene flow may be responsible for persistently incorporating strains with increased insensitivity within the pathogen population (Pavelková et al. 2014). Thus, although the frequency of *P. cubensis* isolates that were insensitive to propamocarb in the present study was relatively low, there may be a risk of their expansion, which could lead to an increase in their frequency in the eastern United States. In addition, the occurrence of propamocarb-resistant isolates observed in this study may also be a reflection of the natural variation in sensitivity to propamocarb within the populations of *P. cubensis* (Thomas et al. 2017a). Thus, constant resistance monitoring programs may be needed to establish the prevailing degree and extent of propamocarb-resistant isolates within pathogen populations in the eastern United States.

P. cubensis is considered a high-risk pathogen for development of resistance to fungicides and has developed resistance to several important fungicides such as phenylamides, carboxylic acid amides, and quinone outside inhibitors (Cohen et al. 2015; Lebeda and Cohen 2011; Pavelková et al. 2014). Thus, use of fungicides from different chemical groups as tank mixes or alternations, is one of the methods recommended to avoid unexpected control failures and sustain the usefulness of new and existing products. While resistance to structurally related chemical compounds (cross resistance) has been reported

in *P. cubensis*, resistance to structurally unrelated compounds (multiple resistance) is less common (Gisi and Sierotzki 2014; Pavelková et al. 2014). In this study, seven out of the eight propamocarb-resistant isolates were also resistant to fluopicolide, thereby exhibiting multiple resistance. Multiple resistance in *P. cubensis* has also been reported in Israel where metalaxyl-resistant isolates were also reported to be resistant to propamocarb (Cohen and Samoucha 1984). Although the mechanism of multiple resistance observed in these isolates is not known, it could be related to increased efflux of the fungicides with the aid of ATP-binding cassette (ABC) transporters that leads to a kind of resistance known as multidrug resistance (de Waard et al. 2006). Cloned ABC transporter genes in *Botrytis cinerea* have been correlated with multidrug resistance to several fungicides used to control gray mold (Kretschmer et al. 2009). In addition, it is possible that these isolates have specific resistance to both fungicides as has been reported for *B. cinerea* causing gray mold in grapevines (Latorre and Torres 2012). The selection of *P. cubensis* isolates with multiple resistance to propamocarb and fluopicolide in cucurbit fields reported in the present study emphasizes the importance of following guidelines of the Fungicide Resistance Action Committee (FRAC 2017) to manage fungicide resistance development in *P. cubensis*. Variability in the fitness of metalaxyl-resistant isolates has been reported for other oomycetes such as *P. infestans* (Cohen and Reuveni 1983; Lee et al. 1999). Although examining fitness of resistant isolates was not a goal of this study, some of the fluopicolide- and propamocarb-resistant isolates had growth on nontreated leaf tissue that was similar to that of sensitive isolates, while other resistant isolates had growth on nontreated leaf tissue that was relatively limited compared with that of the sensitive isolates. Additional field studies, especially with propamocarb, are still needed to determine whether frequency of resistant isolates will change in response to the use or lack of use of propamocarb in cucurbit fields.

Although the number of isolates collected from other host types was relatively small compared with isolates collected from cucumber, a significant association was observed between cucurbit host type and the sensitivity of isolates to fluopicolide. All isolates that were resistant to fluopicolide were collected from cucumber except for one isolate that was collected from pumpkin. Further, 82% of all isolates collected on cucumber were resistant, while all isolates collected on cantaloupe and squash were sensitive to fluopicolide. Although there was no significant association between host type and sensitivity, all propamocarb-resistant isolates were also collected from cucumber. Temporal variation and within-year variability in the efficacy of fluopicolide and propamocarb in controlling CDM has been reported in the eastern United States (Adams and Quesada-Ocampo 2014; Adams et al. 2015; Gugino and Grove 2016, 2017; Keinath 2016). For example, disease severity in plots treated with propamocarb in Pennsylvania in 2015 was similar to that of the nontreated control, while fluopicolide was highly effective (Gugino and Grove 2016). The reverse occurred in 2016 where propamocarb was highly effective, while disease severity in plots treated with fluopicolide was not different from that of the nontreated control (Gugino and Grove 2017). Recent studies on the pathogen population have identified two genetically distinct lineages within *P. cubensis* populations that are specialized on different cucurbit host types in the United States (Thomas et al. 2017a). Isolates that are specialized on cucumber belong to lineage II, while those that are specialized on other cucurbit host types belong to lineage I (Thomas et al. 2017a). Thus, temporal variability in fungicide efficacy could be partly attributed to the lineage of *P. cubensis* prevailing during the growing season. In this study, both A1 and A2 mating types were found among isolates of *P. cubensis* that were resistant and sensitive to fluopicolide and propamocarb and no specific patterns of association between pathotypes identified and fungicide sensitivity were observed among the subset of isolates examined. Similarly, no association has been observed between pathotype and sensitivity to propamocarb in *P. cubensis* populations in the Czech Republic (Urban and Lebeda 2007). A large sample of isolates collected especially from cantaloupe, pumpkin, and squash and characterized for mating type and pathotype will be needed to establish a clear relationship between fungicide sensitivity and mating type or pathotype in *P. cubensis* in the United States.

The high potential of *P. cubensis* to develop resistance to single-site fungicides implies that identification and testing of compounds with new modes of action is critical in the management of CDM. Ethaboxam is currently registered in the United States as a seed treatment against *Phytophthora* and *Pythium* and was recently granted registration for foliar use on cucurbits against CDM. In Korea, ethaboxam was found to be effective under field conditions against several oomycetes including *P. cubensis* (Kim et al. 1999). In this study, the variation in the baseline sensitivity of *P. cubensis* isolates from the eastern United States to ethaboxam was presented. Isolates from New York, North Carolina, and Ohio were among the most sensitive, while those from Georgia and New Jersey were the least sensitive to ethaboxam. In addition, most of the *P. cubensis* isolates that were resistant to fluopicolide and propamocarb were highly sensitive to ethaboxam. Prior to the use of a new fungicide, the baseline sensitivity of individuals in a pathogen population may already differ by a factor of 10 to 100 between the least and the most sensitive isolates (Gisi et al. 1997). This factor, which can be interpreted as the resistance diversity factor (i.e., DvF) within the baseline population, is dependent on the pathogen and the intrinsic activity of the compound of interest. For example, values of DvF ranging from 5 to 20 have been reported for azoxystrobin and cyproconazole for *Mycosphaerella graminicola* (Gisi et al. 1997). Comparatively higher values of DvF have been reported for the baseline sensitivity of *P. capsici* to fluopicolide (Keinath and Kousik 2011) and *P. infestans* to azoxystrobin (Gisi et al. 1997) with values of about 97 and 100, respectively. In this study, the DvF for ethaboxam was about 17, which indicates that the baseline populations of *P. cubensis* were highly sensitive to ethaboxam. Ethaboxam is a single site compound that disrupts the β -tubulin assembly in mitosis (FRAC 2017). The fungicide is classified as having a low to medium risk for resistance development and requires resistance management for sustained efficacy.

Management of CDM requires an integrated approach incorporating host resistance and fungicides from different chemical classes in the overall disease management program. Ethaboxam was recently registered for cucurbits to control CDM in the United States. The introduction of ethaboxam, which has a novel mode of action, is expected to provide cucurbit growers with a needed fungicide option in the management of CDM. Due to the ability of *P. cubensis* to develop resistance to fungicides, the potential of the pathogen to develop resistance to ethaboxam will need to be evaluated and the sensitivity monitored. Fungicide spray programs that combine or alternate ethaboxam with other compounds with different modes of action not known to be affected by resistance yet (e.g., oxathiapiprolin or fluzinam), will need to be evaluated to establish their potential of enhancing CDM control and reducing selection pressure for *P. cubensis* to develop resistance.

Acknowledgments

We wish to thank Taylor Ghiloni and Barbara Stevens for their technical assistance with various aspects of this work and collaborators on the CDM ipmPIPE project for their assistance in collecting field samples used in this study.

Literature Cited

- Adams, M. L., Parada, C. H., and Quesada-Ocampo, L. M. 2015. Evaluation of fungicides for control of downy mildew on cucumber, Kinston I 2014. Plant Dis. Manage. Rep. 9:V081.
- Adams, M. L., and Quesada-Ocampo, L. M. 2014. Evaluation of fungicides for control of downy mildew on cucumber. Kinston 2013. Plant Dis. Manage. Rep. 8:V240.
- Barnes, W. C., and Epps, W. M. 1954. An unreported type of resistance to cucumber downy mildew. Plant Dis. Rep. 38:620.
- Cohen, Y., Meron, I., Mor, N., and Zuriel, S. 2003. A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. Phytoparasitica 31:458-466.
- Cohen, Y., and Reuveni, M. 1983. Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields in Israel. Phytopathology 73: 925-927.
- Cohen, Y., and Samoucha, Y. 1984. Cross-resistance to four systemic fungicides in metalaxyl-resistant strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*. Plant Dis. 68:137-139.
- Cohen, Y., Van den Langenberg, K. M., Wehner, T. C., Ojiambo, P. S., Hausbeck, M., Quesada-Ocampo, L. M., Lebeda, A., Sierotzki, H., and Gisi, U. 2015.

- Resurgence of *Pseudoperonospora cubensis* - the agent of cucurbit downy mildew. *Phytopathology* 105:998-1012.
- de Waard, M. A., Andrade, A. C., Hayashi, K., Schoonbeek, H. J., Stergiopoulos, I., and Zwiers, L. H. 2006. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. *Pest Manag. Sci.* 62: 195-207.
- FRAC. 2017. Fungicide Resistance Action Committee Code List: Fungicides sorted by mode of action (including FRAC Code numbering).
- Gisi, U., Hermann, D., Ohl, L., and Steden, C. 1997. Sensitivity profiles of *Mycosphaerella graminicola* and *Phytophthora infestans* populations to different classes of fungicides. *Pestic. Sci.* 51:290-298.
- Gisi, U., and Sierotzki, H. 2008. Fungicide modes of action and resistance in downy mildews. *Eur. J. Plant Pathol.* 122:157-167.
- Gisi, U., and Sierotzki, H. 2014. Oomycete fungicides – phenylamides, quinone outside inhibitors and carboxylic acid amides. Pages 145-174 in: *Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management*. H. Ishii and D. W. Hollomon, eds. Springer, Japan.
- Gugino, B. K., and Grove, T. L. 2016. Evaluation of selected fungicides and fungicide programs for managing downy mildew of cucumber, 2015. *Plant Dis. Manage. Rep.* 10:V072.
- Gugino, B. K., and Grove, T. L. 2017. Evaluation of selected fungicides and fungicide programs for managing downy mildew of cucumber, 2016. *Plant Dis. Manage. Rep.* 11:V080.
- Hausbeck, M. K., and Linderman, S. D. 2014. Evaluation of fungicides for control of downy mildew of cucumber, 2013. *Plant Dis. Manage. Rep.* 8:V304.
- Holmes, G. J., Ojiambo, P. S., Hausbeck, M. K., Quesada-Ocampo, L., and Keinath, A. P. 2015. Resurgence of cucurbit downy mildew in the United States: a watershed event for research and extension. *Plant Dis.* 99:428-441.
- Holmes, G. J., Wehner, T., and Thornton, A. 2006. An old enemy re-emerges. *Am. Veg. Grow.* 54:14-15.
- Keinath, A. P. 2016. Utility of a cucumber plant bioassay to assess fungicide efficacy against *Pseudoperonospora cubensis*. *Plant Dis.* 100:490-499.
- Keinath, A. P., and Kousik, C. S. 2011. Sensitivity of isolates of *Phytophthora capsici* from the eastern United States to fluopicolide. *Plant Dis.* 95:1414-1419.
- Kim, D. S. 1997. Biological activity of LGC-30473: a novel Oomycetes fungicide. Pages 7-64 in: *Proceedings of International Symposium on Fungicide Resistance and Development of New Fungicides*. Working Group on Fungicide Resistance, Seoul, Korea.
- Kim, D. S., Park, H. C., Chun, S. J., Yu, S. H., Choi, K. J., Oh, J. H., Shin, K. H., Ko, Y. J., Kim, B. S., Hahn, Y. I., and Chung, B. K. 1999. Field performance of a new fungicide, ethaboxam, against cucumber downy mildew, potato late blight and pepper *Phytophthora* blight in Korea. *Plant Pathol. J.* 15:48-52.
- Kitner, M., Lebeda, A., Sharma, R., Runge, F., Dvořák, P., Tahir, A., Choi, Y.-J., Sedláková, B., and Thines, M. 2015. Coincidence of virulence shifts and population genetic changes of *Pseudoperonospora cubensis* in the Czech Republic. *Plant Pathol.* 64:1461-1470.
- Kretschmer, M., Leroy, M., Mosbach, A., Walker, A., Filinger, S., Mernke, D., Schoonbeek, H., Pradier, J., Leroux, P., de Waard, M. A., and Hahn, M. 2009. Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathog* 5:e1000696.
- Langston, D., and Sanders, F. H. 2013. Evaluation of fungicides for control of downy mildew on cucumber in Georgia I, 2012. *Plant Dis. Manage. Rep.* 7:V108.
- Latorre, B. A., and Torres, R. 2012. Prevalence of isolates of *Botrytis cinerea* resistant to multiple fungicides in Chilean vineyards. *Crop Prot.* 40:49-52.
- Lebeda, A., and Cohen, Y. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) - biology, ecology, epidemiology, host-pathogen interaction and control. *Eur. J. Plant Pathol.* 129:157-192.
- Lebeda, A., and Urban, J. 2007. Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Hort.* 731:327-336.
- Lebeda, A., and Widrechner, M. P. 2003. A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* 110:337-349.
- Lee, T. Y., Mizubuti, E., and Fry, W. E. 1999. Genetics of metalaxyl resistance in *Phytophthora infestans*. *Fungal Genet. Biol.* 26:118-130.
- Ojiambo, P. S., Gent, D. H., Quesada-Ocampo, L. M., Hausbeck, M. K., and Holmes, G. J. 2015. Epidemiology and population biology of *Pseudoperonospora cubensis*: a model system for management of downy mildews. *Annu. Rev. Phytopathol.* 53:223-246.
- Ojiambo, P. S., and Holmes, G. J. 2011. Spatiotemporal spread of cucurbit downy mildew in the eastern United States. *Phytopathology* 101:451-461.
- Ojiambo, P. S., Paul, P. A., and Holmes, G. J. 2010. A quantitative review of fungicide efficacy for managing downy mildew in cucurbits. *Phytopathology* 100:1066-1076.
- Pavelková, J., Lebeda, A., and Sedláková, B. 2014. Efficacy of fosetyl-Al, propamocarb, dimethomorph, cymoxanil, metalaxyl and metalaxyl-M in Czech *Pseudoperonospora cubensis* populations during the years 2005 through 2010. *Crop Prot.* 60:9-19.
- Russell, P. E. 2004. Sensitivity Baselines in Fungicide Resistance Research and Management, 3rd Ed. FRAC, Brussels, Belgium.
- Sweigard, J. A., Anreassi, J., Pember, S., Gutteridge, S., Pasteris, R., Hanagan, M. A., Carroll, A. C., Sopha, J., and Nesnow, D. 2014. Discovery of the target site of oxathiapiprolin (DuPont Zorvec disease control). Abstract Agro 911. 13th IUPAC Congress of Pesticide Chemistry, San Francisco, CA.
- Thomas, A., Carbone, I., Choe, K., Quesada-Ocampo, L. M., and Ojiambo, P. S. 2017a. Resurgence of cucurbit downy mildew in the United States: insights from comparative genomic analysis of *Pseudoperonospora cubensis*. *Ecol. Evol.* 7:6231-6246.
- Thomas, A., Carbone, I., Cohen, Y., and Ojiambo, P. S. 2017b. Occurrence and distribution of mating type of *Pseudoperonospora cubensis* in the United States. *Phytopathology* 107:313-321.
- Thomas, A., Carbone, I., Lebeda, A., and Ojiambo, P. S. 2017c. Virulence structure within populations of *Pseudoperonospora cubensis* in the United States. *Phytopathology* 107:777-785.
- Thomas, C., Inaba, T., and Cohen, Y. 1987. Physiological specialization in *Pseudoperonospora cubensis*. *Phytopathology* 77:1621-1624.
- Urban, J., and Lebeda, A. 2007. Variation of fungicide resistance in Czech Republic populations of *Pseudoperonospora cubensis*. *J. Phytopathol.* 155: 143-151.
- USDA-NASS. 2016. Vegetables 2015 Summary. USDA-National Agricultural Statistics Service. <http://usda.mannlib.com/ell/usa/nass/VegeSumm//2010s/2016/VegeSumm-02-04-2016.pdf>
- Zhu, S., Liu, P., Li, J., Yuan, S., and Si, N. 2008. Assessing the risk of resistance in *Pseudoperonospora cubensis* to the fungicide flumorph in-vitro. *Pest Manag. Sci.* 64:255-261.