MANAGEMENT OF CUCUMBER DOWNY MILDEW WITH FUNGICIDES AND HOST RESISTANCE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Pathology – Master of Science

2018

ABSTRACT

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Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is a devastating foliar blight of cucumbers that re-emerged in Michigan in 2005 when host resistance failed. CDM is resistant to several fungicides and Michigan growers rely on fungicide recommendations based on local data. Our goal was to improve Michigan pickling cucumber growers CDM management by: 1) Evaluating fungicides over three field seasons for their ability to protect a susceptible cultivar and 2) Compare intermediately resistant (IR) cultivars 'Peacemaker' and 'Citadel' with susceptible cultivars when treated with three fungicide programs or not treated. CDM severity was visually assessed and the area under disease progress curve calculated. Trials were harvested within the spray interval. Mandipropamid, propamocarb, fluxapyroxad+pyraclostrobin, copper octanoate, and dimethomorph were similar to the control for CDM severity in 2015; cymaxonil was similar to the control in 2016 and in 2017, famoxadone+cymoxanil and fluopicolide were additionally similar to the control. Several fungicides yielded higher than the control, oxathiapiprolin mixed with either chlorothalonil or mandipropamid, mancozeb, dimethomorph+amectoctradin, zoxamide+mancozeb, ethaboxam, cyazofamid, chlorothalonil, and fluazinam. IR cultivar control plots were less diseased and higher yielding than the susceptible cultivar control plots in 2016; 'Citadel' was similar to susceptible cultivar control plots in 2017. In 2016, Program 3 provided disease protection for the susceptible standards that was better than Program 1. Program 3 and 2 provided similar levels of disease control for 'Peacemaker', 'Citadel' and 'Vlaspik' in 2016; all cultivars in 2017.

ACKNOWLEDGEMENTS

Thank you to Dr. Mary Hausbeck for helping me grow and allowing me to follow my passion in applied plant pathology. Her mentorship will follow with me throughout my life and career. I also want to thank the members of my committee Dr. Ray Hammerschmidt and Dr. Chris DiFonzo for their passion towards research and extension in the agriculture industry.

I am thankful for Hausbeck lab members, also referred to as the 'Pickle Pickers', for this work would not have been possible without your help. I appreciate the technical expertise from Sheila Linderman and Alex Cook. Thank you to Jen Foster for her mentorship and guidance in my academic, professional and beer drinking careers.

Finally, I am most thankful for my parents, grandparents and loved ones for their constant support and encouragement throughout my studies and whatever I do in life.

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LITERATURE REVIEW

INTRODUCTION

Cucumbers (*Cucumis sativus* L.) are grown across the world in tropical, subtropical and temperate regions (Lebeda et al., 2011). In the U.S., cucumbers are primarily grown in California, Florida, Georgia, North Carolina and Michigan, for the fresh market and processing industries (USDA, 2016). Michigan is the top producer of pickling cucumber and the majority of the cropping acres are procured under contract (USDA, 2016). In 2015, 35,409 hectares of pickling cucumbers were planted in the U.S.; 12,140 hectares were planted in Michigan (USDA, 2016). Production in 2015 was valued at $172.7 million in the U.S., $37.6 million was produced in Michigan. The state ranks fifth in fresh market cucumber production, valued at $13.5 million of the total $177 million in the U.S. (USDA, 2016).

Many diseases affect the commercial crop including foliar blight, root, crown and fruit rot (Koike et al., 2007). Downy mildew is worldwide the most economically important disease to cucumber production (Palti and Cohen, 1980). Crown and root rot, caused by the oomycete *Phytophthora capsici*, is a soil borne pathogen affecting Michigan's cucurbit crops (Hausbeck and Lamour, 2004). Cucumber vines are tolerant to Phytophthora root rot but fruit rot is a problem for Michigan's growers (Hausbeck and Lamour, 2004). Powdery mildew, caused by the ascomycete *Podosphaera xanthii* (previously *Sphaerotheca fuliginea*) covers the foliage in white fungal growth leading to necrosis (Koike et al., 2007). Anthracnose (*Colletotrichum lagenarium*) can infect the foliage and fruits of cucumber, muskmelon and watermelon (Koike et al., 2007). Angular leaf spot, caused by the bacterial pathogen *Pseudomonas syringae* pv. *lachrymans*, causes angular lesions on the foliage that can spread to the fruit (Koike et al., 2007). These symptoms may be confused with downy mildew (Zitter, 2017).

PSEUDOPERONOSPORA CUBENSIS ON CUCURBIT HOSTS

Downy mildew of cucurbits is caused by the oomycete *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (Lebeda and Cohen, 2011). The pathogen infects approximately 20 different genera in the Cucurbitaceae family (Palti and Cohen, 1980). This plant pathogen belongs to the fungi-like class Oomycota, from the lineage group of Stramenopiles, belonging to the kingdom Chromista (Dick, 2001). Recent studies show that this group is part of the "superkingdom" Chromalveolate (Beakes et al., 2012). All plant pathogens in the Oomycetes are part of the Peronosporales group, including genera *Phytophthora, Pseudoperonospora, Pythium* and *Albugo* (Waterhouse, 1973; Beakes et al., 2012). There are six well-known species of the *Pseudoperonospora* genus, of which *P. cubensis* and *P. humuli* (downy mildew of hop) have significant economic importance (Runge et al., 2011).

There are distinct genotypic and phenotypic differences that separate the oomycetes from true-fungi including bifillagate zoospores, cell walls made up primarily of cellulose and beta-glucans, diploid ploidy, and rare septation of the hyphae (Beakes et al., 2012; Dick, 2001; Gisi and Sierotzki, 2015). *Pseudoperonospora cubensis,* like other downy mildew pathogens, is an obligate biotroph that survives only on living host tissue. Downy mildew can cause yield losses in cucumber, squash, pumpkin and melon crops and is an economic problem for growers in the eastern U.S. and Ontario, Canada (Lebeda and Widrlechner, 2003; Granke et al., 2014; Holmes et al., 2006).

COMPARING *PSEUDOPERONOSPORA CUBENESIS* AND *P. HUMULI*

Commercially grown hops (*Humulus lupulus* L.) have increased in Michigan with 418 hectares of production in 2015 (Sirrine, 2016). Hop downy mildew, caused by the pathogen

Pseudoperonospora humuli, can cause yield loss and reduce the quality of the hop cones (Royle and Kremheller, 1981). *Pseudoperonospora humuli* can overwinter as mycelium in hop crowns, increasing management challenges for this perennial crop (Royle and Kremheller, 1981). *Pseudoperonospora humuli* has been considered a taxonomic synonym to *P. cubensis* (Choi et al., 2005). Morphologically, there appears to be no differences between the two species, thus, a molecular approach has been used to discern genetic differences (Choi et al., 2005). One study found that the amplified internal transcribed spacer (ITS) regions of rDNA of the two species had a similar length, and thus suggesting that they are the same species (Choi et al., 2005).

Other researchers concluded that the two species should remain different. Runge et al. (2011) studied the nrITS, *cox*2 and *ypt*1 of *P. humuli* and *P. cubensis* and found that the characteristics of the *cox*2 nrITS could distinguish between the two species. Mitchell et al. (2011) found that while *P. cubensis* infected hop plants at a low sporangial concentration (5 x 10^3 sporangia ml^{-1}), *P. humuli* only produced a single sporangiophore on one cantaloupe plant. Researchers speculated that this could be due to the aggressive pathogenicity of *P. cubensis* to infect a wide range of hosts, however more studies are needed (Mitchell et al., 2011; Runge et al., 2011). *Pseudoperonospora humuli* isolates collected from the host *Humulus japonicus* were genetically more similar to *P. cubensis* than other *P. humuli* isolates, thus researchers believe that *P. cubensis* may have originated from a host jump of *P. humuli* on *H. japonicus* (Mitchell et al., 2011). Differences in the sexual life cycle of these two species are additional reasons for maintaining separate taxonomic nomenclature (Mitchell et al., 2011). *Pseudoperonospora humuli* oospores overwinter and cause infection in the field (Bressman and Nichols, 1933), whereas *P. cubensis* oospores have not been reported to occur on any host in the field (Thomas et al., 2017).

SIGNS AND SYMPTOMS

On cucumber, downy mildew symptoms first appear on the leaves as angular, water-soaked lesions restricted by the veins best seen during periods of leaf wetness. These water-soaked lesions become chlorotic; pathogen sporulation can be seen on the bottom side of the leaf, also restricted by leaf veins in cucumbers (Hausbeck, 2017). This black growth consists of sporangiophores that have branched out and produced asexual structures known as sporangia at each tip. The lesions expand and coalesce to cause a blighting that covers the entire leaf surface (Savory et al., 2011). Other cucurbits, such as melons, squash and pumpkins, develop irregular shaped lesions of various shapes and sizes (Hausbeck, 2017). As the infection progresses, the foliar, coalesced lesions become necrotic and can lead to plant death. The resulting decrease in canopy cover can subject fruits to deformation and secondary infections (Keinath et al., 2007), thus reducing yield but also fruit quality (Hausbeck, 2017).

Sporangia can only be seen under a microscope and are 20-40 µm long by 14-25 µm wide (Palti and Cohen, 1980). The sporangia are lemon shaped and have a distinct clear cell wall and a dark center with a papilla on one end (Hausbeck, 2017). A dissecting scope can also be used, as the branching on the sporangiophores (180-400 µm long) are visible under minor magnification. Biflagellate zoospores (10-13µm in diameter) are released from the sporangia after at least 2 hours of free moisture (Cohen, 1977).

LIFE CYCLE AND REPRODUCTION

Pseudoperonospora cubensis reproduces asexually by producing dark brown sporangiophores and sporangia (Palti and Cohen, 1980). Once a sporangium infects a susceptible host, it produces sporangiophores on the underside of the leaf within 5 to 7 days under optimal conditions (Cohen, 1981). The sporangium detaches from the sporangiophore when there is a change from high relative humidity to low relative humidity (Neufeld et al., 2013). Sporangia are wind-borne and can survive up to 16 days following dissemination (Cohen, 1981), travelling distances up to 1,000 km and averaging about 10 km per day (Kanetis et al., 2010; Ojiambo and Holmes, 2011). A high quantity of sporangia are needed for dissemination, as many do not reach a susceptible host or are no longer viable (Kanetis et al., 2010). Once the sporangia land on a susceptible host, leaf wetness is needed for 5 to15 zoospores to be released from the sporangium (Colucci and Holmes, 2010). The biflagellate zoospores swim in free water to find open stomata where they encyst and produce a germ tube that enters the intracellular space and develop haustoria within the cell wall (Cohen, 1981). The sporangia of some oomycetes directly infect the plant with a germ tube without releasing zoospores (Fry and Grünwald, 2010), however, the primary means for infection in *P. cubensis* is through zoospores (Colucci and Holmes, 2010).

The sexual stage of *P. cubensis* produces oospores (Zang et al., 2012), but sexual reproduction is rare or undetected for cucurbit downy mildew in the field. Oospores for other downy mildews, including *P. humuli*, are found on leaves, shoots, and cones near the end of the growing season (Royle and Kremheller, 1981). *P. cubensis* is thought to be heterothallic, so the pathogen needs more than one mating type in a population in order for viable oospore formation (Cohen and Rubin, 2012; Thomas et al., 2017).

There are two reported mating types of *P. cubensis* in the world, A1 and A2 (Cohen and Rubin, 2012), and both have been reported in the U.S. (Thomas et al., 2017). Mating type A1 is associated with cucumber or muskmelon hosts, while the A2 mating type is associated with muskmelons, watermelon, pumpkin and squash (Thomas et al., 2017; Cohen et al., 2013; Ojiambo et al., 2015). The A1 mating type was also collected from pumpkin (*Cucurbita maxima*)

(Thomas et al., 2017). Both mating types have been found on *Cucumis melo* (Thomas et al., 2017). Only the A1 mating type has been found in New Jersey, New York, Ohio, Michigan and Ontario, while both mating types have been found in southern states (Thomas et al., 2017).

Viable oospores have been produced in the laboratory by crossing A1 and A2 isolates on *Cucumis sativus* and *Cucumis melo.* Oospores produced on cucumber foliage in the lab were 25-50 µm in diameter, ranging in color from hyaline on cucumber to light brown on melon (Thomas et al., 2017; Cohen and Rubin, 2012). Cohen and Rubin (2012) found that the frequency of viable oospore production was low (0.2%), but Thomas et al. (2017) found the percentage of viable oospores produced using plasmolysis was about 40%.

Oospore production has not been detected in North American fields, but has been reported in Austria, Bulgaria, China, India, Israel, Japan, Italy and Russia (Cohen and Ruben, 2012). Oospore viability is important to disease management (Cohen et al., 2015). Viable oospores were reported in cucumber fields in temperate China, where initial inoculum was thought to be from sporangia migrating north from frost-free locations in southern China as hosts become available (Zhang et al., 2012). These oospores may overwinter and serve as initial inoculum (Zhang et al., 2012).

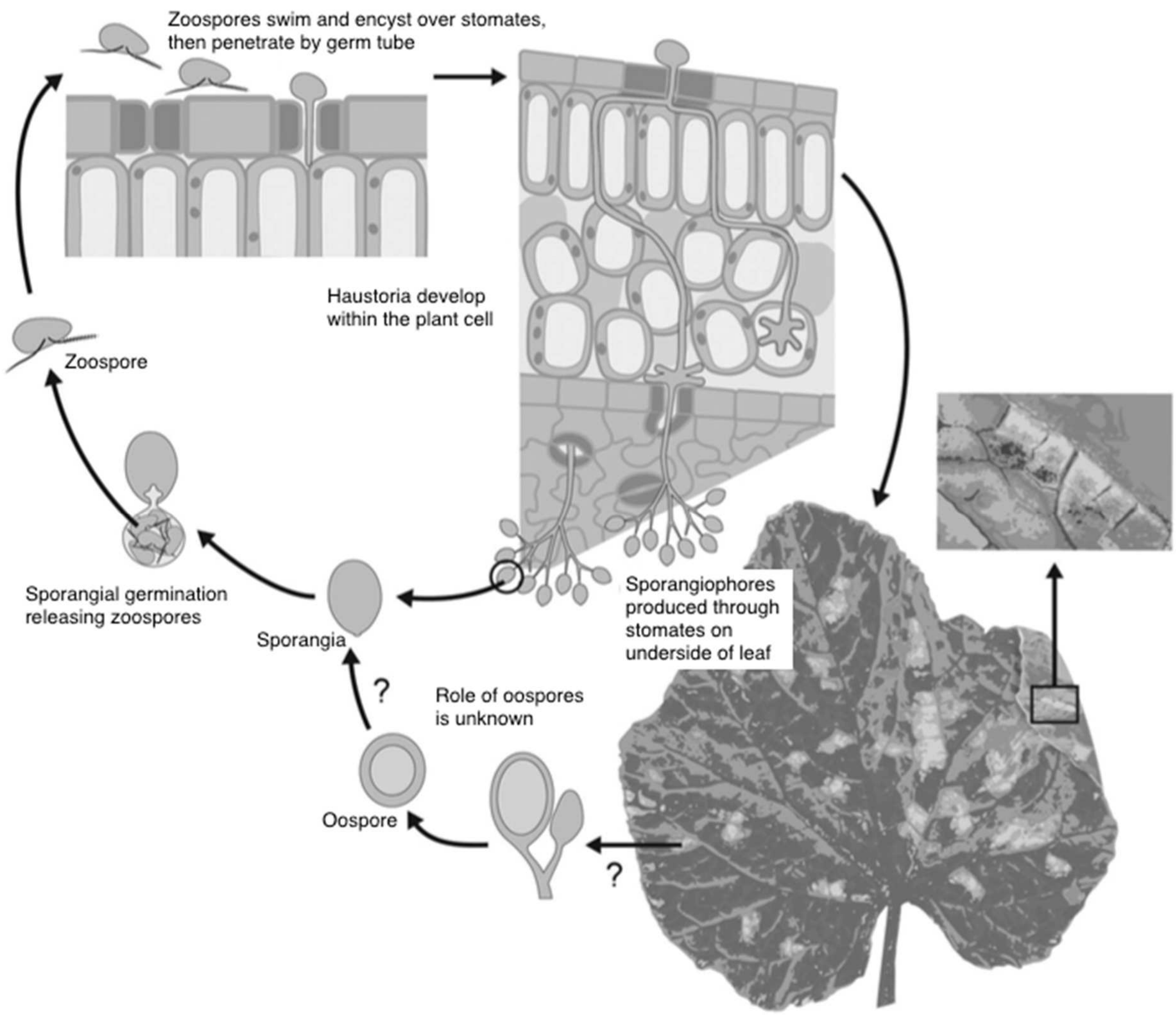

Figure 1. Life cycle of *Pseudoperonospora cubensis* on cucumber (adapted from Savory et al., 2011).

EPIDEMIOLOGY

Since the asexual spores of *P. cubensis* must survive and reproduce on living host tissue, the pathogen cannot overwinter in areas without year around cucurbit production in the U.S. (Holmes et al., 2015). The initial inoculum may originate from frost-free cucurbit production regions of the southern U.S. and Mexico and travel north once the weather warms and hosts are available (Ojiambo and Holmes, 2011). Inoculum also originates from greenhouses (Ojiambo and Holmes, 2011; Granke et al., 2014). For example, there are 315 hectares of year-round greenhouse cucumber production in Ontario, Canada (Ontario Greenhouse Vegetable Growers, 2015). Quesada-Ocampo et al. (2012) found a high level of genetic similarity among isolates collected in fields in Ontario, Michigan and Ohio. There was a higher degree of genetic differentiation among Ontario isolates and isolates from Georgia and Louisiana, suggesting that greenhouse inoculum plays a role in epidemics of north-eastern U.S. and Canadian field grown cucurbits. Naegele et al. (2016) found that all populations sampled in Ontario were also found in Michigan, but there was a unique pathogen population only found in Michigan. This could be due to isolate migration throughout the season or host selection (Naegele et al., 2016). Population genetics of *P. cubensis* were studied across the world by both geographic regions and host species (Quesada-Ocampo et al., 2012). Six genetic clusters were found, with certain clusters predominanting in certain areas. However, there was a low genetic differentiation among continents, suggesting isolates move among populations (Quesada-Ocampo et al., 2012). A higher degree of diversity is more commonly found from isolates collected from *C. sativus* hosts than on other cucurbits (Quesada-Ocampo et al., 2012).

A study in Israel found that *P. cubensis* infected cucurbits had a seed infection rate less than 10% (Cohen et al., 2014). Cucurbit fruits that tested positive for downy mildew were sliced and able to infect healthy leaves of cucurbits (Cohen et al., 2014). The seeds from these infected fruits were also able to transmit *P. cubensis* to the hypocotyls of germinating plants in a lab at a rate of 1.6%. This was the first reported case of successful seed transmition *P. cubensis* and it is important to study further the potential impacts of seed transmission on agricultural production and trade (Cohen et al., 2014).

HOST RESISTANCE AND PATHOGENICITY

Fungicides alone may not be adequate to protect cucumbers from downy mildew such that yields are optimized. Resistant varieties could optimize yield potential and provide an improved downy mildew management system for cucurbit growers (Call et al., 2012). Complete host resistance to downy mildew was first reported in the cucumber line PI 197087 that possessed the *dm1* gene (Barnes and Epps, 1954) as the source of disease resistance (Call et al., 2013). This gene provided complete host resistance in cucumber crops in the U.S. until 2004 when it was no longer effective, and the pathogen caused severe damage to cucumber crops (Holmes et al., 2006). It is speculated that a change in climate or in the downy mildew populations may be responsible (Savory et al., 2011; Holmes et al., 2015). Cultivars with the *dm1* gene demonstrate resistance when compared to the cultivars without the gene, but the pathogen can cause severe damage (Savory et al., 2011). Call et al. (2013) found that the cultivar line with the highest resistance was PI 197088, which still required weekly applications of a protectant fungicide to produce high yields. Plant breeding efforts have introduced moderate levels of resistance to downy mildew, but cultivars are not immune to infection (Call et al., 2012).

Thomas et al. (1987) proposed five pathotypes of *P. cubensis* based on host preference and geographic region. Pathotypes 1, 2 and 3 were found in Japan, pathotype 3 was also found in Israel, and able to infect *Cucumis sativus, C. melo* (vars. *reticulatus*, *conomon* (only pathotypes 2,3), *acidulus* (only pathotype 3)). Pathotypes 4 and 5 were from the U.S. and could infect *C. sativus, C. melo* and *Citrullus lanatus;* only pathotype 5 could infect *Cucurbita* species (Thomas et al. 1987). A sixth pathoype was reported in Israel in 2002 and infecting *Cucurbita pepo*, *C. moschata* and *C. maxima* (Cohen et al., 2003). The origin of this pathotype is unknown (Cohen and Rubin, 2012). Recent research showed that pathotypes was not as important as previously thought in understanding the population structure of *P. cubensis* (Quesada-Ocampo et al., 2012; Ojiambo et al., 2015; Thomas et al., 2017). Population structure was instead associated primarily with host, and two linages have been suggested in the U.S. (Thomas et al., 2017; Bello et al., 2016).

Pseudoperonospora cubensis is most virulent on pickling cucumber cultivars (Cespedes-Sanchez et al., 2015). This may be due to the increased virulence of the lineage found primarily on cucumbers, but more studies are needed (Thomas et al., 2017). Neufeld and Ojiambo (2012) observed that disease severity was greater on cucumber than on cantaloupe or acorn squash. A recent study showed that *P. cubensis* can infect wild cucurbit species either grown as ornamentals or naturally occurring as weeds in North Carolina (Wallace et al., 2016), but the mating type is not known.

INFLUENCE OF LEAF WETNESS

Leaf wetness is necessary for infection (Neufeld and Ojiambo, 2012) and is a limiting factor in downy mildew epidemics (Palti and Cohen, 1980). The longer the leaf wetness period, the larger the range of temperature at which infection can occur (Cohen, 1977). Under optimal conditions, a minimum two-hour leaf wetness period was necessary (Cohen, 1977). A recent study showed that infection occurs with a minimum leaf wetness period of 60 and 45 minutes at 20°C and 25°C, respectively (Sun et al., 2017). Different cucurbit hosts infected with *P. cubensis* have varying rates of optimal leaf wetness and inoculum concentration (Neufeld and Ojiambo, 2012). *Pseudoperonospora cubensis* on cucumbers requires fewer hours of leaf wetness at any given temperature to develop the same disease severity as squash or muskmelon (Neufeld and

Ojiambo, 2012). Germination of sporangia on muskmelon was greatest when moisture was not limited, however, even though less sporangial germination occurred on cucumbers, there was still greater disease severity on cucumbers compared to squash.

Sporangia are released following a change in relative humidity, normally in the late morning and early afternoon (Granke et al., 2014). Granke and Hausbeck (2011) measured airborne sporangia concentration and found the peak sporangial release was between 0800 to 1300 hr. Leaf wetness was negatively correlated with airborne sporangial concentrations and reductions in moisture typically lead to an increased level of sporangia (Granke and Hausbeck, 2011).

INFLUENCE OF TEMPERATURE

Temperature is not the limiting factor for a downy mildew infection to occur but is the main factor that influences the duration and virulence of the infection (Neufeld and Ojiambo, 2012). Optimum temperature for infection is 15°C to 20°C, with a range from 5°C to 30°C (Cohen, 1977; Neufeld and Ojiambo, 2012; Savory et al., 2011; Sun et al., 2017). *P. cubensis* isolates collected after the re-emergence in 2004 in the U.S. are more virulent and infect at higher temperatures (Neufeld and Ojiambo, 2012). It was previously shown that at 28°C there was little or no infection, with 25°C being the upper limit for most *P. cubensis* infections (Cohen, 1977). The optimum temperature for infection is not dependent on the cucurbit host (Neufeld and Ojiambo, 2012). Cool temperatures, while favorable for infection, can delay disease symptoms. High temperatures decrease the pathogen’s ability to successfully infect the host, but symptoms are more rapidly seen (Cohen, 1977). Granke and Hausbeck (2011) found that increases in temperature are positively correlated with an increase in airborne sporangia concentration.

INFLUENCE OF SOLAR RADIATION

Kanetis et al. (2010) found that increasing solar radiation reduced viability of *P. cubensis* sporangia and thus decreased disease severity. While solar radiation cannot decrease viability completely, a level of 29.5 MJ m^{-2} can reduce viability to about 5%. On sunny days, solar radiation has a greater impact on sporangia viability than lack of moisture (Kanetis et al., 2010). Granke et al. (2014) found that airborne sporangia concentration is negatively correlated with solar radiation. This is similar to the study by Kanetis et al. (2010) although they were unable to monitor if the solar radiation was on a sunny or cloudy day (Granke et al., 2014). Similar phenomena have been noted in other aerially dispersed Oomycete systems. Viability of sporangia from the oomycete *Phytophthora infestans* decreased by 95% after 1.1 hours of intense sunlight, but viability was not significantly reduced after 3 hours on a cloudy day (Mizubuti et al., 2000).

CULTURAL, BIOLOGICAL AND CHEMICAL MANAGEMENT

After host plant resistance became ineffective in 2004, management of *P. cubensis* included cultural and chemical tactics. In addition to yield loss, a lack of foliage resulting from pathogen infection can increase sun scald, allowing secondary fruit rots and misshapen fruits (Keinath et al., 2007). Since *P. cubensis* overwinters in regions without a frost and sporangia are spread aerially, crop rotation does not limit the disease in northern regions. Cultural control methods include decreasing planting density to allow airflow within the canopy, thereby reducing moisture and infection potential (Hausbeck and Lamour, 2004). Michigan growers adapted this cultural method to limit *Phytophthora capsici* fruit rot as it ensures better spray coverage of the fruits (M. Hausbeck, *personal communication*). Other methods include using intermediately resistant cultivars in combination with fungicides. Monitoring the northern spread of downy mildew can help growers decide on the level of protection for their crop (Ojiambo and Holmes, 2011).

Using Organic Materials Review Institute (OMRI) approved products to control *P. cubensis* is important for organic production in the U.S., where synthetic fungicides cannot be used. In the U.S. biopesticides make up 3% of the total fungicide market but, can be valuable tools for an integrated pest management program in conventional production (Thakore, 2006). Organic production does not allow the use of synthetically-derived active ingredients but allows for some naturally occurring chemicals such as copper (Marine et al., 2016). Using copper-only products has been of concern to organic growers due to the impact of soil health (Adrees et al., 2015). Marine et al. (2016) found that extending the intervals between copper products while alternating with a biorational product like *Streptomyces lydicus* WYEC 108 (Actinovate AG) or *Bacillus subtilis* QST713 (Serenade Soil), can give similar results to a copper-only program. However, the efficacy of biorational products can be variable and should not be relied on for the control of downy mildew in organic productions (Marine et al., 2016).

Global sales of fungicides for control of downy mildew is approximately $1.2 billion, of which $144 million is used specifically for *P. cubensis* (Gisi and Sierotzki, 2008). Cucumber growers in Michigan use an aggressive approach to control downy mildew by applying fungicides every 5 to 7 days (Savory et al., 2011). In Michigan, approximately $6 million is spent on protecting pickling cucumbers from downy mildew annually (Granke et al., 2014;

Savory et al., 2011). There are 16 chemical groups for control of oomycetes (Gisi and Sierotzki, 2015) and the most predominant of single-site compounds for control of the downy mildews are the phenylamides, Quinone outside inhibitors, carboxylic acid amides and the cyanoacetamid-oximes (Gisi and Sierotzki, 2008). Fungicides are assigned numbers by the Fungicide Resistance Action Committee (FRAC) based on how the fungicide targets the pathogen.

Many fungicide classes with multi-site modes of action are used against downy mildews including the dithiocarbamates, phthalimides, chloronitriles, and copper compounds; they make up about half of all fungicides sold for oomycete control (Gisi and Sierotzki, 2008). Most of the multi-site fungicides act by contact and must be used preventively. The multi-site fungicides employ multiple modes of action and pathogen populations are less likely to develop resistance compared to other chemistry classes (FRAC, 2016). They are commonly used in conjunction with single-site fungicides to provide plant protection (Gisi and Sierotzki, 2008).

Phenylamides, such as metalaxyl and metalaxyl-M, are thought to inhibit oomycete infection by stopping the production of ribosomal RNA (Davidse, 1995). The first reported *P. cubensis* resistance to phenylamides was in 1980, 2 years after this chemistry class was commercialized in Israel (Reuveni et al. 1980). Resistance to metalaxyl was reported in 1987 in the U.S. (Moss, 1987) and metalaxyl was ineffective when the pathogen re-emerged in 2004 (Holmes et al., 2015). Quinone outside inhibitors inhibit mitochondrial respiration by attaching to the ubiquinol oxiziding pocket (Gisi et al., 2002). Commonly referred to as strobilurins, the class includes azoxystobin, fenamidone, pyraclostrobin and famoxadone (Gisi and Sierotzki, 2008). Resistance to the strobilurins has been reported in the U.S. since 2000 (Sierotzki et al., 2000) and this class was ineffective in controlling downy mildew since the re-emergence (Holmes et al., 2015). Carboxylic acid amides (CAA) include dimethomorph, iprovalicarb, benthiavalicarb and mandipropamid, and the main target for CAA is cellulose synthase (Gisi and Sierotzki, 2015). There have been numerous reports of *P. cubensis* resistance to this class, and these fungicides are no longer recommended (Gisi and Sierotzki, 2015; Ojiambo et al., 2015). The fungicide cymoxanil is a cyanoacetamide-oxime fungicide and the target site is unknown (Gisi and Sierotzki, 2008). Oomycetes resistant to this fungicide have been reported in Europe since 1997 (Gisi and Sierotzki, 2008) along with reports on *P. cubensis* reduced sensitivity in the U.S. (Keinath, 2016). Benzamides (Group 43), include fluopicolide which was especially effective against *P. cubensis* from 2000 to 2008 (Ojiambo et al., 2010), but there have been numerous reports of resistance and reduced efficacy in Michigan since 2013 (Hausbeck and Linderman, 2014; Ojiambo et al., 2015).

New active ingredients have been developed for use against *P. cubensis*. The novel active ingredient, oxathiapiprolin, became available to manage *P. cubensis* in 2016. Oxathiapiprolin inhibits the OSBPI-homologue which stops lipid transfer between cell membranes. This inhibition causes massive rearrangement of organelles and disrupts cell function. This disruption prevents zoospore release, sporangia germination, lesion formation, lesion expansion, sporangiophore development and sporangial production (Cohen, 2015). Ethaboxam has been tested for efficacy against oomycetes including cucurbit down mildew since the late 1990s (Kim et al., 1999). The fungicide was tested for efficacy against *P. cubensis* since the late 1990s (Kim et al., 1999; Miller and Mera, 2011; Adams and Ojiambo, 2012; Hausbeck et al., 2015) and was registered in 2017.

Many populations of *P. cubensis* in the U.S. and worldwide are resistant to metalaxyl-M, azoxystrobin, pyraclostrobin, cymoxanil, propamocarb, dimenthomorph, mandipropamid and fluopicolide (Cohen, 2015). Keinath (2016) found reduced efficacy of propamocarb, cymoxanil, flupicolide, dimethomorph, azoxystrobin and mandipropamid in pickling cucumber bioassays conducted in South Carolina. Since 2014, there has been reduced efficacy to propamocarb in Michigan (Hausbeck and Linderman, 2014), while it is effective in the southern U.S (Keinath, 2016).

DISEASE FORECASTING

Disease forecasting systems may allow growers to minimize fungicide use while optimizing crop protection. The potential of this is widely believed to be underutilized by growers (Gent et al., 2013). The occurrence of downy mildew in the U.S. is tracked via a website that also forecasts risk levels based on weather conditions (Ojiambo et al., 2011). Spore trapping has been used for cucurbit downy mildew in Michigan since 2006. Granke et al. (2014) monitored airborne sporangia concentration and found relationships with *P. cubensis* risk factors including solar radiation, leaf wetness and temperature. Sporangia concentration, measured using a volumetric spore sampler, was positively correlated with disease occurrence (Granke et al., 2014). This is useful for growers deciding when to time their initial and subsequent sprays, but more research is needed to develop this predictive model. Choudhury et al. (2016) used rotating-arm spore trapping for early detection of spinach downy mildew (*Peronospora effusa*) in California, and successfully detected spores nine days before infection was seen in commercial fields. Gent et al. (2009) used spore trapping and polymerase chain reaction (PCR) techniques to detect *P. humuli* in commercial hop yards, and detected sporangia before symptoms occurred, leading to improved fungicide management. Summers et al. (2015) used real-time PCR to differentiate air borne spores from *P. cubensis* and *P. humuli* and to predict disease incidence. However, they were unable to detect *P. cubensis* sporangia on the traps before the symptoms appeared in the field. Using inoculum concentrations as well as monitoring favourable weather patterns can be combined for a more precise disease management system.

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LITERATURE CITED

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CHAPTER 1. EVALUATION OF FUNGICIDES FOR DOWNY MILDEW (*PSEUDOPERONOSPORA CUBENSIS*) ON CUCUMBER

ABSTRACT

Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is a devastating foliar blight of cucumbers that re-emerged in Michigan in 2005. The pathogen is resistant to several fungicides and Michigan growers rely on fungicide recommendations based on local data to reduce the risk posed by CDM. The objective of this study was to evaluate fungicides in field studies over three seasons for their ability to protect a susceptible pickling cucumber cultivar against *P. cubensis* in Michigan. Three field trials were conducted from 2015 to 2017 using the CDM-susceptible pickling cucumber 'Vlaspik'. Fungicide sprays were initiated prior to disease symptoms and re-applied weekly. According to rAUDPC data from 2015, treatments of mandipropamid, propamocarb, fluxapyroxad + pyraclostrobin, copper octanoate, and dimethomorph were similar to the control for foliar plant area diseased *P. cubensis*. These fungicide treatments along with the cymaxonil treatment were similar to the control in 2016. In 2017, the rAUDPC data indicated the fungicide treatments that were similar to the control in 2016 were also ineffective in 2017 along with treatments of famoxadone + cymoxanil or fluopicolide. Each year of the study, oxathiapiprolin applied alone (2015 only) or in combination with chlorothalonil or mandipropamid (2016, 2017), dimenthomorph + amectoctradin, fluazinam, zoxamide + mancozeb, cyazofamid, and ethaboxam were similar for the foliar plant area diseased based on rAUDPC data. An exception occurred in 2017 when ethaboxam was less effective than fluazinam, and oxathiapiprolin mixed with either chlorothalonil or mandipropamid. Mancozeb and chlorothalonil were similar in their level of CDM protection in 2015 and 2017, according to rAUDPC data. In 2015, the yield from the control plot was similar to all of the fungicide treatments with the exception of mancozeb and fluopicolide, which were higher. In 2017, yields were significantly greater than the control for the following treatments:

oxathiapiprolin mixed with either chlorothalonil or mandipropamid, mancozeb, dimethomorph + amectoctradin, zoxamide + mancozeb, ethaboxam, cyazofamid, chlorothalonil, and fluazinam.

INTRODUCTION

Michigan is the top producer of pickling cucumbers in the U.S.. In 2015, 12,140 hectares were planted in the state with a value of nearly $38 million dollars (United States Department of Agriculture, 2016). *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew (CDM), infects the foliage of over 20 cucurbit genera (Palti and Cohen, 1980) but is especially destructive on pickling cucumber (*Cucumis sativus* L.) (Cespedes-Sanchez et al., 2015). In the 1950s and 1960s, a resistance gene (*dm1*) was introduced into commercial cucumber cultivars (Barnes and Epps, 1954; Call et al., 2013), allowing growers to produce the crop without fungicides (Call et al., 2012). In 2004, a severe CDM outbreak occurred in the southern U.S. causing severe economic losses. Growers in the northeastern U.S. and Ontario observed the disease in the 2005 growing season (Holmes et al., 2006). That year, Michigan pickling cucumber growers experienced devastating crop loss. 'Vlaspik' pickling cucumber, favored by growers utilizing a once-over mechanical harvester, is highly susceptible to CDM (Cespedes-Sanchez et al., 2015). Lesions are restricted by the leaf veins and appear as chlorotic necrotic spots on the upper surface with pathogen sporulation underneath (Holmes et al., 2015; Savory et al., 2011).

Populations of *P. cubensis* vary across the world and within the U.S. (Quesada-Ocampo et al., 2012; Thomas et al., 2017). Two lineages are found in the southern U.S. whereas only one lineage is found in the northern U.S. and Ontario (Thomas et al., 2017). Although the pathogen cannot overwinter in northern climates such as Michigan (Holmes et al., 2015), *P. cubensis* has reoccurred each year in the state since 2005, costing pickling cucumber growers an estimated six million dollars or more annually in fungicide sprays to protect their crop (Savory et al., 2011; Granke et al., 2014). Prior to the re-emergence of CDM in Michigan, pickling cucumber growers focused primarily on limiting fruit rot caused by *Phytophthora capsici*, another oomycete pathogen (Hausbeck, *personal communication*). Today, these growers apply fungicides frequently to limit CDM (Savory et al., 2011) timing sprays to coincide with an observed influx of *P. cubensis* sporangia into the growing region or initial disease symptoms in the crop detected through intensive scouting.

Pseudoperonospora cubensis is a pathogen at high risk for developing fungicide resistance (FRAC, 2013). Resistance has documented for certain fungicides including those in the FRAC groups 4 (phenylamides), 11 (Quinone outside inhibitors) and 40 (carboxylic acid amides) (Gisi and Sierotzki, 2015; Holmes et al., 2015). Fluopicolide and propamocarb were effective against CDM when introduced commercially in 2008 and 2006, respectively, (Ojiambo et al., 2010; Hausbeck, 2011; Raid, 2013; Wyenandt, 2013), but since 2013 their efficacy appears to be reduced in Michigan CDM trials (Hausbeck and Linderman, 2014; Goldenhar and Hausbeck, 2016). Mandipropamid was registered in 2008 (EPA, 2008), but was determined to be ineffective against CDM in Michigan by 2010 (Hausbeck and Cortright, 2010). Cymoxanil showed reduced efficacy against CDM according to a bioassay in the southern U.S. (Keinath, 2016) and Michigan field studies (Hausback and Cortright, 2011).

Oxathiapiprolin, registered in late 2015 (EPA, 2015), inhibits *P. cubensis* by targeting oxysterol-binding proteins (Cohen, 2015) and has been effective in Michigan studies (Hausbeck et al., 2016; Goldenhar and Hausbeck, 2016). Ethaboxam was registered in 2017 (EPA, 2017) and has demonstrated efficacy against CDM (Miller and Mera, 2011; Adams and Ojiambo, 2012; Adams and Quesada-Ocampo, 2014; Trueman, 2014; Gugino, 2015; Hausbeck et al., 2015).

Each year, fungicides are recommended to protect Michigan's pickling cucumber crop based on local trial data to ensure that this destructive pathogen is effectively managed. The objective of this study was to evaluate fungicides in field studies over three seasons for their ability to protect a susceptible pickling cucumber cultivar against *P. cubensis* in Michigan.

MATERIALS AND METHODS

Field experiments were conducted during July to September in 2015, 2016, and 2017 at the Michigan State University (MSU) Plant Pathology Farm in Lansing, Michigan. Temperature and precipitation variables were recorded from July to October using the Michigan Automated Weather Network approximately 3 kilometers from the plant pathology farm. The 2015 and 2017 trials were planted in Capac loam soil, previously cropped to cucumber. The 2016 trial was established in Houghton muck soil, previously planted to carrots. Glyphosate (Roundup PowerMax at 2.34 L/ha, Monsanto Company, St. Louis, MO) was applied prior to planting for weed control. Soil was plowed, cultivated, and fertilized with ammonium nitrate (112 kg/ha).

Plots were prepared as raised plant beds covered with black plastic (15 cm high by 60 cm wide) with drip tape down the center. Single rows, spaced 1.68-m center to center, were seeded with the CDM susceptible 'Vlaspik' pickling cucumber (Seminis Vegetable Seeds, Inc., St. Louis, MO) on 24, 19, and 14 July for 2015, 2016, 2017, respectively. Seed was commercially treated with azoxystrobin, fludioxonil, mefenoxam, and thiamethoxam (Farmore FI400, Syngenta Crop Protection, Greensboro, NC) to protect against seedling pathogens and insects. Throughout the growing season, fertilizer (20-20-20 at 2.8 kg/ha) was applied weekly via drip tape, weeds were managed by hand, and insects were controlled with imidacloprid (Admire Pro at 0.58 L/ha, Bayer CropScience, Research Triangle Park, NC) applied through the drip tape four weeks after seedling emergence. Cucumbers were trickle irrigated as needed.

Fungicide treatments and a control (untreated) were arranged in a randomized complete block design with four replicates. Each treatment replicate consisted of a 6.1-meter plant bed with a 0.61-meter buffer between beds within a plant row. Fungicides (Table 1) were applied as sprays to the foliage using a carbon dioxide backpack boom sprayer (Bellspray Inc. R&D Sprayers, Opelousas, LA) equipped with XR8003 flat-fan nozzles spaced 45.7 cm apart, operating at 275.8 kilopascals (2016) or 344.7 kilopascals (2015 and 2017 trials), delivering 467.7 L/ha. Two nozzles were used to apply the fungicide at the onset of the trial with a third nozzle added once the plants grew to the edges of the plant bed.

CDM symptoms were first identified on 27 July 2015, 9 August 2016, and 3 August 2017, on earlier planted, untreated cucumbers not in the trial area, located at the MSU plant pathology farm. Fungicide treatments were applied to the plots as follows: 7, 14, 21, 28 August, and 4 September (2015); 10, 17, 24, 31 August, and 7, 14 September (2016); and 2, 9, 18, 25 August, and 2, 9, 16 September (2017). Although treatments were applied on each date in each year, some treatments were adjusted during the course of the study. Fluxapyroxad + pyraclostrobin was applied only in August 2016 due to product availability. Due to a change in registration, oxathiapiprolin was tested in 2015 whereas newly available pre-packs of oxathiapiprolin+mandipropamid and oxathiapiprolin+chlorothalonil were tested in 2016 and 2017.

Disease was visually assessed on the following dates: 19, 31 August and 4 September (2015); 6, 9, 14, 19, 22 September (2016); and 18, 24, 31 August and 6, 11, 18, 26 September

(2017). The percentage of foliar plant area diseased with CDM was assessed for each treatment plot using the Horsfall-Barratt scale (Horsfall and Barratt, 1945) (1=0%, 2=0 to 3%, 3=3 to 6%, 4=6 to 12%, 5=12 to 25%, 6=25 to 50%, 7=50 to 75%, 8=75 to 87%, 9=87 to 94%, 10=94 to 97%, 11=97 to <100%, 12=100%). The area under the disease progress curve (AUDPC) was used to represent the disease severity progression throughout the disease epidemic. AUDPC values were calculated as described by Madden et al. (2007);

$$\text{AUDPC}= = \sum_{i=1}^{n-1} \frac{y_i+y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where y_i is the assessment of the disease (using the mid-point percentage from the Horfall-Baratt scale) at the ith observation, t_i is time (days) at the ith observation, and n is the total number of ratings. To compare among sites or years, the relative or absolute AUDPC (rAUDPC) has been useful (Fry, 1978). This is calculated by dividing each AUDPC value by the maximum potential AUDPC for that specific trial. Plots were hand harvested on 10 September 2015, and on 1, 12, 20 September 2017. Fruit larger than 7.5 centimeters in length were collected from each plot and weighed. The 2016 trial was not harvested due to an uneven plant stand.

Statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC). A global analysis of variance (ANOVA) F-test was calculated (using PROC MIXED) for each trial, to determine significant differences among treatments. Fungicide treatments were considered as fixed effects and blocks as a random effect. The treatment by year interaction was significant for the calculated rAUDPC values, and therefore years could not be combined. Years were then analyzed individually using the PROC GLIMMIX in SAS version 9.4 Normality was checked using residual plots and Levene's test was performed to test homogeneity of the variances for each trial. No trials violated the analysis of variance (ANOVA) test. If the F-test was significant, all pair-wise comparisons were assessed with Tukey's Honestly Significant Difference (HSD) test.

Table 1. Fungicide treatments applied to 'Vlaspik' pickling cucumber to control *Pseudoperonospora cubensis* in field trials established from 2015 to 2017 at the Michigan State University Plant Pathology research farm.

Active ingredient	Commercial product name	Registrant[a]	Chemical Group Name (FRAC)	FRAC[b] code	Rates per hectare
copper octanoate	Cueva SC	Certis	Multi-site inhibitor	M1	4.68 L
fluazinam	Omega SC	Syngenta	Phenyl-pyridinamine	29	1.17 L
fluopicolide	Presidio SC	Valent	Benzamide	43	0.29 L
propamocarb	Previcur Flex SL	Bayer	Carbamate	28	1.4 L
cyazofamid	Ranman SC	FMC	Quinone inside inhibitor	21	0.2 L
fluxapyroxad+pyraclostrobin	Priaxor SC	BASF	Succinate- dehydrogenase inhibitors+Quinone outside inhibitor	7+11	0.58 L
famoxadone+cymoxanil	Tanos DF	DuPont	Quinone outside inhibitor+ Cyanoacetamide-oxime	11+27	0.56 kg
cymoxanil	Curzate DG	DuPont	Cyanoacetamide-oxime	27	0.35 kg
chlorothalonil	Bravo WeatherStik SC	Syngenta	Multi-site inhibitor	M5	2.34 L
oxathiapiprolin	Orondis OD[c]	Syngenta	Oxysterol binding protein homologue inhibition	49	0.12 L
oxathiapiprolin+chlorothalonil	Orondis Opti SC[c]	Syngenta	Oxysterol binding protein homologue inhibition+Multi-site inhibitor	49+M5	2.5 L
oxathiapiprolin+mandipropamid	Orondis Ultra SC[c]	Syngenta	Oxysterol binding protein homologue inhibition+Carboxylic-acid amide	49+40	0.7 L
dimethomorph	Forum SC	BASF	Carboxylic-acid amide	40	0.44 L
mandipropamid	Revus SC	Syngenta	Carboxylic-acid amide	40	0.58 L
dimethomorph+ametoctradin	Zampro SC	BASF	Carboxylic-acid amide+ Triazolopyrimidine	40+45	1.03 L
ethaboxam	Elumin SC	Valent	Thiazole-carboxamide	22	0.58 L
zoxamide+mancozeb	Gavel DF	Gowan	Benzamides+Multi-site inhibitor	22+M3	2.24 kg
mancozeb	Koverall DG	Cheminova	Multi-site inhibitor	M3	2.24 kg

[a]Certis USA (Colombia, MD), Syngenta Crop Protection (Greensboro, NC), Valent USA (Walnut Creek, CA), Bayer CropScience (Research Triangle Park, NC), FMC Corporation (Philadelphia, PA), BASF Cooperation (Research Triangle Park, NC), DuPont Crop Protection (Wilmington, DE), Gowan Company (Yuma, AZ), Cheminova (Research Triangle Park, NC)

[b]Fungicide Resistance Action Committee (FRAC), codes given to fungicides based on their activity on pathogens. M=multisite inhibitors http://www.frac.info

[c]Orondis OD was tested in 2015 only, Orondis Ultra SC and Orondis Opti SC were tested in 2016 and 2017

RESULTS

Disease severity was assessed for 16 days in 2015 and 2016, and 47 days in 2017. In 2015, the control reached a maximum of 70% foliar plant area diseased 38 days after planting and then decreased (Fig. 2). The foliar plant area diseased reached nearly 100% in the control plot in 2016 and 2017, 65 days after planting (Figs. 3, 4).

Based on rAUDPC in 2015, treatments of mandipropamid, propamocarb, fluxapyroxad + pyraclostrobin, copper octanoate, and dimethomorph were similar to the control for foliar plant area diseased *P. cubensis* (Table 4). These fungicides along with the cymaxonil treatment were also similar to the control in 2016. In 2017, the rAUDPC data indicated the fungicide treatments that were similar to the control in 2016 were also ineffective in 2017 along with treatments of famoxadone + cymoxanil or fluopicolide (Table 4).

Each year of the study, oxathiapiprolin applied alone (2015 only) or in combination with chlorothalonil or mandipropamid (2016, 2017), dimenthomorph + amectoctradin, fluazinam, zoxamide + mancozeb, cyazofamid, and ethaboxam were better than the control and similar for the foliar plant area diseased based on rAUDPC data (Table 4). An exception occurred in 2017 when ethaboxam was less effective than fluazinam, and oxathiapiprolin mixed with either chlorothalonil or mandipropamid. CDM symptoms were not observed in oxathiapiprolin-treated plots in 2015 (Figure 2). In 2016 and 2017, plots treated with oxathiapiprolin + chlorothalonil exhibited a low (<6% leaf area) level of CDM symptoms (Figures 3, 4). The multisite fungicides mancozeb and chlorothalonil were similar in their level of CDM protection in 2015 and 2017. In 2016, chlorothalonil was more effective than mancozeb. The two treatments containing cymoxanil, alone or in combination with famoxadone had similar rAUDPC values and neither were better than the control in 2017.

In 2015, the yield from all fungicide treatments did not differ from the control with the exception of mancozeb and fluopicolide, which were higher (Table 5). In 2017, yields were significantly greater than the control for the following treatments: oxathiapiprolin mixed with either chlorothalonil or mandipropamid, mancozeb, dimethomorph + amectoctradin, zoxamide + mancozeb, ethaboxam, cyazofamid, chlorothalonil, and fluazinam.

The average monthly temperatures were coolest in 2015, with August averaging 25°C. While 2016 and 2017 temperatures were warmer, more rain occurred in August 2016, with 147 mm total, compared to the same time period in 2015 (117 mm) and 2017 (41 mm). Average rainfall is 80 mm for Michigan (NOAA, 2018).

Table 2. Average monthly maximum temperature (°C) and precipitation (mm) from 2015, 2016 and 2017 from a location approximately 3 kilometers from the Michigan State University plant pathology farm in Lansing, Michigan (https://mawn.geo.msu.edu)

Month	**2015**		**2016**		**2017**	
	Precipitation	Average Temperature	Precipitation	Average Temperature	Precipitation	Average Temperature
July	57.1	26.7	73.7	28.3	46.5	27.5
August	116.8	25.4	146.6	28.3	41.1	25.3
September	83.3	24.8	74.7	23.8	22.1	25.0

Table 3. Fixed effect analysis of variance table for the relative area under the disease progress curve (rAUDPC) caused by *Pseudoperonospora cubensis* in 2015, 2016 and 2017.

Source	N	DF	F ratio	Prob > F
Year	2	2	29.3511	<0.0001
Treatment	17	17	63.7135	<0.0001
Treatment*Year	34	34	16.4168	<0.0001

Table 4. Foliar plant area diseased by *Pseudoperonospora cubensis* on 'Vlaspik' cucumber when treated with fungicides or not treated during 2015 to 2017 reported as the relative area under disease progress curve (rAUDPC).

Treatment	**Relative AUDPC**		
	2015	**2016**	**2017**
untreated control	0.41 a[y]	0.59 a	0.57 a
oxathiapiprolin	0.00 g	NT	NT
oxathiapiprolin+chlorothalonil	NT[z]	0.01 d	0.01 d
oxathiapiprolin+mandipropamid	NT	0.01 d	0.01 d
ethaboxam	0.02 g	0.15 d	0.29 bc
dimethomorph+amectoctradin	0.04 g	0.14 d	0.14 cd
fluazinam	0.04 g	0.07 d	0.02 d
mancozeb	0.05 fg	0.40 b	0.22 c
zoxamide+mancozeb	0.05 fg	0.18 cd	0.16 cd
cyazofamid	0.11 e-g	0.07 d	0.11 cd
chlorothalonil	0.19 ef	0.20 cd	0.14 cd
cymoxanil	0.21 de	0.46 ab	0.49 a
famoxadone+cymoxanil	0.24 c-e	0.36 bc	0.50 a
fluopicolide	0.25 b-e	0.36 bc	0.45 ab
mandipropamid	0.34 a-d	0.62 a	0.56 a
propamocarb	0.36 a-c	0.51 ab	0.52 a
fluxapyroxad+pyraclostrobin	0.37 a-c	0.45 ab	0.45 ab
copper octanoate	0.38 a-c	0.45 ab	0.44 ab
dimethomorph	0.41 a	0.50 ab	0.45 ab

[y]Means within a column with the same letter are not significantly different, Tukey's honestly significant difference, α=0.05.

[z]NT=Not tested in column year

Table 5. Marketable yield of 'Vlaspik' cucumbers when treated with fungicides or not treated for *Pseudoperonospora cubensis* in 2015 and 2017.

Treatment	**Marketable[v] yield (kg/plot)**	
	2015[w]	**2017[x]**
untreated control	4.75 c[y]	22.30 ef
oxathiapiprolin+chlorothalonil	NT[z]	55.27 a
oxathiapiprolin+mandipropamid	NT	54.96 a
mancozeb	8.91 a	48.68 a-d
fluopicolide	8.88 ab	36.94 b-e
dimethomorph+amectoctradin	7.42 a-c	53.10 a
ethaboxam	7.30 a-c	46.04 a-d
fluapyroxad+pyraclostrobin	7.13 a-c	33.56 d-f
zoxamide+mancozeb	7.10 a-c	53.65 a
cyazofamid	7.04 a-c	49.23 a-c
chlorothalonil	6.78 a-c	53.35 a
fluazinam	6.76 a-c	50.85 ab
cymoxanil	6.68 a-c	28.97 ef
famoxadone+cymoxanil	6.43 a-c	25.47 ef
dimethomorph	6.41 a-c	30.44 ef
propamocarb	6.15 a-c	26.68 ef
oxathiapiprolin	5.92 a-c	NT
copper octanoate	5.73 a-c	35.10 c-f
mandipropamid	4.89 c	19.77 f

[v]Marketable yield was comprised of fruit >7.5 cm in length.
[w]Harvested on 10 September 2015.
[x]Harvested on 1, 12 and 20 September 2017.
[y]Means within a column with the same letter are not significantly different, Tukey's honestly significant difference, α=0.05.
[z]NT=not tested in column year.

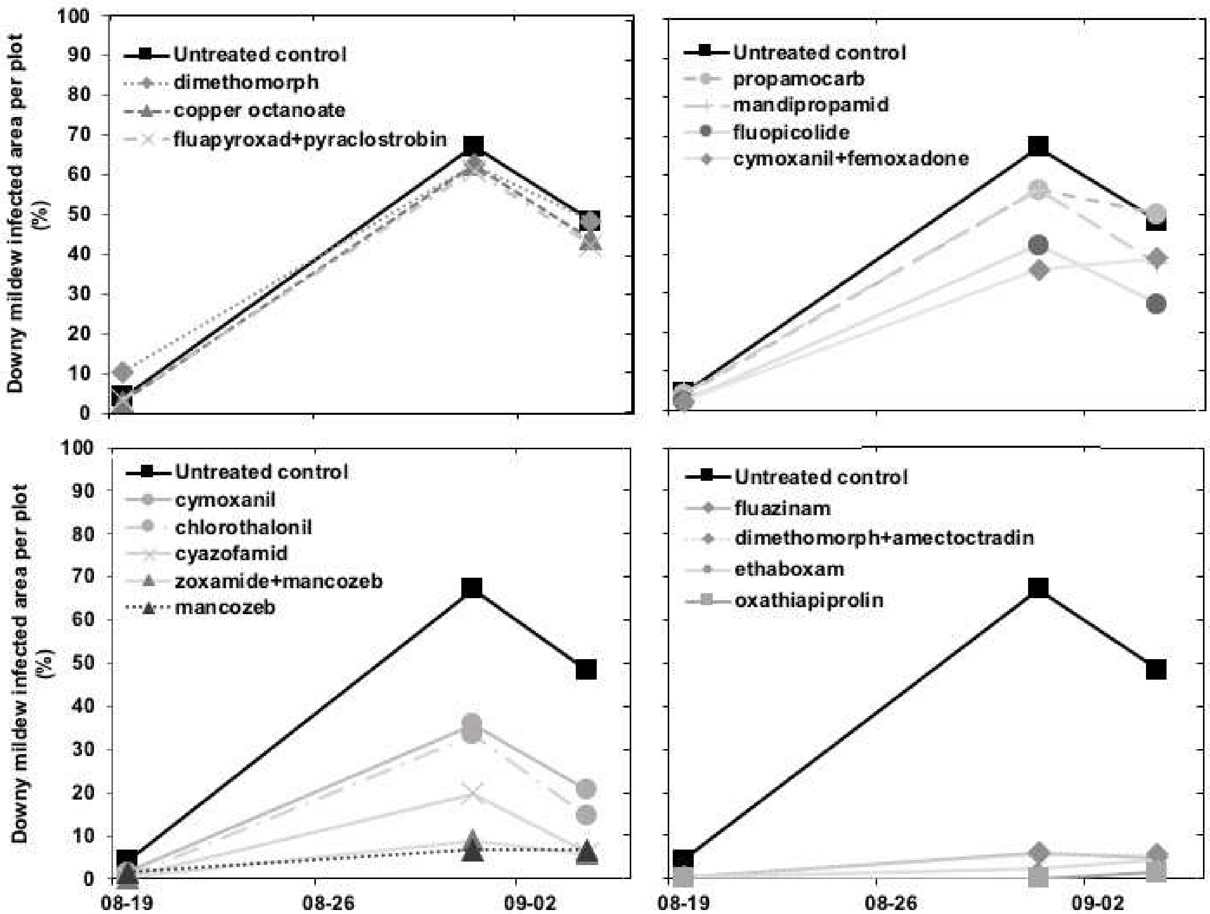

Figure 2. *Pseudoperonospora cubensis* infected area over time in 2015 on 'Vlaspik' cucumber when treated with fungicides or not treated.

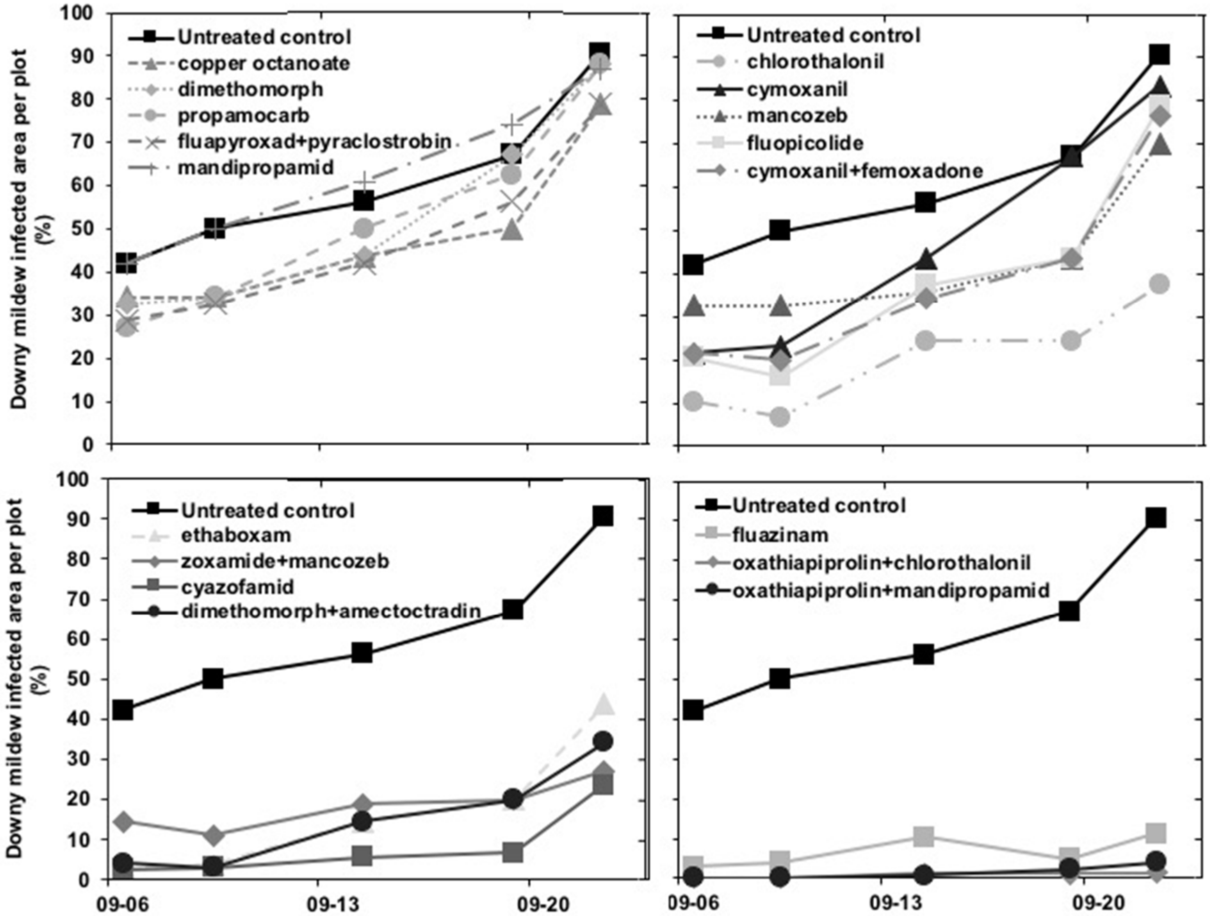

Figure 3. *Pseudoperonospora cubensis* infected area over time in 2016 on 'Vlaspik' cucumber when treated with fungicides or not treated.

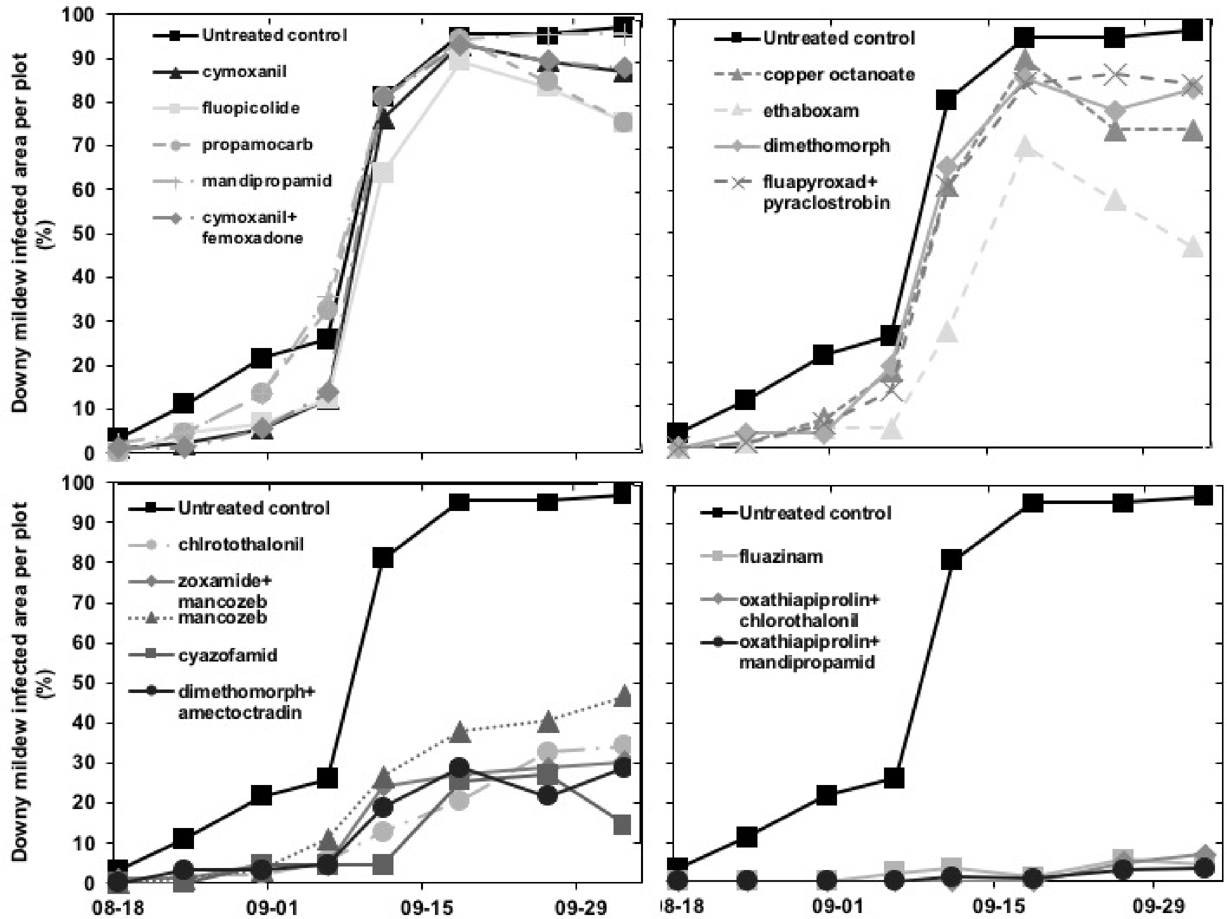

Figure 4. *Pseudoperonospora cubensis* infected area over time in 2017 on 'Vlaspik' cucumber when treated with fungicides or not treated.

DISCUSSION

In Michigan, the importance of fungicides for CDM protection was established after the pathogen re-emerged and host resistance was no longer adequate (Holmes et al., 2006). Michigan growers now manage CDM at significant cost and rely on fungicide recommendations based on local data to reduce the risk posed by CDM. Results from this three-year field study identified the following treatments as effective against CDM: ethaboxam, cyazofamid, dimethomorph + amectoctradin, zoxamide + mancozeb, mancozeb, chlorothalonil, and oxathiapiprolin alone or mixed with either chlorothalonil or mandipropamid. Resistance management is key to maintaining efficacy of single-site fungicides and growers are encouraged to rotate among fungicides from different Fungicide Resistance Action Committee (FRAC) groups (FRAC, 2018). Mixing high risk fungicides with low risk protectant fungicides is also recommended to delay resistance (Hobbelen et al., 2011).

The broad-spectrum fungicides, mancozeb and chlorothalonil consistently controlled CDM in this study. They are considered to be at low risk for resistance (Gisi and Sierotzki, 2015; FRAC, 2018); tank mixing them with high risk fungicides is an important resistance management approach (Hobbelen et al., 2011). In 2016, when rainfall in August was high, chlorothalonil reduced CDM based on rAUDPC data compared to mancozeb. Plots treated with chorothalonil or mancozeb produced similar yields in 2017. However, the yield for mancozeb was similar to fluopicolide, fluapyroxad + pyraclostrobin, and copper octanoate.

For each year of this three-year study, several fungicides were similar to the control for CDM and included mandipropamid, propamocarb, fluxapyroxad + pyraclostrobin, copper octanoate, and dimethomorph. Cymoxanil was added to the list of treatments that was similar to the control in years two and three of the study. For year three, two additional fungicides were similar to the control and included famoxadone + cymoxanil and fluopicolide. Prior to the completion of this study, growers had routinely used fungicides containing cymoxanil, propamocarb, and fluopicolide. Mandipropoamid was not effective when used alone and thus the oxathiapiprolin + mandipropamid treatment is effective due to the inclusion of oxathiapiprolin.

Oxathiapiprolin is a relatively new active ingredient that became available to Michigan growers in 2016, and has proven efficacy against *P. cubensis* (Cohen, 2015; Goldenhar and Hausbeck, 2016). This active ingredient is in the novel FRAC group 49 and is considered at high risk of pathogen resistance (Cohen, 2015; FRAC, 2018), thus, resistance management is important. While both oxathiapiprolin-based treatments performed similarly for CDM control and yield in this study, oxathiapiprolin + chlorothalonil is recommended for use since chlorothalonil is a multisite, contact fungicide with proven efficacy in our study. This is an effective way to manage fungicide resistance management (Hobbelen et al., 2011).

Other single site active ingredient provided better control of CDM than the untreated in all years. Fluazinam (FRAC group 29) is effective against CDM and is registered for use against other oomycete pathogens, including *Phytophythora infestans*. However, this fungicide is not widely used by Michigan growers due to its high cost. Ethaboxam is in FRAC group 22 and was effective against CDM in 2015 and 2016, similar to previous studies conducted in the U.S. (Miller and Mera, 2011; Adams and Ojiambo, 2012; Miller and Mera, 2013; Adams and Quesada-Ocampo, 2014; Gugino, 2015; Hausbeck et al., 2015) and Canada (Trueman, 2014). In 2017, ethaboxam was significantly less effective than the best performing fungicides but performed similarly to zoxamide + mancozeb; ethaboxam and zoxamide are in the same FRAC group. Ethaboxam became registered late in 2017 and should be closely monitored for pathogen resistance. Tank mixing with chlorothalonil or mancozeb, as well as alternating with effective

CDM fungicides, will reduce the risk of resistance to maintain this effective fungicide. Zoxamide is available as a premixture with chlorothalonil (Zing!, Gowan Company, Yuma, AZ) or mancozeb (Gavel, Gowan Company, Yuma, AZ). While only the zoxamide + mancozeb premix was tested in this study, the zoxamide + chlorothalonil premix also provides protection from CDM (Hausbeck et al., 2016). Both premixes provide good options for resistance management (Hobbelen et al., 2011). Cyazofamid, dimethomorph + amectoctradin, and zoxamide + mancozeb consistently provided good control of CDM and were among the most efficacious products in all three years. This is consistent with other studies across the U.S. on cucurbits (Adams and Quesada-Ocampo, 2014; Gugino, 2015; Keinath, 2016). Amectoctradin is the only active ingredient in FRAC group 45 that is registered for CDM, available as a premix with dimethomorph. Cyazofamid is the only FRAC group 21 fungicide registered and has shown consistent efficacy.

Isolates of *P. cubensis* have been reported to exhibit a reduced sensitivity to cymoxanil, propamocarb, mandiproamid, fluopicolide, and dimenthomorph (Keinath, 2016). In this study, mandipropamid, propamocarb, copper octanoate, dimenthomorph and fluxapyroxad + pyraclostrobin were similar to the control each year of this study. Cymoxanil was not different from the control in 2016 and 2017, and fluopicolide and famoxadone + cymoxanil were not effective in 2017. Commercial fungicides that only contain the following FRAC groups are not recommended for Michigan growers to manage CDM; benzamides (FRAC group 43), carbamates (FRAC group 28), Quinone outside inhibitors (FRAC group 11), cyanoacetamide-oximes (FRAC group 27) and carboxylic-acid amides (FRAC group 40). Local fungicide testing should continue annually based on the dynamic fungicide market for Michigan pickling cucumber growers.

APPENDIX

CUCUMBER (*Cucumis sativus* 'Vlaspik')
Downy mildew; *Pseudoperonospora cubensis*

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Evaluation of fungicides for control of downy mildew of cucumber when applied after pathogen establishment, 2016.

The trial was established at the MSU Plant Pathology Farm in Lansing, MI, in a field previously planted to cucumber. Plots were prepared as raised plant beds. Drip tape was established on each bed, and the beds were covered with black plastic. Single rows spaced 1.68-m at center were seeded with 'Vlaspik' cucumber on 25 Jul. Each treatment replicate was a 6.1-m bed for each of four replicates with a 0.61-m buffer between beds within a planting row. Treatments were arranged in a randomized complete block design. The plot was fertilized throughout the growing season with weekly applications of 20-20-20 via drip tape at 2.8 kg/ha. Weeds were removed mechanically on 2 Sep. Insects were controlled with Admire Pro 0.59 L/ha applied through the drip 4 weeks after plant emergence. Foliar fungicide sprays were applied to cucumber foliage with obvious symptoms of downy mildew with a CO_2 backpack boom sprayer equipped with two then three XR8003 flat-fan nozzles, operating at 40 psi, delivering 468 L/ha. Treatments were applied at 4-day intervals on 2, 7, 11, 15, 19, 23, and 27 Sep. Cucumber leaves were evaluated for downy mildew severity using the Horsfall-Barratt scale on 6, 19, 22, 26, 30 Sep, 4, 7, and 14 Oct. Yields were harvested from the entire 20-ft row on 9, 16, and 22 Sep and only total yield reported.

Disease was allowed to develop prior to the first fungicide application. On 6 Sep, all treatments including the untreated were uniformly diseased with a rating of 6.0-6.3 (6=25 to 50% disease). Applications of Orondis Opti SC or Orondis Ultra SC immediately limited disease development significantly compared to all other treatments and the untreated control on 19 and 22 Sep. By 26 Sep, the untreated plants received a rating of 9.8 (9=87 to 94% disease). Cueva SC, Previcur Flex SL, Presidio SC, and Forum SC allowed disease progression that was similar to the untreated control. From 19 Sep to 4 Oct, the plots treated with Orondis Opti SC or Orondis Ultra SC did not show any disease progression from the time that the sprays had begun as the ratings remained the same. While all other treatments were significantly better than the untreated plots, the level of disease development would be considered commercially unacceptable. On 7 Oct (10 days after last fungicide application), the Orondis-based treatments showed only limited disease progression from the original disease rating of 6.0 with ratings of 6.3 to 6.5; these treatments were the most effective fungicides. Plants treated with Ranman SC received a rating of 7.8 (7=50 to 75% disease). On 14 Oct (17 days after the last fungicide application), plots treated with either Orondis Opti SC or Orondis Ultra SC were similar to the V-10208 SC treatments and were the most effective treatments in limiting downy mildew disease. In comparison, the untreated plot was almost entirely diseased (11.8; 12=100% disease) on the last evaluation date; treatments of Cueva SC, Presidio SC, Previcur Flex SC, Revus SC, and Forum SC were similar to the untreated control. While all other treatments included in this study were better than the untreated control the disease ratings were high. The untreated plot yielded similarly to those treated with Cueva SC, Koverall DG, Bravo WS SC, Presidio SC, Previcur Flex SL, Gavel DF, Tanos DF, Curzate DF, Omega SC, Revus SC, and Forum SC. The highest yields (>14 kg/plot) were achieved with treatments of Ranman SC, Zampro SC, Orondis Opti SC, or Orondis Ultra SC. Although this trial provides helpful information to growers facing established downy mildew, fungicides are best applied preventively for maximum control and to delay the development of fungicide resistance to the downy mildew pathogen.

Table 6. Foliar ratings and yield from a post-infection of *Pseudoperonospora cubensis* trial on Michigan State University plant pathology farm in 2016

Treatment and rate/ha,	Foliar ratings[*]								Total yield
applied at 4-day intervals	9/6	9/19	9/22	9/26	9/30	10/4	10/7	10/14	(kg/ row)
Untreated control	6.0 b[**]	8.0 a	8.5 ab	9.8 a	10.5 a	10.8 a	10.8 a	11.8 a	8.0 f
Bravo Weatherstik 2.34L	6.0 b	7.5 ab	7.5 c-e	7.8 e-h	7.8 gh	8.0 fg	8.8 d-g	9.0 c-e	10.5 b-f
Koverall 2.24 kg	6.0 b	8.0 a	8.0 a-d	8.5 c-e	8.5 e-g	8.5 e-g	8.8 d-g	9.3 cd	12.8 a-f
Cueva 4.68 L	6.0 b	8.0 a	8.8 a	9.8 a	10.3 ab	10.5 ab	10.5 ab	11.5 a	8.6 ef
Presidio 0.29 L	6.0 b	7.8 ab	8.3 a-c	9.0 a-c	9.5 b-d	9.5 b-e	9.8 a-d	11.0 ab	11.4 b-f
Previcur Flex 1.4 L	6.3 a	7.8 ab	8.8 a	9.8 a	9.8 a-c	10.0 a-d	10.8 a	11.0 ab	10.1 c-f
Ranman 0.3 L	6.0 b	7.3 bc	7.0 e	7.3 gh	7.5 h	7.5 g	7.8 g	9.3 cd	17.5 a
Zampro 1.03 L	6.0 b	7.5 ab	7.3 de	7.5 f-h	7.5 h	8.0 fg	8.8 d-g	9.5 cd	14.2 a-d
Gavel 2.24 L	6.0 b	7.8 ab	8.3 a-c	8.8 b-d	8.8 d-f	9.0 d-f	9.3 c-f	10.0 bc	10.4 b-f
Tanos 0.56 kg	6.0 b	7.3 bc	7.5 c-e	8.3 c-f	8.0 f-h	8.5 e-g	8.5 e-g	10.0 bc	12.2 b-f
Curzate 0.35 kg	6.0 b	6.8 cd	7.3 de	8.0 d-g	8.0 f-h	8.5 e-g	9.0 d-f	10.0 bc	11.6 b-f
Omega 1.17 L	6.0 b	7.8 ab	7.8 b-e	8.3 c-f	7.5 h	8.0 fg	9.0 d-f	9.3 cd	10.0 c-f
Revus 0.58 L	6.0 b	7.5 ab	7.5 c-e	8.3 c-f	9.0 c-e	9.3 c-e	9.5 b-e	10.8 ab	11.1 b-f
Forum 0.44 L	6.0 b	7.8 ab	8.0 a-d	9.5 ab	10.0 ab	10.3 a-c	10.3 a-c	11.3 a	9.3 def
Orondis Opti 2.5 L	6.0 b	6.0 e	6.0 f	6.0 i	6.0 i	6.0 h	6.3 h	8.0 ef	15.4 ab
Orondis Ultra 0.7 L	6.0 b	6.5 de	6.0 f	6.0 i	6.0 i	6.0 h	6.5 h	7.8 f	14.8 a-c
Elumin 0.58 L	6.3 a	7.3 bc	7.0 e	7.0 h	7.3 h	7.5 g	8.3 fg	8.5 d-f	13.5 a-e

[*]Rated on the Horsfall-Barratt scale of 1 to 12, where 1=0% plant area diseased, 2=>0 to 3%, 3=>3 to 6%, 4=>6 to 12%, 5=>12 to 25%, 6=>25 to 50%, 7=>50 to 75%, 8=>75 to 87%, 9=>87 to 94%, 10=>94 to 97%, 11=>97 to <100%, 12=100% plant area diseased.

[**]Column means with a letter in common are not statistically different (LSD t Test; *P*=0.05).

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CHAPTER 2. EVALUATION OF CUCUMBER DOWNY MILDEW (*PSEUDOPERONOSPORA CUBENSIS*) MANAGEMENT PROGRAMS USING CULTIVAR RESISTANCE COMBINED WITH FUNGICIDE PROGRAMS

ABSTRACT

Michigan is the top producer of pickling cucumbers in the U.S. and annually since 2005, its growers have had to manage cucurbit downy mildew (CDM), a devastating foliar blight. Previously, host resistance had provided protection from the oomycete *Pseudoperonospora cubensis*, the causal agent of CDM. Pickling cucumber cultivars ‘Peacemaker’ and ‘Citadel’ have been released recently and display intermediate resistance (IR) to CDM. This objective of this study was to compare IR cultivars with CDM susceptible cultivars ‘Expedition’ and ‘Vlaspik’ for their response to Michigan’s *P. cubensis* populations when treated with three fungicide programs or not treated (control). Field trials were conducted in 2016 and 2017 and treated weekly as follows: 1) chlorothalonil, 2) cyazofamid + chlorothalonil alternated (alt) with propamocarb + chlorothalonil alt with dimethomorph+amectoctradin (premix) + chlorothalonil; and 3) oxathiapiprolin + chlorothalonil (premix) alt with propamocarb + chlorothalonil, alt with cyazofamid + chlorothalonil. Foliar blight severity was visually assessed and the area under disease progress curve calculated. Trials were harvested and graded within the spray interval.

‘Citadel’ and ‘Peacemaker’ control plots were less diseased than ‘Vlaspik’ and ‘Expedition’ control plots in 2016; ‘Citadel’ was similar to ‘Vlaspik’ and ‘Expedition’ control plots in 2017. In 2016, Program 3 provided disease protection for ‘Vlaspik’ and ‘Expedition’ that was better than Program 1. Program 3 and 2 provided similar levels of disease control for ‘Peacemaker’, ‘Citadel’ and ‘Vlaspik’ in 2016; all cultivars in 2017. When untreated ‘Peacemaker’ yields were greater than other untreated cultivars; ‘Citadel’ yielded more marketable fruit than ‘Vlaspik’ and ‘Expedition’ in 2016 but was similar in 2017. In 2016 all fungicide programs increased yields in ‘Vlaspik’; only program 2 and 3 for ‘Expedition’, and ‘Citadel’; in 2017 all programs increased yields of ‘Vlaspik’ and ‘Expedition’.

INTRODUCTION

Pseudoperonospora cubensis infects cucurbits, causing foliar blighting and reduced yields (Palti and Cohen, 1980); *Cucumis* spp. are especially susceptible (Cespedes-Sanchez et al., 2015). Cucurbit downy mildew (CDM) lesions on cucumber (*Cucumis sativus* L.) foliage are restricted by leaf veins, appearing initially as water-soaked areas that become chlorotic and then necrotic (Koike et al., 2007). *Pseudoperonospora cubensis* is an obligate oomycete pathogen that survives on cucurbits in regions without a killing frost including the southern U.S. and Mexico (Holmes et al., 2015). Greenhouse cucumber production in northern regions may also allow the pathogen to overwinter (Ojiambo and Holmes, 2011).

Since the 1950s, the *dm-1* gene has conferred resistance to CDM in cucumber cultivars (Barnes and Epps, 1954; Call et al., 2013) and fungicides were not needed to protect the crop from this disease (Call et al., 2012). In 2004, there was an outbreak of CDM in the southern U.S. resulting in significant economic losses; an outbreak occurred in the northeast U.S. and the Great Lakes growing region in 2005 (Holmes et al., 2006). Since 2005, CDM has been an annual problem for Michigan cucumber growers. Fungicides are now needed to protect cucumber yields in Michigan and all U.S. production regions (Holmes et al., 2006; Call et al., 2012). Fungicides containing the active ingredients mefenoxam, pyroclostrobin, and azoxystrobin have not controlled *P. cubensis* since its re-emergence in 2005 (Holmes et al., 2015). Recently, the fungicides cymoxanil, propamocarb, mandiproamid, fluopicolide and dimethomorph showed reduced efficacy in South Carolina (Keinath, 2016), Michigan (Hausbeck and Linderman, 2014), Pennsylvania (Gugino, 2015), and Ontario (Trueman, 2014; Goldenhar, *unpublished data*). Propamocarb, registered in 2006 is considered to be an effective fungicide (Ojiamdo et al., 2010;

Raid, 2017) but reduced efficacy has been noted in Michigan (Hausbeck and Linderman, 2014) even though it has remained effective for growers in Florida (Raid, 2017).

Resistance management recommendations include tank mixing single site fungicides with contact, multi-site fungicides, and alternating fungicides with different FRAC codes (Brent and Holloman, 1995; FRAC, 2018). The multi-site fungicides mancozeb and chlorothalonil provide limited CDM protection (Adams et al., 2015; Hausbeck and Linderman, 2014; McGrath and Hunsburger, 2012; Trueman, 2014) and are commonly used alone or in combination with other CDM fungicides (Hausbeck, *personal communication*). Cyazofamid and dimethomorph + ametoctradin (premix) became registered in 2014 and 2012 (EPA, 2014; EPA, 2012), respectively, and provide good protection against CDM (Adams et al., 2015; Hausbeck and Linderman, 2014; McGrath and Hunsburger, 2012). Fungicides containing oxathiapiprolin are highly effective against CDM (Adams et al., 2015; Cohen, 2015; Miller et al., 2016) and became available to U.S. growers in late 2015 (EPA, 2015).

Pickling cucumber cultivars ‘Vlaspik’ and ‘Expedition’ are industry standards in Michigan and are susceptible to CDM. The commercially available cucumber cultivars ‘Peacemaker’ and ‘Citadel’ have an intermediate level of CDM resistance but fungicides are still needed for optimal control and yield (Holdsworth et al., 2014; Call et al., 2013; Adams and Quesada-Ocampo, 2017). When exposed to pathogen populations in North Carolina, ‘Peacemaker’ cucumber exhibited reduced CDM severity compared to ‘Citadel’; both cultivars had reduced disease compared to the susceptible ‘Vlaspik’ and ‘Expedition’ (Adams and Quesada-Ocampo, 2017).

This objective of this study was to compare ‘Peacemaker’, ‘Citadel’, ‘Expedition’, and ‘Vlaspik’ for their response to Michigan’s *P. cubensis* populations when treated with three different fungicide programs or not treated.

MATERIALS AND METHODS

Trials were established in 2016 and 2017 on the Michigan State University (MSU) plant pathology farm, Lansing, Michigan, on Capac loam soil previously planted with cucumber. Glyphosate (RoundUp PowerMax at 2.34 L/ha, Monsanto Company, St. Louis, MO) was applied before trial establishment for weed control. Soil was plowed, cultivated and fertilized with ammonium nitrate at a rate of 112 kilograms per hectare. Plots were prepared as raised plant beds covered with black plastic (15 cm high by 60 cm wide) with drip tape. Single rows spaced 1.68-m center to center were seeded on 22 July 2016 and 21 July 2017. Two, single bed rows 6.1-m long were planted with one of the four cultivars, which was designated as a subplot. Each cultivar was planted in four subplots within each of the four main plots which was one of the three fungicide programs or untreated control. This split block design had a four by four factorial treatment, with 16 sub-plots within each replication. This was replicated three times resulting in 48 subplots (cultivar) and 12 main plots (fungicide program or control). A cucumber pollinator cultivar (Seminis Vegetable Seeds, Inc., St. Louis, MO) was planted at the ends of each plant bed to ensure adequate pollination. The CDM-susceptible ‘Vlaspik’ was planted along the trial border. Cucumbers were irrigated as needed throughout the season.

Treatments were arranged as a four by four factorial design with four pickling cucumber cultivars and three fungicide programs plus the untreated control. The cultivars were CDM susceptible (‘Vlaspik’, ‘Expedition’) and intermediately resistant (IR) (‘Citadel’, ‘Peacemaker’).

All seed was obtained from Seminis Vegetable Seeds, Inc. (St. Louis, MO) and commercially treated with azoxystrobin, fludioxonil, mefenoxam and thiamethoxam (Farmore FI400, Syngenta Crop Protection, Greensboro, NC) to limit damping off and early season insect damage. Throughout the growing season, fertilizer (20-20-20 at 2.8 kg/ha) was applied weekly via drip tape, weeds were managed by hand, and insects were controlled with imidacloprid (Admire Pro at 0.58 L/ha, Bayer CropScience, Research Triangle Park, NC) applied through the drip tape four weeks after seedling emergence. Three fungicide treatment programs and an untreated control were assigned to each cultivar and included the following: 1) chlorothalonil (Bravo WeatherStik SC at 2.34 litres/hectare (L/ha), Syngenta Crop Protection, Greensboro, NC), 2) cyazofamid (Ranman SC at 0.20 L/ha, FMC Corporation, Philadelphia, PA) tank mixed with chlorothalonil alternated (alt) with propamocarb (Previcur Flex SL at 1.40 L/ha, Bayer CropScience, Research Triangle Park, NC) tank mixed with chlorothalonil alt with dimenthomorph+amectoctradin (premix) (Zampro SC at 1.03 L/ha, BASF Corporation, Research Triangle Park, NC) tank mixed with chlorothalonil, and 3) oxathiapiprolin+chlorothalonil (premix) (Orondis Opti SC at 2.50 L/ha, Syngenta Crop Protection, Greensboro, NC) alt with cyazofamid tank mixed with chlorothalonil alt with propamocarb tank mixed with chlorothalonil.

Spray treatments were initiated on 10 (2016) and 3 August (2017) prior to CDM symptoms within the plot but in response to CDM symptoms observed in an adjacent field of the CDM susceptible 'Straight Eight' cucumber that was not treated with fungicides. Within the treatment plot, fungicides were applied to the foliage with a CO_2 backpack boom sprayer (Bellspray Inc. R&D Sprayers, Opelousas, LA) equipped with two XR8003 flat-fan nozzles spaced 45.7 cm apart, operating at 275.8 kilopascals, delivering 467.7 L/ha. A third nozzle was added to the boom as the canopy increased and filled the treatment row. Fungicides were applied as follows; 10, 17, 24, 31 August and 7, 14 September 2016 and 3, 10, 18, 25 August and 1, 9, 16 September 2017.

Foliage was evaluated for plant area diseased with CDM on 2, 6, 9, 14, 19 and 22 September 2016 and 18, 24, 31 August and 6, 12, 18 and 26 September 2017 using the Horsfall-Barratt scale (1=0%, 2=0 to 3%, 3=3 to 6%, 4=6 to 12%, 5=12 to 25%, 6=25 to 50%, 7=50 to 75%, 8=75 to 87%, 9=87 to 94%, 10=94 to 97%, 11=97 to <100%, 12=100%) (Horsfall and Barratt, 1945). The area under the disease progress curve (AUDPC) was calculated to express CDM progression throughout the epidemic. AUDPC values were calculated as described by Madden et al. (2007);

$$\text{AUDPC} = = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where, y_i is the severity assessment of the disease (using the mid-point percentage from the Horfall-Baratt scale) at the *i*th observation, t_i is time (days) at the *i*th observation, and n is the total number of ratings. The relative AUDPC (rAUDPC) were then calculated by dividing the AUDPC in the given year by the maximum potential AUDPC.

In 2016, fruit were harvested from both plot rows on 9 September, stored in a controlled atmosphere cooler, and graded on 12 September. In 2017, fruit were harvested on 19 September and graded the next day. Fruit from each plot were weighed and graded into the following pickling cucumber categories (USDA, 1997); 1 (<2.75 cm dia), 2A (2.75-3.25 cm dia), 2B (3.25-4.0 cm dia), 3A (4.0-4.5 cm dia), 3B (4.5-5.25 cm dia), 4 (5.25-5.75 cm dia), 4+ (>5.75 cm dia), and culls (misshapen and/or with an angle >45°). Total marketable yield was calculated by determining the difference between total yield and cull fruit.

Statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC). A global analysis of variance (ANOVA) F-test was calculated (PROC MIXED) for each trial to determine significant differences among fungicide by cultivar treatments. Fungicide program and cultivars were considered fixed effects, and blocks as a random effect, with the block by fungicide program also being considered a random effect. The rAUDPC differed significantly by year. Data from 2016 and 2017 were then analyzed separately using the PROC GLIMMIX. Normality was checked using residual plots and Levene's test was performed to test homogeneity of the variances for each trial. No AUDPC or yield data violated the assumption of the ANOVA test. The interaction between fungicide program and cultivars was significant for both years according to rAUDPC values and thus each cultivar by fungicide program was looked at individually. All AUDPC and yield pair-wise comparisons for the sixteen fungicide by cultivar combinations were assessed with Tukey's Honestly Significant Difference (HSD) test.

RESULTS

CDM was first observed in the untreated 'Straight Eight' plots on the MSU plant pathology farm on 9 (2016) and 3 August (2017). CDM diseased area in the untreated plots reached nearly 100% in both years by mid-September (Figure 6). During August 2016, rainfall was 147 mm total, which was above average (80 mm) for Michigan (NOAA, 2018).

In both years, according to the rAUDPC data, 'Vlaspik' was similar to 'Expedition' in CDM disease severity whether or not fungicides were applied according to one of the three fungicide programs. In 2016, the rAUDPC data showed that the IR cultivars were significantly less diseased than the susceptible cultivars when untreated or treated with chlorothalonil (Program 1). In 2017, the IR cultivar 'Peacemaker' had significantly less CDM than the susceptible cultivars when untreated; 'Peacemaker' and 'Citadel' exhibited similar levels of disease but 'Citadel' was similar to the industry standards. In the same year, when treated with chlorothalonil, 'Peacemaker' was less diseased than 'Vlaspik' but similar to the other cultivars according to rAUDPC data. When the fungicide programs that included additional CDM fungicides (Programs 2 and 3) were used, the cultivars within each program displayed similar levels of CDM each year regardless of CDM resistance. An exception occurred in 2016 when fungicide Program 2 resulted in similar CDM levels among the IR cultivars and 'Vlaspik', which had lower CDM than 'Expedition'.

In 2016, the susceptible cultivars yielded less in total marketable yield than the IR cultivars when untreated. In 2017, only 'Peacemaker' yielded significantly more than the susceptible cultivars when CDM was not limited with fungicides. In both years, the 'Citadel' yield was increased when treated with fungicide Program 2 compared to the untreated. In 2017, 'Citadel' total marketable yields were also increased when using Program 1. The 'Peacemaker' total marketable yields were not increased with the fungicide programs used in this study for either year.

In both years, marketable yields were similar for the IR and susceptible cultivars when treated with fungicides. Differences were observed for fruit grades 2B (2016, 2017) and 3A (2016) within the treatment programs. In 2016, when cultivars were untreated, the IR 'Peacemaker' yielded significantly more 2B and 3A fruit than the susceptible cultivars Expedition and Vlaspik, respectively. In 2017, when plots were untreated, the yield of 'Peacemaker' 2B was greater than that of 'Vlaspik'.

Table 7. Analysis of variance table for area under the disease progress curve response to fixed effects; year, fungicide, cultivar and their interaction. Experiments were conducted in 2016 and 2017 on four cultivars 'Vlaspik,' 'Expedition,' 'Citadel' and 'Peacemaker' untreated and with three fungicide programs for control of *Pseudoperonospora cubensis*.

Fixed Effect	**F**	***P*>F**
Year	34.90	0.0041
Fungicide	1033.78	<0.0001
Cultivar	86.45	<0.0001
Fungicide*cultivar	8.00	<0.0001
Year*fungicide	147.72	<0.0001
Year*cultivar	10.59	<0.0001

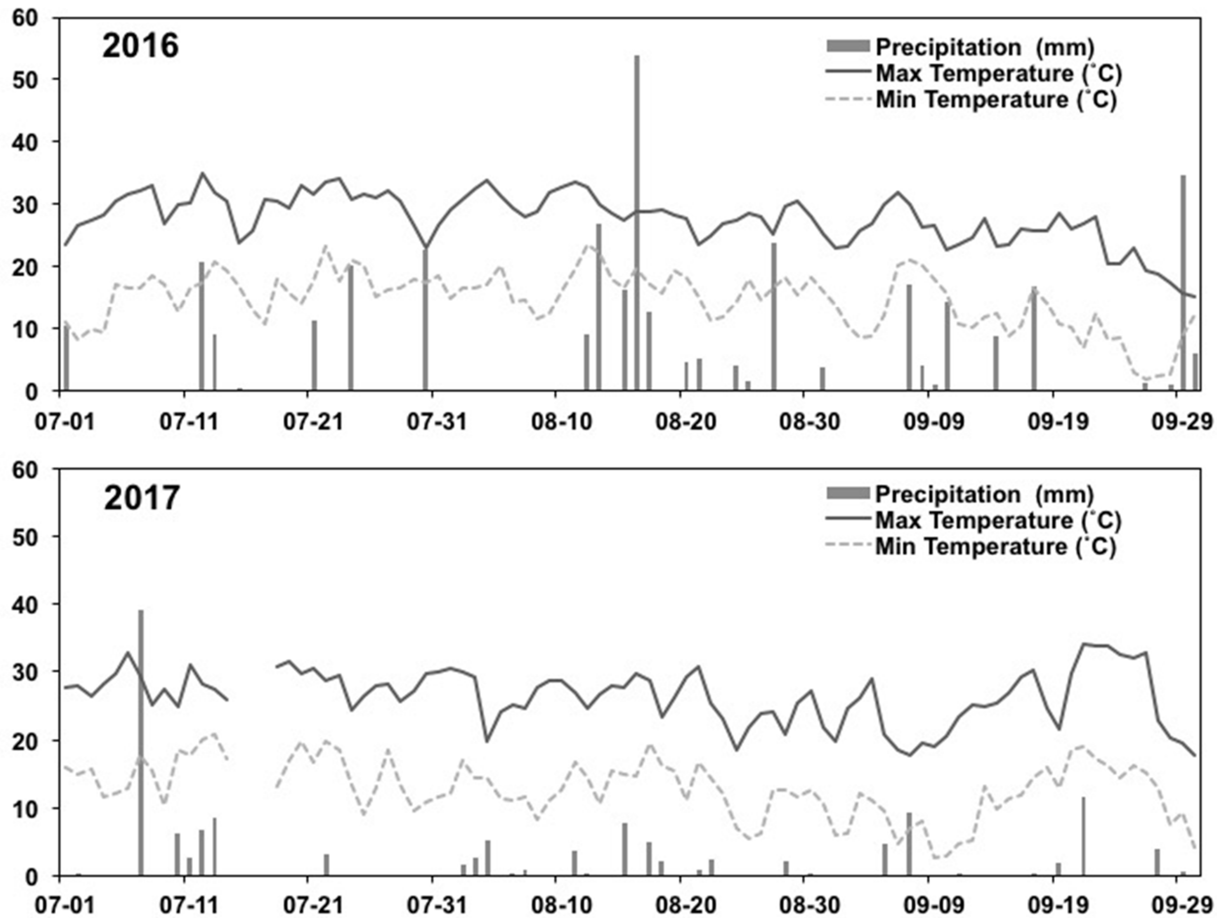

Figure 5. Daily temperature maximum and minimum (°C) and precipitation (mm) from July to September in 2016 and 2017 from the Michigan State University Hancock Turfgrass Research Center in East Lansing, Michigan (https://mawn.geo.msu.edu)

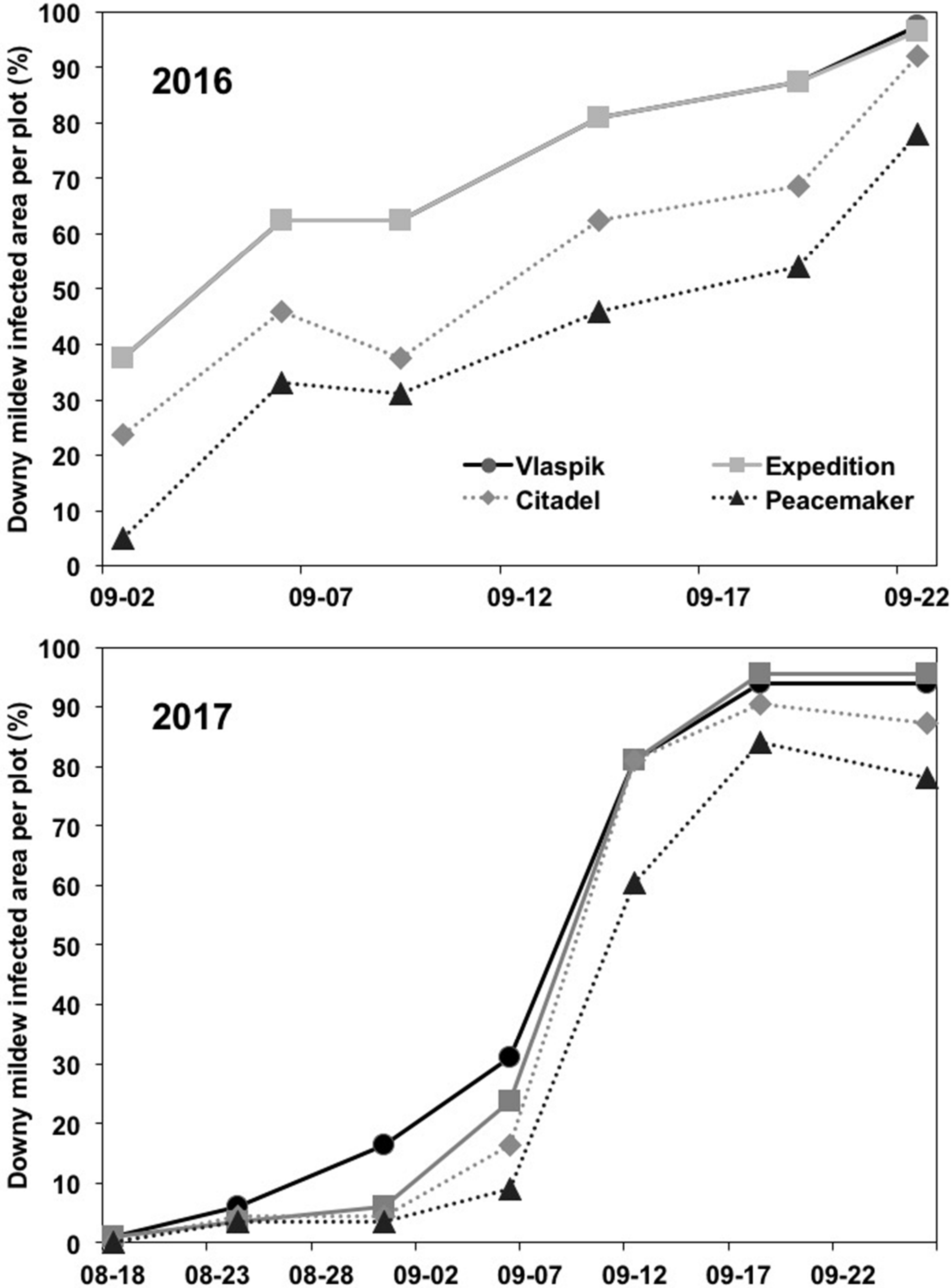

Figure 6. Plot area infected with *Pseudoperonospora cubensis* in 2016 and 2017 for the duration of the ratings on untreated pickling cucumber varieties 'Vlaspik', 'Expedition', 'Citadel' and 'Peacemaker'.

Table 8. *Pseudoperonospora cubensis* disease severity on 'Vlaspik', 'Expedition', 'Citadel' and 'Peacemaker' reported as the relative area under disease progress curve (rAUDPC), for untreated and fungicide programs for 2016 and 2017.

Cultivar	2016	2017
Untreated control		
Vlaspik	0.72 a[z]	0.46 a
Expedition	0.72 a	0.44 a
Citadel	0.54 b	0.41 ab
Peacemaker	0.41 cd	0.34 b
Program 1: chlorothalonil		
Vlaspik	0.46 bc	0.11 c
Expedition	0.49 bc	0.09 cd
Citadel	0.33 de	0.05 c-e
Peacemaker	0.23 ef	0.03 de
Program 2: cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil *alt* dimenthomorph+amectoctradin (premix) + chlorothalonil		
Vlaspik	0.33 de	0.07 c-e
Expedition	0.42 b-d	0.07 c-e
Citadel	0.26 ef	0.05 c-e
Peacemaker	0.20 ef	0.03 de
Program 3: oxathiapiprolin+chlorothalonil (premix) *alt* cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil		
Vlaspik	0.21 ef	0.02 de
Expedition	0.22 ef	0.02 e
Citadel	0.16 f	0.01 e
Peacemaker	0.14 f	0.01 e

[z]Column means with a letter in common are not significantly different (Tukey's honestly significant difference test, N=3, α=0.05).

Table 9. Total marketable yield and fruit size of 'Vlaspik', 'Expedition', 'Citadel' and 'Peacemaker' when graded according to USDA standards for 2016 when treated with fungicide programs or not treated.

Cultivar	Grading Category (bushels per hectare)								
	Total Marketable	1A/B	2A	2B	3A	3B	4	4+	Cull
Untreated control									
Vlaspik	46.52 d[z]	0.8	2.4	20.8 a-c	21.8 d	34.1 f	29.5	2.6 bc	19.5
Expedition	46.56 d	2.2	3.5	16.2 c	30.6 cd	37.7 ef	22.3	1.2 c	18.2
Citadel	87.89 bc	0.7	5.1	25.3 a-c	45.4 b-d	46.4 d-f	51.2	40.7 a-c	13.0
Peacemaker	120.24 a-c	2.5	7.1	41.3 a	66.9 a-c	52.1 c-f	83.1	41.5 a-c	11.4
Program 1: chlorothalonil									
Vlaspik	99.80 a-c	2.8	4.7	32.1 a-c	60.6 a-d	69.3 a-f	57.3	15.6 a-c	17.2
Expedition	83.12 cd	1.3	2.6	17.7 bc	49.6 a-d	74.4 a-d	46.7	11.3 a-c	21.2
Citadel	102.31 a-c	3.2	8.1	26.0 a-c	56.0 a-d	81.4 a-c	44.9	30.7 a-c	10.0
Peacemaker	115.18 a-c	0.8	7.4	38.8 ab	68.2 a-c	74.2 a-e	55.6	36.4 a-c	3.3
Program 2: cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil *alt* dimenthomorph+amectoctradin (premix) + chlorothalonil									
Vlaspik	105.38 a-c	1.9	6.1	32.0 a-c	53.9 a-d	59.3 b-f	65.0	41.0 a-c	22.7
Expedition	103.40 a-c	1.8	6.2	31.6 a-c	55.6 a-d	57.3 b-f	59.0	38.2 a-c	18.2
Citadel	138.14 a	2.8	5.8	30.4 a-c	70.2 a-c	70.9 a-f	84.7	74.2 ab	13.5
Peacemaker	134.98 a	1.5	5.6	31.1 a-c	58.6 a-d	66.0 a-f	88.6	79.3 a	19.9
Program 3: oxathiapiprolin+chlorothalonil (premix) *alt* cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil									
Vlaspik	122.96 a-c	1.0	3.8	25.0 a-c	68.0 a-c	90.5 ab	81.7	31.9 a-c	19.7
Expedition	133.32 a	2.8	5.0	40.6 a	86.1 ab	102.8 a	68.8	20.2 a-c	17.1
Citadel	118.70 a-c	1.3	7.2	39.3 a	64.4 a-c	68.0 a-f	66.1	46.8 a-c	10.3
Peacemaker	125.47 ab	0.7	4.8	38.8 a	87.2 a	79.3 a-d	47.9	41.3 a-c	16.8

[z]Column means with no letters or share a letter in common are not statistically different (Tukey's honestly significant difference, N=3, α=5%)

Table 10. Total marketable yield and fruit size of 'Vlaspik', 'Expedition', 'Citadel' and 'Peacemaker' when graded according to USDA standards for 2017 when treated with fungicide programs or not treated.

Cultivar	Grading Category (bushels per hectare)								
	Total Marketable	1A/B	2A	2B	3A	3B	4	4+	Cull
Untreated control									
Vlaspik	45.99 c[z]	1.1	0.2	3.0 d	31.8	56.8	20.6	0.0	6.4
Expedition	37.00 c	2.9	1.9	7.2 b-d	30.3	31.4	14.9	2.9	14.3
Citadel	68.30 bc	2.0	2.4	4.0 cd	35.3	70.2	53.7	1.1	6.6
Peacemaker	105.79 ab	3.2	2.3	13.9 a-c	54.3	116.6	65.7	5.4	7.2
Program 1: chlorothalonil									
Vlaspik	124.05 ab	5.4	3.1	17.9 a	52.1	98.9	101.7	27.2	5.9
Expedition	128.18 ab	3.9	3.2	13.8 a-c	51.6	111.8	108.0	24.3	6.6
Citadel	140.61 a	5.3	3.9	17.3 ab	44.0	119.6	130.5	26.7	3.5
Peacemaker	134.09 a	3.5	1.7	10.6 a-d	51.0	135.7	113.8	14.9	5.0
Program 2: cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil *alt* dimenthomorph+amectoctradin (premix) + chlorothalonil									
Vlaspik	150.77 a	7.6	4.2	14.1 a-c	57.6	111.1	144.2	33.7	5.8
Expedition	135.67 a	4.1	3.6	18.1 a	61.2	136.9	94.4	16.7	5.0
Citadel	143.77 a	3.2	4.3	13.2 a-d	50.7	125.7	124.0	34.1	3.2
Peacemaker	147.65 a	6.0	3.7	14.5 ab	70.7	140.1	114.3	15.4	4.8
Program 3: oxathiapiprolin+chlorothalonil (premix) *alt* cyazofamid + chlorothalonil *alt* propamocarb + chlorothalonil									
Vlaspik	130.12 ab	4.2	4.3	16.0 ab	47.1	112.2	106.9	30.7	6.2
Expedition	146.68 a	4.2	3.1	13.2 a-d	62.0	124.3	128.3	27.2	8.3
Citadel	126.92 ab	3.6	4.2	13.2 a-d	45.6	108.4	123.0	15.4	2.1
Peacemaker	150.57 a	4.7	3.0	16.4 ab	53.1	150.8	130.8	13.1	5.5

[z]Column means with no letters or share a letter in common are not statistically different (Tukey's honestly significant difference, N=3, α=5%)

DISCUSSION

CDM has occurred annually in Michigan for more than a decade although the timing of initial infection and disease severity varies. Cucumbers are especially susceptible to CDM and resistant cultivars are needed to reduce growers' reliance on fungicides and their risk of crop loss. Integrating commercially-available resistant cultivars with effective fungicides is a disease management approach used successfully in other agricultural industries (Agrios, 2005). Results from this study show that combining fungicides and IR cultivars reduced CDM foliar blighting but did not always result in significantly increased yields.

Overall, rAUDPC values were higher in 2016 than in 2017. *Pseudoperonospora cubensis* requires free moisture to produce zoospores and infect the foliage (Palti and Cohen, 1980); infection can occur from 5°C to 30°C (Cohen, 1977; Savory et al., 2011). Total precipitation was higher in August 2016 than in August 2017 (Figure 5). Increased precipitation favors CDM, especially in August when *P. cubensis* sporangia airborne concentrations are likely to be high in Michigan (Granke et al., 2014).

Growers desire a level of genetic resistance to CDM that would eliminate or decrease the need for fungicides. The results of this study indicate that while fungicides were important in reducing foliar blighting from CDM, combining fungicides with IR cultivars did not always significantly increase yields in this study. Our trials were planted each year so as to ensure that the plants were young at a time when *P. cubensis* inoculum are expected to be high. Yet, using fungicides in conjunction with the IR cultivars did not consistently boost yields suggesting that 'Peacemaker' and 'Citadel' can withstand even a significant amount of foliar blight without sacrificing yields.

Fungicide Program 1 included the multi-site fungicide chlorothalonil only and provided CDM control compared to the untreated. Mancozeb is also a multi-site fungicide that often provides a level of CDM control similar to chlorothalonil (Trueman, 2014; Adams and Quesada-Ocampo, 2017b). Chlorothalonil and mancozeb are recommended for use as a tank mixing partner for CDM-targeted fungicides. Utilizing a multi-site fungicide in combination with alternating effective CDM specific products reduces the risk of fungicide resistance (Brent and Holloman, 1995).

Fungicide Programs 2 and 3 included propamocarb, a highly effective fungicide when first registered and popular among Michigan growers. Although propamocarb continues to be effective against CDM in the southern U.S. (Adams and Quesada-Ocampo, 2017b; Raid, 2017), it is no longer effective in the state (Hausbeck and Linderman, 2013; Goldenhar and Hausbeck, 2016) or in Ontario (Goldenhar, *unpublished data*). However, tank mixing propamocarb with chlorothalonil and alternating it with products belonging to different FRAC groups ensured that the overall programs limited CDM.

Program 3 was the only program with the newly-registered fungicide oxathiapiprolin. Studies showed oxathiapiprolin to be especially effective against CDM (Adams et al., 2015; Cohen, 2015; Miller et al., 2016). The most profitable grades of pickling cucumbers in Michigan are the 2B and 3A categories (M. Hausbeck, *personal communication*). In the 3A category, Program 3 was the only program in 2016 where all cultivars yielded better than untreated 'Vlaspik'. 'Peacemaker' treated with Program 3 was better than 'Citadel' and the susceptible cultivars.

Untreated, IR cultivars had higher marketable yields than untreated susceptible cultivars in 2016. In 2017, only untreated 'Peacemaker' yielded higher than the untreated susceptible cultivars. A similar pattern was observed for foliar disease levels. Similar to a study conducted in North Carolina (Adams and Quesada-Ocampo, 2017a) where untreated ‘Peacemaker’ was more resistant to foliar blight caused by CDM than untreated ‘Citadel’ in 2016. ‘Citadel’ and ‘Peacemaker’ were similar in CDM levels in 2017 in this study.

An integrated management program for CDM that uses both CDM-specific and multi-site fungicides and IR cultivars may aid Michigan’s pickling cucumber growers. However, the costs of the IR cultivars plus the fungicides should be considered. Yearly monitoring of the response of fungicides and IR cultivars to Michigan *P. cubensis* populations is important for effective grower recommendations.

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FUTURE WORK

Michigan cucumber growers benefit from the fungicide efficacy work conducted locally. Single product efficacy work should be continued annually and compared to previous years' trials to monitor changes. Resistant cultivars should also be tested locally to evaluate the benefit to growers. The cost of fungicides and seed for resistant cultivars should be considered when developing CDM management programs. Evaluating the cost of an integrated program can assist growers with choosing the most cost-effective approach for their operation. Since yield and quality are important data for growers, larger research plots could be considered when testing fungicide programs in combination with resistant cultivars to reduce variability and strengthen the relationship between foliar blighting and marketable yield.