MANAGING CUCURBIT DOWNY MILDEW ON PICKLING CUCUMBER USING DISEASE FORECASTERS AND FUNGICIDES

By

Matthew Ray Uebbing

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ABSTRACT

Michigan is the nation's leading producer of cucumbers (*Cucumis sativus*) for pickling, valued at $45.4 million in 2019. Cucurbit downy mildew (CDM) caused by oomycete *Pseudoperonospora cubensis* is an annual threat to pickling cucumber production in the state. Prior to 2004, CDM on cucumber in the U.S. was controlled via host resistance that was integrated into commercial cultivars in the 1950s. In 2004, a new population of *P. cubensis* appeared that was highly virulent on previously resistant cucumber cultivars resulting in a CDM epidemic throughout the southeast and Atlantic coast; Michigan reported a CDM outbreak in 2005. Currently, prophylactic fungicides are relied on for disease control, but the pathogen has become resistant to key products. Our goal was to improve CDM disease management for Michigan's pickling cucumber growers by 1) evaluating 13 currently labelled fungicides for efficacy under field conditions and 2) evaluating the TOM-CAST, BLITE-CAST, and DM-CAST forecasters at different thresholds for their application in scheduling fungicide applications. Trials were conducted in 2021 and 2022 at the Michigan State University Plant Pathology Research Farm in Lansing, Michigan. Disease severity was assessed by visually estimating the foliar area showing symptoms (%) and calculating the area under the disease progress curve (AUDPC). Oxathiapiprolin + chlorothalonil was the most effective fungicide whereas fluopicolide, dimethomorph, and pyraclostrobin were ineffective. Chlorothalonil and mancozeb provided a moderate level of control but are not recommended as stand-alone treatments. When fungicide applications were scheduled using BLITE-CAST or DM-CAST models, CDM levels were similar to those observed with the 7-day program but did not consistently reduce total fungicide applications. These results provide Michigan growers with information to implement effective and sustainable CDM management strategies

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LITERATURE REVIEW

Introduction

Cucumber, *Cucumis sativus* L., is a member of the *Cucurbitaceae* family which includes commercially important crops such as cantaloupe, squash, pumpkin, and watermelon (Keinath et al. 2017). Cucumbers are an important vegetable crop in the U. S. with 48,422 hectares harvested in 2017 alone (USDA, NASS 2017a). Fresh markets accounted for 16,397 of the hectares harvested in 2017 while cucumber harvested for processing (pickling cucumber) accounted for 32,025 of the total hectares harvested in 2017 (USDA, NASS 2017a). Michigan is the leading producer of pickling cucumbers in the country with a total of 11,843 hectares harvested in 2017 (USDA, NASS 2017b). The value of utilized production for the 2019 pickling cucumber crop in Michigan was around $45.4 million with 219,050 metric tons utilized (USDA, NASS 2020).

Most popularly grown varieties in Michigan are either susceptible or only moderately resistant to cucurbit downy mildew (CDM) (Call et al. 2012a). As a result, CDM is a constant threat to the yield potential of pickling cucumbers in Michigan and anywhere cucurbits are grown (Cespedes-Sanchez et al. 2015). CDM affects cucurbit leaves, resulting in reduced photosynthetic potential and in severe cases, defoliation (Cespedes-Sanchez et al. 2015; Lindenthal et al. 2005). Current management of CDM in pickling cucumber requires timely fungicide applications (Savory et al. 2011). In Michigan, these applications are initiated by spore trap identification coupled with an observation of symptoms; then applications are repeated on a 7-day schedule thereafter (Granke et al. 2014). Fungicide costs associated with CDM control cost Michigan cucumber producers around $6 million annually (Granke et al. 2014; Goldenhar and Hausbeck 2019). Based on the high costs associated with its control, CDM is one of the most

important diseases of cucumber (Holmes et al. 2015); CDM is caused by the obligate, biotrophic oomycete *Pseudoperonospora cubensis* (Savory et al. 2011).

Pseudoperonospora cubensis

History. *Pseudoperonospora cubensis* (Berk. and M.A. Curtis) Rostovzev 1903, is a member of kingdom Chromista; phylum Oomycota; class Peronosporea; order Peronosporales; family *Peronosporaceae*; genus *Pseudoperonospora* (Salcedo et al. 2020). It was first described in Cuba in 1868 on a non-cultivated cucurbit and named *Peronospora cubensis* by Berkley and Curtis (1868). It was reclassified and named *Pseudoperonospora cubensis* in 1903 (Rostovzev 1903). The first documentation of *P. cubensis* in the United States occurred in a New Jersey greenhouse on cucumber in 1889 (Halsted 1889a). In that same year, *P. cubensis* was also documented on field-grown cucumber, squash, and pumpkin in New Jersey and on cucumber in Florida and Texas (Galloway 1889; Halsted 1889b).

P. cubensis and *P. humuli* (the causal agent of hop downy mildew) are morphologically indistinguishable and initial investigations found no genetic basis for maintaining them as two different species based on analysis of the Internal Transcribed Sequence (ITS) data (Choi et al. 2005). Runge et al. (2011) analyzed the ITS, cytochrome c oxidase subunit 2 (*cox*2), and *ras*-related GTP-binding protein 1 (*ypt*1) loci of *P. humuli* and *P. cubensis*. A combined phylogenetic analysis using the ITS, *cox*2, and *ypt*1 loci revealed that while *P. humuli* occupied a monophyletic group with *P. cubensis* distinct from other *Pseudoperonospora* species in the study (*P. cannabina*, *P. celtitis*, *P. urticae*), they grouped into different clades (Runge et al. 2011). The monophyletic group consisted of one clade of *P. humuli* (isolated from *Humulus lupulus*) which was termed clade 3 and two clades of *P. cubensis*, termed clades 1 and 2, placed sister to clade 3. This observation indicates a separation of *P. cubensis* and *P. humuli* as two distinct species as the

two clades of *P. cubensis* were placed together distinct from *P. humuli* (Runge et al. 2011). Interestingly, *P. cubensis* clade 1 appears to be more closely related to *P. humuli* than to *P. cubensis* clade 2 (Kitner et al. 2015). Also, two cryptic lineages along with an isolate of *P. humuli* (isolated from *Humulus japonicus*) placed basally to all the clades in the *P. cubensis-P. humuli* cluster indicating that a possible host transfer from hop to *Cucurbitaceae* gave rise to *P. cubensis* (Runge et al. 2011). Mitchell et al. (2011) also concluded that *P. cubensis* and *P. humuli* are separate species. The phylogenetic analysis using the ITS region, the nuclear β-tubulin gene, and the *cox* or cytochrome *c* oxidase cluster (includes partial *cox*2, *cox*2-*cox*1 spacer and partial *cox*1) supported the separation of *P. cubensis* and *P. humuli* into different clades (Mitchell et al. 2011). Phylogenies constructed using both single locus (*cox* cluster or ITS) and multiple loci (ITS and β-tubulin) supported this separation (Runge et al. 2011). Furthermore, Mitchell et al. (2011) found that *P. humuli* was unable to infect two highly susceptible hosts of *P. cubensis* (*Cucumis sativus* cultivar Straight 8 and *Cucumis melo* cultivar Ananes Yokneam) and *P. cubensis* was only able to infect two highly susceptible hop ('Nugget' and 'Pacific Gem') at very low levels. Other cross infection experiments have produced similar, yet unidentical, results with *P. cubensis* being able to infect susceptible hop in 6 out of 25 trials conducted and *P. humuli* being able to infect susceptible cucumber in 7 out of 25 trials conducted (Runge and Thines 2012). These results provided further evidence to keep the current species delineations.

Signs and Symptoms. *P. cubensis* has a broad host range, able to cause disease on twenty different genera of cucurbits (Savory et al. 2011). In cucumber, symptoms begin as small (3 to 10mm), water-soaked lesions on the upper leaf surface (Hausbeck 2017). Lesions may occur anywhere between 4 to 12 days after infection of cucumber depending on inoculum

concentration, temperature, and leaf wetness duration (Cohen 1977) and are typically bound by leaf veins which is not always observed in other cucurbit species (Hausbeck 2017). Lesions are chlorotic in early stages of colonization and later in the colonization process these chlorotic lesions expand, turning necrotic and coalescing to form large, blighted areas on the leaf surface (Hausbeck 2017; Oerke et al. 2006). Infected leaves can also dry considerably and curl upward; in severe cases, defoliation can occur. (Palti and Cohen 1980; Hausbeck 2017). Secondary symptoms that are a result of this defoliation include decreased fruit size and yield and occasional sun scalding of exposed fruit (Goldenhar and Hausbeck, 2019; Keinath et al. 2007; Salcedo et al. 2020). On cucurbits other than cucumber, symptoms include small, irregularly shaped chlorotic lesions not bound by leaf veins (Hausbeck 2017). Differences in leaf temperature and transpiration rates, which are inversely correlated, have been observed in leaves colonized by *P. cubensis* (Lindenthal et al. 2005; Oerke et al. 2006). These differences correspond to the stage of infection with a decrease in leaf temperature (and increase in transpiration) corresponding to the early stages of infection and an increase in leaf temperature (and decrease in transpiration) corresponding to the later stages of infection (Lindenthal et al. 2005; Oerke et al. 2006). Stomatal aperture of leaves colonized by *P. cubensis* is higher than in healthy leaves (Lindenthal et al. 2005). It has been noted that warmer weather can increase the rate of chlorotic and necrotic lesions in infected tissue (Cohen and Rotem 1971). On the contrary, cooler weather can delay the onset of visible symptoms while simultaneously leading to increased *P. cubensis* colonization of the leaf tissue (Cohen and Rotem 1971).

Sporangia production on the abaxial leaf surface produces a characteristic sign of *P. cubensis*: a dark brown, gray, or violet-black covering that resembles “fluff” or “down” (Salcedo et al. 2020; Hausbeck 2017). Sporangia (20-40 × 14-25 μm) are ovoid to ellipsoidal, thin walled,

with a papilla at the distal end. They are olivaceous-purple and develop on the end of hyaline sporangiophores, 180-400 µm in length and 5-10 µm in width, which emerge from stomata on the abaxial leaf surface in groups of 1 to 5 (Choi et al. 2005; Palti 1975). Sporangia germinate to form flagellate zoospores which are 10-13 µm in diameter (Palti 1975). Hyaline hyphae that develop in the intercellular spaces of the leaf mesophyll are coenocytic; hyphae also form small, ovate haustoria that penetrate leaf palisade tissues sometimes containing finger-like processes from their swollen tips (Fraymouth 1956; Palti 1975). Oospores (22-42 µm in diameter) are light yellow or hyaline and globular in appearance (Palti 1975). Oospores have been observed in Russia, Japan, Austria, India, Italy, Israel, and China (Bains et al. 1977; Bedlan 1989; Cohen et al. 2003; D'Ercole 1975; Hiura and Kawada 1933; Palti and Cohen 1980).

Infection and Colonization. Airborne sporangia are the primary and secondary inoculum source throughout the growing season leading to multiple rounds of infection, a polycyclic disease cycle (Hausbeck 2017). Sporangia production occurs on the lower leaf surface after at least 6 hours of leaf wetness (Cohen and Rotem 1969) and is dependent on the diurnal cycle with sporangia production amplified by increased day lengths (Cohen and Rotem 1971). Additionally, a minimum of 6 hours of continuous darkness (coinciding with leaf wetness) is needed for sporangia to differentiate and light, especially blue light, is inhibitory towards sporangia production (Cohen and Eyal 1977). The ideal temperature for sporangia production is 20° C; although, sporulation can occur anywhere between 5 and 25° C (Cohen and Rotem 1969, 1971). Sporulation for biotrophic pathogens like *P. cubensis* occurs most prolifically on living tissue, i.e. chlorotic lesions (Cohen and Rotem 1969; Rotem et al. 1978). Sporangia are released during periods of decreasing relative humidity where hygroscopic twisting of drying sporangiophores releases sporangia into the air (Lange et al. 1989). Consequently, airborne sporangia

concentrations at approximately 0.5 meters above the crop canopy are highest at 0900 hours and between 1100 and 1400 hours in low or high and moderate disease severity, respectively (Granke et al. 2014). Once sporangia have been released into the atmosphere, their survival depends upon environmental conditions such as temperature, relative humidity (Cohen and Rotem 1971; Hausbeck 2017) and most importantly solar radiation intensity (Kanetis et al. 2010). Sporangial survival is higher when both temperature and relative humidity are low, at or below 30 °C and 55% relative humidity, respectively (Cohen and Rotem 1971) and on cloudy days when solar radiation intensity is low (Kanetis et al. 2010).

Following inoculation, the sporangium germinates after a minimum of 2 hours of leaf wetness at an ideal temperature (20 °C) (Cohen 1977). A sporangium can germinate at temperatures ranging from 5 to 25 °C although longer leaf wetness periods are needed at 5 °C (12 hours) (Cohen 1977). Sun et al. (2017) reported *P. cubensis* germination occurring at temperatures up to 30 °C. Germination occurs via cytoplasmic cleavage, releasing up to 15 motile, biflagellate zoospores that preferentially swim to open stomata where they encyst (Iwata 1949; Hausbeck 2017). Germ tubes from encysted zoospores form an appressoria (Hausbeck 2017; Savory et al. 2012). From the appressoria, a penetration hypha forms and enters the stomatal opening (Hausbeck 2017; Savory et al. 2012). Intercellular growth and colonization of the mesophyll and palisade tissue follows infection and nutrient uptake occurs via specialized structures called haustoria, which are clavate-branched and establish within mesophyll cells by invaginating the plant cell membrane (Fraymouth 1956; Voglmayr et al. 2004). Haustoria release effector proteins which are thought to redirect host metabolism and suppress the host's defense response (Tian et al. 2011; Whisson et al. 2007).

Infection and colonization by *P. cubensis* have multiple effects on the physiology of the host plant (Lindenthal et al. 2005). Many of these effects result from the redirection of nutrients into the haustoria; the discovery of nuclear localized effectors provides a possible mechanism for this host reprogramming (Tian et al. 2011). The formation of a transpiration sink in the areas of infected leaf tissue with actively growing pathogen is also thought to aid in the redirection of nutrients to the growing pathogen (Lindenthal et al. 2005). Lindenthal et al. (2005) also reported that *P. cubensis* infection significantly lowered net photosynthetic rate in infected cucumber leaves while they were chlorotic. In tissues that had become necrotic, net photosynthetic rate became negative, with the respiration rate exceeding the assimilation rate (Lindenthal et al. 2005). Infection by *P. cubensis* causes a decrease in the photosynthetic potential of infected leaf tissues (and therefore yield) and in severe cases can result in the complete defoliation of infected cucumber plants. It has been shown that *Cucumis* is the cucurbit genus most susceptible to *P. cubensis* (Cespedes-Sanchez et al. 2015). Other cucurbit genera like *Citrullus*, *Cucurbita Lagenaria*, and *Luffa* are less susceptible to *P. cubensis*, although symptoms and yield loss can still occur (Cespedes-Sanchez et al. 2015). Outside of the family *Cucurbitaceae*, while infection and sporulation can occur on hop under laboratory conditions, hop is not considered a viable host in nature (Mitchell et al. 2011).

Host Range. CDM caused by *P. cubensis* has only been observed in nature on plant species in the *Cucurbitaceae* family; cucumber, cantaloupe, squash, and pumpkin are the four most important commercial crops affected (Palti and Cohen 1980; Savory et al. 2011). In Japan, *P. cubensis* isolated from *Cucumis sativus* was pathogenic on *C. sativus*, *C. melo*, and *Lagenaria vulgaris* but not pathogenic on C*ucurbita maxima*, *C. moschata* and *Luffa cylindrica* (Iwata 1941). In contrast, *P. cubensis* isolated from *C. moschata* was pathogenic on all the species

mentioned above (Iwata 1941). In Israel before 2003, *P. cubensis* was only observed causing disease on cucumber and cantaloupe, but in 2003 a new pathotype of *P. cubensis* was observed that caused disease on summer squash and pumpkin (and very low levels of disease on watermelon) in addition to cucumber and cantaloupe (Cohen et al. 2003). In the Czech Republic, under natural infection conditions, CDM is observed mostly on *Cucumis sativus*, the most commonly grown cucurbit in the country, with rare occurrences of disease noted on *C. melo* and *Cucurbita moschata*, which are more rarely grown in the country (Lebeda et al. 2011). In the United States, Doran (1932) and Hughes and Van Haltern (1952) reported *P. cubensis* isolated from *Cucumis sativus* severely infecting *C. sativus* and *C. melo*, causing a low level of disease on *Luffa cylindrica* and *Lagenaria leucantha*, and not causing disease on *Cucurbita pepo, C. moschata, C. maxima, Melothria scabra*, and *Momordica balsamina*. In 2015, a field study in Michigan using natural inoculum found that *Cucumis* spp. were most susceptible to CDM while *Citrullus*, *Lagenaria* and *Luffa* spp. were less susceptible (Cespedes-Sanchez et al. 2015). *Cucurbita* spp. showed very little to no disease symptoms (Cespedes-Sanchez et al. 2015). It is apparent that physiological specialization occurs within *P. cubensis* (Cespedes-Sanchez et al. 2015; Palti and Cohen 1980) with *Cucumis sativus* and *C. melo* being the two most severely affected hosts and other cucurbit species being less affected (Cespedes-Sanchez et al. 2015; Doran 1932; Hughes and Van Haltern 1952; Iwata 1941; Lebeda et al. 2011).

Host Resistance. In the 1950s, a series of resistant cucumber cultivars was developed using resistance from plant introduction (PI) ascension 197087 including ‘Polaris’, ‘Poinsett’, ‘Pixie’, and ‘Chipper’ (Cohen et al. 2015). While these resistant cultivars could become infected by *P. cubensis*, the pathogen was restricted, causing small, circular, yellowish-green lesions; pathogen sporulation was reduced compared to susceptible cultivars (Cohen 1976). After the integration of

PI 197087 into resistant cultivars, cucumber growers controlled CDM with minimal fungicide applications if any at all (Cohen et al. 2015). However, in 2004 the genetic resistance from PI 197087 was overcome by *P. cubensis* (Cohen et al. 2015). After 2004, cultivars with PI 197087 derived resistance were only moderately resistant to CDM (Call et al. 2012a), resulting in an epidemic that devastated growers from Georgia, South Carolina, North Carolina, Virginia, Maryland, Delaware, and New Jersey (Holmes et al. 2015). Michigan reported a significant outbreak of CDM in 2005 (Holmes et al. 2015). Post 2004, high yielding, tolerant cultigens have been identified (Call et al. 2012b) as well as some moderately resistant cultigens, but none are highly resistant (Call et al. 2012b). The PI 197088 and PI 330628 cultigens are the most promising, showing similar responses to artificial inoculation of *P. cubensis* to those cultivars with PI 197087 derived resistance (Chen et al. 2020). They show a similar response specifically in how they limit pathogen growth; although the pathogen establishes initial mycelium and haustoria, the haustoria are quickly encased in callose and infected cells quickly accumulate lignin-like, phloroglucinol-positive materials which likely impact the pathogen's ability to extract nutrients out of host cells (Chen et al. 2020). In cantaloupe, the resistance interaction is very similar: initial pathogen colonization is slowed by callose deposition, accumulated lignin-like materials and even a hypersensitive response (Reuveni 1983). Lesions are typically brown in resistant plants and sporulation is greatly reduced while susceptible plants have greenish or yellow lesions and abundant sporulation (Reuveni 1983).

Epidemiology. Importantly, *P. cubensis* is an obligate pathogen meaning it cannot survive outside of a living host (Hausbeck 2017; Palti and Cohen 1980; Savory et al. 2011). Oospores, which are important overwintering structures to some oomycetes (Cohen and Rubin 2012), have yet to be proven as an important source of inoculum under field conditions (Palti and Cohen

1980; Savory et al. 2011) and will be discussed in more detail in the following paragraph. *P. cubensis* has not been found to overwinter above 30° latitude in North America (Ojiambo et al. 2015). Consequently, *P. cubensis* is thought to only overwinter in the Eastern U.S. in Florida and along the coast of the Gulf of Mexico where cucurbits are grown year-round (Quesada-Ocampo et al. 2012). During the summer months, airborne *P. cubensis* sporangia are transported from the southeastern U.S. to northern cucumber growing regions like Michigan via wind currents (Ojiambo et al. 2015). This is thought to be the primary inoculum source for CDM in Michigan; however, it has also been noted that greenhouses in Canada that grow cucurbits throughout the winter months could also serve as a primary inoculum source for cucumber growers in Michigan and Canada (Naegele et al. 2016). Closer examinations into the population structures of *P. cubensis* in Michigan and Canada have shown that there are differences between the two populations and that *P. cubensis* population diversity can vary even at county and field level (Naegele et al. 2016). These findings seem to indicate the possibility of inoculum entering the state from southern states initially, along with subsequent migrations of inoculum into the state from other regions (Naegele et al. 2016). Clarifying the potential sources of this inoculum will be an area for future research.

It has been shown that *P. cubensis* is able to produce oospores under laboratory conditions that can germinate and infect cucurbits (Cohen and Rubin 2012; Thomas et al. 2017). Cucumber and cantaloupe are the two hosts that *P. cubensis* produces the most oospores on, with oospores rarely being formed on squash, pumpkin, and watermelon (Cohen and Rubin 2012). Oospores are formed in the leaves of the host plant and have the potential to persist in the field, on plant debris, or in the soil (Cohen and Rubin 2012) and occurrence in the field in the U.S. was only recently confirmed on cucumber and cantaloupe in North and South Carolina (Kikway et al.

2022). Importantly, *P. cubensis* is heterothallic meaning that for oospore production to occur both the A1 and A2 mating types must be present (Cohen and Rubin 2012; Thomas et al. 2017). Furthermore, it has been noted that mating type A1 and mating type A2 preferentially infect different cucurbit species (Thomas et al. 2017). Wallace et al. (2020) found that each mating type is grouped into one of two host-adapted clades. Mating type A1 is predominately found within clade 2 which is host-adapted to the genus *Cucumis* while mating type A2 is predominately found within clade 1 and is host adapted to the genera *Cucurbita* and *Citrullus* (Wallace et al. 2020). Therefore, the likelihood of finding oospores in the field would presumably be increased in areas where *Cucumis* and either *Cucurbita* or *Citrullus* are grown in close proximity (Thomas et al. 2017; Wallace et al. 2020). The mechanism that dictates host preference among mating types is currently unknown; however, what is known is that both the A1 and A2 mating types are present in the U.S. (Thomas et al. 2017) and under certain laboratory conditions can form oospores that are able to germinate and cause infection (Thomas et al. 2017). Further research needs to be done to elucidate the importance of oospores in the CDM disease cycle, especially their formation and viability for infection under field conditions.

Population Genetics. A global analysis of *P. cubensis* population structure identified six genetic clusters and found that some genotypes are widespread throughout the world (Quesada-Ocampo et al. 2012). All six genetic clusters are present in each continent: Asia, Europe, and North America. However, the relative abundance of these clusters varies; cluster 1 is more common in Europe while clusters 2, 3, and 5 are more common in North America.

Populations of *P. cubensis* from different geographic regions in Europe show signs of genetic differentiation; pathogen populations in Greece (Crete) are different than those in Central (Czech Republic) and Western Europe (France and the Netherlands) (Sarris et al. 2009). Similar

population structure in a given geographic region is also possible. Central and Western Europe have a similar cluster composition (Quesada-Ocampo et al. 2012; Sarris et al. 2009) and so does Southern Europe (Quesada-Ocampo et al. 2012). Alternatively, population studies in Turkey and the Czech Republic showed two *P. cubensis* populations with a similar genetic structure. Population structure was similar in these two geographic regions, illustrating a potential common inoculum source and distribution mechanism such as prevailing winds (Polat et al. 2014).

In the U.S., cluster composition cannot be explained exclusively by geographic region. Quesada-Ocampo et al. (2012) showed similar cluster composition between the geographically close Great Lakes states (Michigan, Ohio, and Wisconsin) and the geographically distant Virginia. Furthermore, Ontario, Canada showed similar cluster composition to the geographically close Great Lakes states, and with geographically distant California (Quesada-Ocampo et al. 2012). Genetic diversity in the U.S., like cluster composition, cannot be exclusively explained by geography, with the highest diversity estimates most often occurring in southern states like Georgia and North Carolina, but also occurring in the Midwest including Iowa (Quesada-Ocampo et al. 2012). Many clusters may be present within a smaller geographic region (state or province) with differing abundances (Naegele et al. 2016). However, in an individual field, diversity is quite low with one cluster usually dominating (Naegele et al. 2016).

P. cubensis population structure is influenced not only by geography but time (Naegele et al. 2016). Over the course of a growing season, diversity in a given region (state/province, county, or field) increases and genetic differentiation decreases because of new inoculum being introduced (Naegele et al. 2016). Migration also shapes *P. cubensis* population structure (Naegele et al. 2016; Polat et al. 2014; Quesada-Ocampo et al. 2012). Genetic structure within different geographic regions can be similar (Polat et al. 2014; Quesada-Ocampo et al. 2012;

Sarris et al. 2009); differing genetic structures may be found within one geographic region (Naegele et al. 2016; Quesada-Ocampo et al. 2012). Regions may share inoculum sources through similar migration patterns (Polat et al. 2014; Quesada-Ocampo et al. 2012; Sarris et al. 2009) with one region receiving inoculum from many different sources during a growing season (Naegele et al. 2016; Quesada-Ocampo et al. 2012).

Another factor driving population structure in *P. cubensis* is host preference (Quesada-Ocampo et al. 2012; Wallace et al. 2020). Certain genetic clusters are more commonly isolated from particular cucurbit host(s) than others (Quesada-Ocampo et al. 2012). Two genetically distinct clusters (clade 1 and clade 2) have been identified in the U. S. and high differentiation between these two groups has been observed (Quesada-Ocampo et al. 2012; Runge et al. 2011; Wallace et al. 2020). These clades have also been identified in Europe and Asia (Kitner et al. 2015; Runge et al. 2011). Rahman et al. (2021) and Wallace et al. (2020) found that distribution and abundance of *P. cubensis* in North Carolina is not affected by location but can be influenced by time with clade 2 being more abundant in the summer and clade 1 being more abundant in the fall. Clade 2 preferentially infects commercially important cucurbits *Cucumis sativus* and *C. melo* while clade 1 infects commercially important cucurbits *Cucurbita pepo*, *C. maxima*, *C. moschata*, and *Citrullus lanatus* (Wallace et al. 2020). Since both clades are able, at low levels, to infect common cucurbit hosts, it has been proposed that genetic differentiation existing between the two clades could be a result of more than a host preference barrier (Wallace et al. 2020). Another proposed reason for genetic differentiation between the two clades is the expansion of a single clonal lineage (clade 2) or a speciation event between the two clades with established reproductive barriers, either pre and or post-zygotic (Wallace et al. 2020). Wallace et al. (2020) noted that the index of association analyses provided evidence of

random mating within clade 1, indicative of a sexually reproducing population, but not within clade 2, indicative of an asexually reproducing (clonal) population. It is possible that this new phylogenetic clade (clade 2) represents a new subspecies of *P. cubensis* or even a new species of *Pseudoperonospora* (Wallace et al. 2020).

Clade 2 contains only *P. cubensis* isolates from East Asia (Japan and Korea) except for post-epidemic isolates from Germany, the Czech Republic, and the U.S. (Runge et al. 2011). Clade 1 contains *P. cubensis* isolates mainly from the U.S. but also a few isolates from Europe and Asia (Runge et al. 2011). Since clade 2 originated in East Asia and was only isolated in Europe and the U.S. after epidemics in the 1980's and 2004, it is likely that this clade was responsible for the breakdown of host resistance in cucumber noted in the U. S. in 2004, probably introduced via anthropological transport (Runge et al. 2011).

Chemical Control

Fungicide Resistance. Since the breakdown of host resistance in 2004, pickling cucumber growers have relied on foliar applied fungicides for control of CDM (Ojiambo et al. 2010). Ojiambo et al. (2010) concluded from a meta-analysis of the effects of fungicides on CDM, using data from 2000 to 2008, that fungicide use has a large impact on disease suppression and that fungicide response is 27% greater on cucumber than other cucurbit species. However, fungicide resistance in *P. cubensis* populations in the United States and Michigan is an ongoing concern as this pathogen has a high risk of developing fungicide resistance (FRAC 2019). Fungicide resistance in *P. cubensis* has been documented in the Fungicide Resistance Action Committee (FRAC) groups 4 (Phenylamides), 11 (Strobilurins), and 40 (Carboxylic Acid Amides) (Holmes et al. 2015). FRAC groups are categorized based on

their specific mode of action or what process within the cell is inhibited (Schumann and D'Arcy 2010).

Phenylamide (PA) fungicides have been used to control diseases caused by oomycetes for over 40 years (Gisi and Sierotzki 2015). PA fungicides inhibit several life stages of oomycetes by disrupting rRNA biosynthesis (Gisi and Sierotzki 2015); however, resistance to PA fungicides has been noted in several plant pathogenic oomycetes and in *Phytophthora infestans* is caused by a mutation in the RNA*pol*I gene Y382F (Gisi and Sierotzki 2015). Recently, several candidate genes have been identified in *Phytophthora capsici* relating to mefenoxam sensitivity, including a homolog of yeast ribosome synthesis factor, Rrp5 (Vogel et al. 2021). *P. cubensis* was the first oomycete shown to be resistant to an important PA active ingredient (metalaxyl); a resistant *P. cubensis* isolate was found 2 years after metalaxyl was introduced in Israel (Reuveni 1980). Documentation of metalaxyl resistance has also been noted in Greece, Italy, the Czech Republic, Russia, Australia, and the U.S. (Lebeda and Cohen 2011). Metalaxyl (and other PA fungicides) are no longer included among products recommended for the control of *P. cubensis* (Goldenhar and Hausbeck 2019).

Strobilurins are a class of fungicides first discovered when analyzing antimicrobial compounds produced by the basidiomycete fungus *Strobilurus tenacellus* (Sauter et al. 1999). Only three strobilurins have proven effective in controlling oomycetes and include azoxystrobin, fenamidone, and famoxadone (Gisi and Sierotzki 2015). The strobilurins control oomycetes through a disruption of mitochondrial respiration, interrupting electron transport in complex III of cytochrome b (Gisi and Sierotzki 2015). Reduced efficacy of strobilurins was observed in 1999 in Japan, three years after their introduction in 1996 (Ishii et al. 2001; Sauter et al. 1999). The resistance mechanism to strobilurins is based on a substitution of glycine by alanine at

position 143 of the cytochrome b gene (Ishii et al. 2001). Current fungicide recommendations in the U.S. largely exclude strobilurins because they have been found to be ineffective in controlling *P. cubensis* (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Keinath et al. 2019).

Carboxylic acid amide (CAA) fungicides control plant pathogenic oomycetes by inhibiting cellulose synthesis, targeting the *cellulose synthase 3* (*CesA3*) gene (Blum et al. 2011; Gisi and Sierotzki 2015). Dimethomorph and mandipropamid are the two most common active ingredients used for the control of *P. cubensis* in this fungicide class (Goldenhar and Hausbeck 2019). Resistance to CAAs by *P. cubensis* was first reported in China in 2004 (Zhu et al. 2007). Resistance in *P. cubensis* to CAAs is conferred by a mutation at position 1105 of the *Ces*A3 gene (Blum et al. 2011). Resistance development in phenylamines, strobilurins, and CAAs provide an example of how quickly fungicide resistance can develop in *P. cubensis* populations.

Resistance Management. In order to minimize the risk and rate of fungicide resistance development in currently used fungicides, it is recommended that growers rotate modes of action and use mixtures of lower-risk, protectant fungicides with higher-risk, systemic fungicides (Hobbelen et al. 2011). Moreover, mixing a protectant with a systemic fungicide is more efficacious against *P. cubensis* than using only one or the other (Ojiambo et al. 2010).

Protectant fungicides, also known as contact fungicides, are applied to and remain on plant surfaces where they tend to be effective against a broad range of fungal/oomycete pathogens. Protectant fungicides do not promote the development of resistant pathogen populations because their modes of action target multiple metabolic sites (Schumann and D'Arcy 2010). The two most commonly used protectants for the control of *P. cubensis* are chlorothalonil

and mancozeb (Goldenhar and Hausbeck 2019). Protectants used alone tend to decrease in efficacy over the course of a growing season (D'Arcangelo et al. 2021). Additionally, protectants tend to wash off with rain or be degraded over time by solar radiation, so repeated applications are usually needed to achieve adequate disease control; they do not provide curative action in diseased tissues (Schumann and D'Arcy 2010). In contrast, systemic fungicides are absorbed into plant tissues where they cannot be washed off or degraded, providing some level of curative effect in diseased tissues killing or inhibiting fungal/oomycete growth (Schumann and D'Arcy 2010). The mode of action of these fungicides tends to be quite specific, usually only inhibiting a single enzyme system in the fungus/oomycete, leading to an increased risk of resistance development with repeated use (Schumann and D'Arcy 2010).

Single-Site Fungicides. Some common systemic fungicides used for control of CDM include fluopicolide (FRAC 43), propamocarb (FRAC 28), ethaboxam and zoxamide (FRAC 22), cymoxanil (FRAC 27), cyazofamid (FRAC 21), fluazinam (FRAC 29), and oxathiapiprolin (FRAC 49) (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Keinath et al. 2019).

Bioassays can be helpful for resistance screening, providing a snapshot of pathogen fungicide sensitivity at a single point in time and may not be ideal for developing recommendations (Keinath et al. 2019; Thomas et al. 2018). Field trials provide a picture of pathogen fungicide sensitivity over a growing season (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020). A decrease in the efficacy of fluopicolide has been noted in both field trials and bioassays (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Keinath et al. 2019; Thomas et al. 2018). Insensitivity to fluopicolide is more prevalent in *P. cubensis* from cucumber than from other cucurbits and only present among isolates of the A1 mating type (Thomas et al. 2018). Propamocarb was effective

in field trials in North Carolina (D'Arcangelo et al. 2021) but inconsistent in Michigan trials (Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020). While Goldenhar and Hausbeck (2019) found that propamocarb was ineffective in field trials from 2015-2017, efficacy was noted from 2018 to 2019 (Hausbeck et al. 2019; Kenny et al. 2020). Multiple resistance of *P. cubensis* to propamocarb and fluopicolide has been noted (Thomas et al. 2018).

Ethaboxam has proven efficacy in controlling CDM (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Thomas et al. 2018). In bioassays, zoxamide has proven effective against CDM (Keinath et al. 2019). However, in North Carolina field trials, zoxamide premixed with chlorothalonil did not show a statistically different area under the disease progress curve (AUDPC) or marketable yield compared chlorothalonil alone from 2015-2016 (D'Arcangelo et al. 2021). Zoxamide premixed with mancozeb did not show statistically different AUDPC or marketable yield compared to mancozeb alone from 2013-2016 (D'Arcangelo et al. 2021). In Michigan, zoxamide premixed with mancozeb also showed no statistical difference in AUDPC and marketable yield compared with mancozeb alone in 2018 (Goldenhar and Hausbeck 2019; Hausbeck et al. 2019). Zoxamide premixed with chlorothalonil did show a statistical difference in AUDPC with chlorothalonil alone in 2019 Michigan field trials (Kenny et al. 2020).

Efficacy of cymoxanil from 2013-2017 has varied among states and years (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Keinath et al. 2019). Although it was effective in a 2015 Michigan trial, efficacy was not observed in subsequent field trials (2016-2018) (Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020). Cyazofamid, fluazinam and oxathiapiprolin all have proven efficacy against CDM with oxathiapiprolin consistently providing the highest level of control (D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Keinath et al. 2019).

It is important to evaluate the fungicides against local *P. cubensis* populations due to the genetic diversity noted across different cucumber growing regions (Naegele et al. 2016; Quesada-Ocampo et al. 2012). *P. cubensis* populations in different states may have varied sensitivity to the same fungicide in a given year (Holmes et al. 2015; Keinath et al. 2019).

Yearly, local fungicide trials can be used to monitor the efficacy of fungicides used alone or in alternation with other fungicides in a program. These trials can also be used to evaluate new chemistries for integration into existing fungicide programs. Finally, they can provide up to date recommendations, protecting growers from in-season control failures.

Oxathiapiprolin. Oxathiapiprolin is a systemic fungicide that became available to Michigan growers in 2016. It has been effective in controlling CDM in lab experiments and in Michigan field trials both alone and in mixtures with protectant fungicides (Cohen et al. 2018; Goldenhar and Hausbeck 2019). It is a piperidinyl thiazole isooxazoline fungicide with FRAC code 49 and is at a high-risk for pathogen resistance (Cohen 2015). Mixtures of oxathiapiprolin with mefenoxam, chlorothalonil, or mandipropamid effectively control *P. cubensis* (Cohen et al. 2018; Goldenhar and Hausbeck 2019). Cohen (2015) found that oxathiapiprolin effectively inhibited zoospore release from sporangia and prevented cystospore germination in vitro and in vivo. He also found that extremely low concentrations of the compound could prevent symptoms when applied preventively (0.0001 mg/L). When applied curatively at one- or two-days post-inoculation (dpi), Cohen (2015) found that oxathiapiprolin reduced lesion expansion, sporangiophore formation and sporangial yield. However, Cohen (2015) found that an increased dose was required to achieve these results and symptoms were still observed below a 0.01 mg/L concentration. The same was true for a curative application at 3 dpi; the fungicide dose needed to achieve disease suppression was greater, 0.1 mg/L (Cohen 2015). Finally, Cohen (2015) showed

that oxathiapiprolin has translaminar and acropetal systemic activity in cucumber plants. However, for translaminar systemic activity to control CDM, high doses were required, between 0.001 and 1 mg/L. For acropetal systemic activity to control CDM, concentrations had to be between 50 and 100 mg/L.

Oxathiapiprolin targets an oxysterol binding protein (OSBP)- related protein (ORP) which is a novel mode of action (Li et al. 2020). Until 2020, its interaction with OSBP remained unclear. Li et al. (2020) described how oxathiapiprolin interacts with OSBP in *Phytophthora capsici*, another important oomycete pathogen.

Point mutations in oomycetes have conferred resistance to oxathiapiprolin in a lab setting (Miao et al. 2018). Additionally, resistant isolates of grape downy mildew collected from field trials sprayed with oxathiapiprolin for 4 years showed corresponding point mutations (N837I and L863W) (Miao et al. 2018). Understanding the target protein of oxathiapiprolin and the genetic basis for resistance provides the foundation for responsible resistance management (Miao et al. 2018). Developing and testing fungicide programs that minimize the risk of *P. cubensis* populations developing resistance to oxathiapiprolin is important to maintain the efficacy of oxathiapiprolin for CDM control.

Spore Trapping

Spore Traps for Monitoring. Air-sampling instruments are an important tool used for monitoring aerially dispersed pathogens. Two commonly used air-sampling instruments in the U.S. are the Rotorod sampler (impaction spore trap) and the Burkard spore trap (Frenz 1999). Rotorod samplers function by rapidly rotating two plastic rods (1.52 × 1.52 × 32 mm) coated in silicone grease around a fixed circumference via an electric motor (Frenz 1999). Airborne particles are captured on the collector rods (Frenz 1999). Burkard spore traps are wind-oriented,

time-discriminating, suction samplers. They capture airborne particles on a melanix tape, mounted on a clockwork mechanism and coated in a silicone adhesive, moving past an air intake orifice at a rate of 2 mm/h (Frenz 1999). In Michigan, Burkard spore traps have been used to alert growers that *P. cubensis* sporangia have arrived in production regions and a fungicide program should be initiated (Granke et al. 2014; Granke and Hausbeck 2011). Initially, these monitoring efforts relied entirely on microscopy (Granke et al. 2014). Quantification of sporangia by microscopy is difficult because of morphological similarities between *P. humuli* and *P. cubensis* (Choi et al. 2005). New polymerase chain reaction (PCR) protocols are now available that allow researchers to distinguish between sporangia belonging to *P. humuli*, *P. cubensis* clade 1, and *P. cubensis* clade 2 (Rahman et al. 2021). Previously, when a sporangium was identified via microscopy as *Pseudoperonospora* spp., fungicide programs were initiated for all cucurbit crops (Hausbeck 2021). Now, upon a positive PCR test for *P. cubensis,* fungicide programs can be initiated only for the cucurbits preferentially affected by the specific clade detected (Rahman et al. 2021). Currently in Michigan, Burkard spore traps are placed in key commercial cucurbit fields representing major production regions in the state. The reels from the spore trap are collected weekly and the new PCR protocols used to monitor the influx of *P. cubensis* sporangia and identify them to clade (Bello et al. 2021b), providing crop specific recommendations.

Impaction Spore Traps. Impaction spore traps are used to monitor atmospheric pollen concentrations (Frenz and Boire 1999) and to detect and monitor inoculum concentrations of airborne plant pathogens (Carisse et al. 2008; Choudhury et al. 2016; Klosterman et al. 2014; Thiessen et al. 2016; Van der Heyden et al. 2014). Carisse et al. (2008) used impaction spore traps to analyze spatiotemporal trends in epidemic development of Botrytis leaf blight of onion.

They collected rods weekly and obtained airborne conidial concentrations (ACC) by counting the number of conidia per rod using a microscope. Van der Heyden et al. (2014) used impaction spore traps to measure ACC which they used to analyze the spatiotemporal structure of powdery mildew epidemics on strawberry. They also collected rods weekly and obtained ACC by counting the number of conidia per rod using a microscope. Additionally, both studies ran the impaction spore traps only during periods when the ACC of each pathogen was known to be highest.

Other studies have run impaction spore traps continously throughout the sampling period (Choudhury et al. 2016; Klosterman et al. 2014; Thiessen et al. 2016). Thiessen et al. (2016) ran traps continously and placed sampling rods in traps every three to four days. Klosterman et al. (2014) ran traps continously and collected rods at 48 or 72 hour intervals. Choudhury et al. (2016) ran traps continously and collected rods for spore quantification three times a week: the first after 48 hours, the second after 96 hours, and the third after 168 hours. Bello et al. (2021a) used impaction spore traps for the collection of *P. cubensis* sporangia to be analyzed using PCR, collecting rods four times a week (after 24, 48, 72, and 168 hours). They found that rods collected after 96 hours had a higher proportion of positive samples than rods collected after 24 hours. Additionally, they found that when atmospheric sporangial concentrations were below 10 sporangia/day Burkard spore traps were more sensitive than rotorod samplers. One possible reason for the impaction spore trap having a lower sensitivity than the Burkard spore trap could be that they have a smaller sampling surface for sporangia to be collected over a 24 hour period, 84 mm^2 compared to 432 mm^2, for impaction and Burkard spore traps, respectively (Bello et al. 2021a).

Spore Traps for Timing Fungicide Applications. For aerially dispersed pathogens, it is possible to time fungicide applications based on airborne inoculum concentrations. Dhar et al. (2020) used impaction spore traps to measure airborne inoculum concentrations of *Bremia lactucae*, the lettuce downy mildew pathogen via qPCR. They used a threshold Cq value, that coincided with the first occurrence of visible disease symptoms, to time preventative fungicide applications. Final AUDPC was then compared to an untreated control and a calendar based program; they found that the spore-trap based system did not have a significantly different AUDPC from the untreated control while the calendar based program was significantly different from both the untreated control and the spore-trap based system.

Dhar et al. (2020) also compared airborne sporangial concentrations to key weather parameters (temperature, relative humidity, and wind speed 2 m above the ground). They found that the two lowest Cq values recorded were associated with increases in temperature and wind speed. However, fungicide applications were only initiated using a threshold Cq value and did not incorporate weather conditions favorable to disease progression.

Spore Traps for Predicting Airborne Inoculum Concentration. Spore traps have also been used to develop models to predict airborne inoculum concentrations. Rodríguez et al. (2020) used Burkard spore traps to monitor airborne inoculum concentrations of three grapevine pathogens (*Botrytis*, *Erysiphe*, and *Plasmopora*) at two locations in northwest Spain: Cenlle and O Mato. Spearman's correlation test was used to analyze the importance of maximum, minimum and mean temperature, relative humidity, daylight hours, rainfall, and wind speed. Correlations for the same day and the previous 1-7 days were considered. Additionally, principal component analysis (PCA) was used to analyze the effect of the environmental conditions as a whole. Parameters with the highest correlation coefficients were used to develop a predictive model of

the airborne inoculum concentration for each of the three pathogens. The predictive model for *Botrytis* explained 71% and 59.6% of the variability in the data for Cenlle and O Mato, respectively. Parameters used included spore concentration of the previous day, mean temperature, and relative humidity three days prior for Cenlle and spore concentration the previous day and relative humidity three days prior for O Mato. The predictive model for *Erysiphe* explained 61% and 57.8% of the variability in the data for Cenlle and O Mato, respectively. Parameters used included spore concentration of the previous day for Cenlle and spore concentration the previous day and relative humidity three days prior for O Mato. The predictive model for *Plasmopora* explained 40% and 56% of the variability in the data for Cenlle and O Mato, respectively. Parameters used included sporangial concentrations and wind speed the day prior for Cenlle and sporangial concentrations and relative humidity the day prior for O Mato. An internal validation compared observed spore concentrations against predicted spore concentrations and revealed that the models fit well but are less accurate when high spore concentrations occur.

Disease Forecasting

Since fungicides are necessary for CDM control, there have been efforts to help growers make appropriately timed applications to maximize the effectiveness of each application and limit the number of applications in a season. Disease forecasters can provide guidance for timing fungicide applications by using information about the pathogen-host interaction and environmental conditions to determine the risk of an epidemic developing (Campbell and Madden 1990).

Cucurbit Downy Mildew impPIPE System. The CDM Integrated Pest Information Platform for Extension and Education (impPIPE) system is an early warning system for CDM, launched in

2008 (Ojiambo et al. 2011). It allows growers, crop consultants, and extension educators to have near real-time information regarding the temporal and spatial progress of the CDM epidemic throughout the growing season (Ojiambo et al. 2011). It provides current disease forecasts, management information and a platform for communication (Ojiambo et al. 2011). The introduction of the ipmPIPE system saved growers in Georgia, North Carolina, and Michigan a combined estimated of $6 million compared to the calendar-based system by reducing the number of fungicide applications by two or three over the course of the growing season (Ojiambo et al. 2011).

The impPIPE system provides information to growers, crop consultants, and extension educators so that they can determine the risk of the onset of a CDM epidemic in their growing region, allowing them to initiate a spray program at the proper time (Holmes et al. 2015). In Michigan, initiation of spray programs is based on the initial detection of *P. cubensis* sporangia, measured by spore trapping (Holmes et al. 2015).

After the first fungicide application, the ipmPIPE system (and estimating airborne sporangia concentrations) is limited in that does not provide guidance for continued fungicide application timings. Upon initiation of a fungicide program, subsequent fungicide applications are applied in a prophylactic manner regardless of whether the environmental conditions are conducive to disease or not (Neufeld et al. 2017). Timing applications in such a manner may increase input costs for growers while also unnecessarily exposing the environment to pesticides. Furthermore, these unnecessary applications may increase the risk and rate of *P. cubensis* developing resistance to important systemic fungicides (Neufeld and Ojiambo 2012).

TOM-CAST. TOM-CAST (TOMato disease foreCASTer) is a spray timing program originally developed to control Septoria leaf spot, early blight, and anthracnose fruit rot on tomatoes in Ontario, Canada (Pitblado 1992). It is a modified version of the FAST model that was developed in 1978 at Pennsylvania State University for the control of early blight of tomato (Madden et al. 1977). In the TOM-CAST model, leaf wetness duration and temperature during this leaf wetness period are used to compute a daily DSV (Disease Severity Value) which can be between 0 and 4, zero being the least favorable for disease development and 4 being the most favorable to disease development (Pitblado 1992) These DSV values are accumulated daily until a threshold is reached (for example 15 or 20 DSVs); once the threshold is reached, a fungicide application is triggered and the accumulated DSV total is reset to zero (Pitblado 1992). TOM-CAST is a program that is adaptable and can be modified to other pathosystems. In addition to its use in controlling selected tomato diseases, it has also been used to manage purple spot of asparagus as well as foliar blight of carrot (Bounds et al. 2007; Meyer et al. 2000). To date, there have been no published papers addressing the effectiveness of TOM-CAST in managing *P. cubensis*.

BLITE-CAST. BLIGHT-CAST is a forecasting system developed to control late blight of potato caused by *Phytophthora infestans* (Raposo 1993). Using weather conditions from the previous 7 days, BLIGHT-CAST establishes a fungicide spray schedule (Raposo 1993). Weather conditions used to establish this fungicide spray schedule include average daily temperature, rainfall, hours of RH greater than 90%, and average temperature during periods with RH greater than 90%. These environmental factors are important in predicting the secondary cycles of the pathogen, including the production of secondary inoculum and secondary infection (Raposo 1993). To date, there have been no published papers addressing the effectiveness of BLITE-CAST in managing *P. cubensis*.

DM-CAST. DM-CAST (Downy Mildew foreCAST model) was developed to manage downy mildew in grapes caused by *Peronospora viticola* (Park et al. 1997). It is designed to estimate the level of oospore maturation, determine the date of primary infection, and predict the occurrence and severity of secondary infection cycles (Park et al. 1997). In order to run the model, the following environmental data are needed: daily average temperature and rainfall from September 21st of the previous year to the first primary infection, hourly temperature, relative humidity, and wetness period data and hours of darkness throughout the growing season (Park et al. 1997). Based on these inputs, the DM-CAST model outputs an hourly risk value. This hourly risk value can range between 0 and 100 with higher values corresponding to a greater magnitude of severity in the given infection period and a non-zero value corresponding with a predicted infection period (Park et al. 1997). There has yet to be a paper published regarding DM-CAST's effectiveness in controlling *P. cubensis*.

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CHAPTER 1. FIELD STUDIES COMPARING FUNGICIDES FOR CONTROL OF *PSEUDOPERONSPORA CUBENSIS* ON PICKLING CUCUMBER

Abstract

Michigan ranks first in the nation in cucumber production for processing (pickling), contributing over $45 million to the state's economy in 2019. Cucurbit downy mildew, caused by oomycete *Pseudoperonospora cubensis*, is the most important disease of cucumber and an annual threat to yields. Fungicides are the most important means to control *P. cubensis*. Our goal was to monitor currently labelled products used singly for efficacy over the course of an entire cropping period under high pathogen pressure. Field trials were conducted at the Michigan State University Plant Pathology Research Farm in Lansing, MI in 2021 and 2022. Products were applied weekly and disease severity was evaluated by visually assessing the percentage of foliar area showing downy mildew symptoms. At the end of the season, area under the disease progress curve (AUDPC) was calculated using the disease severity data. Based on AUDPC results, oxathiapiprolin (OXTP) premixed with chlorothalonil was the most effective product at controlling *P. cubensis* and was significantly better than all other products in both years tested. On the final assessment dates in both years all treatments had significantly less foliar disease than the untreated control except pyraclostrobin in both years tested and dimethomorph in 2021.

Introduction

Cucumber, *Cucumis sativus* L., is an important cucurbit vegetable crop in the U.S. In 2017, 48,422 ha of cucumbers were harvested nationally with approximately 32,025 ha used for processing (e.g. pickles) and 16,397 ha going to fresh market (e.g. slicers) (USDA, NASS 2017). In 2019, Michigan used more than 240,000 tons of pickling cucumbers valued at $45.4 million in 2019 (USDA, NASS 2020) and leads the nation in production. That same year, Michigan used more than 61,500 tons of cucumber for fresh market valued at $25.1 million (USDA, NASS 2020). Combined, the value of fresh market and pickling cucumbers in 2019 accounted for

approximately 33% of the total value of vegetable crops produced in Michigan which was approximately $213.5 million (USDA, NASS 2020).

Cucurbit downy mildew (CDM), caused by the obligate, biotrophic oomycete *Pseudoperonospora cubensis*, is an annual threat to pickling cucumber production in Michigan (Cespedes-Sanchez et al. 2015) and other regions where cucumbers are grown (Hausbeck 2017). *Cucumis* spp. (*Cucumis sativus* and *Cucumis melo*) are more susceptible to CDM than other cucurbit species (Cespedes-Sanchez et al. 2015) and most pickling cucumber cultivars grown in Michigan are either susceptible or moderately resistant to CDM (Call et al. 2012). CDM symptoms on cucumber begin as small (3-10 mm), water-soaked lesions on the adaxial leaf surface (Hausbeck 2017) occurring between 4 to 12 days after infection depending on inoculum concentration, temperature, and leaf wetness duration (Cohen 1977). Disease symptoms on cucumber are distinct from other cucurbits (cantaloupe, squash, pumpkin, and watermelon) as lesions are angular and bordered by leaf veins. In other cucurbits, lesions are typically smaller and are not bound by leaf veins (Hausbeck 2017; Savory et al. 2011). Initially, lesions appear chlorotic and then become necrotic, coalescing to form large, blighted areas (Hausbeck 2017; Oerke et al. 2006). Infection by *P. cubensis* lowers yield by reducing photosynthetic potential; severe disease causes defoliation (Cespedes-Sanchez et al. 2015; Lindenthal et al. 2005). Michigan cucumber growers limit CDM by applying fungicides (Goldenhar and Hausbeck 2019; Savory et al. 2011) when alerted to an influx of airborne sporangia (Hausbeck 2022). The estimated cost of fungicides in Michigan to control CDM is estimated to be approximately $6 million annually (Goldenhar and Hausbeck 2019; Granke et al. 2014).

The Fungicide Resistance Action Committee (FRAC) classifies *P. cubensis* at high risk of developing resistance to fungicides (FRAC 2019). Resistance in *P. cubensis* populations has

been documented to phenylamine, strobilurin, and carboxylic acid amine (CAA) fungicides corresponding to FRAC groups 4, 11, and 40, respectively (Gisi and Sierotzki 2015). Resistance to fluopicolide (FRAC group 43) was noted among *P. cubensis* isolates collected from Michigan in 2013 and 2014; one 2013 *P. cubensis* isolate was resistant to propamocarb (FRAC group 28) (Thomas et al. 2018). These fungicides did not effectively protect pickling cucumbers from CDM in Michigan field trials from 2015-2017 (Goldenhar and Hausbeck 2019).

Combining fungicides lowers the risk of fungicide resistance developing in the pathogen population (Hobbelen et al. 2011). Multisite fungicides are favored as tank mix partners for higher-risk, systemic fungicides (Hobbelen et al. 2011). Multisite fungicides, also known as contact or protectant fungicides, are generally effective against a broad range of plant pathogens. Since multisite fungicides target multiple metabolic sites, they do not promote the development of resistant pathogen populations. In contrast, systemic fungicides target a single metabolic site thereby promoting the development of pathogen resistance (Schumann and D'Arcy 2010). Multisite fungicides commonly used to control *P. cubensis* in Michigan include chlorothalonil and mancozeb (Goldenhar and Hausbeck 2019).

Fungicide efficacy against *P. cubensis* can vary within a growing season and among growing regions (Holmes et al. 2015; Keinath et al. 2019). For instance, a *P. cubensis* isolate collected from Michigan in 2013 was resistant to propamocarb in a lab study but four other isolates collected the same year and one collected in 2014 were sensitive (Thomas et al. 2018). In Michigan field trials, efficacy of propamocarb has been inconsistent. The fungicide was ineffective in research field trials from 2015 to 2017 (Goldenhar and Hausbeck 2019) but effective in 2018 and 2019 (Hausbeck et al. 2019; Kenny et al. 2020). Yearly field trials evaluating fungicide efficacy under high pathogen pressure indicate pathogen fungicide

sensitivity during the growing season and aid in developing grower recommendations. The objective of this study was to evaluate fungicides for efficacy against *P. cubensis* during a cucumber cropping period under high pathogen pressure.

Materials and Methods

Plot Establishment and Experimental Design. In both years, the trials were located at the Plant Pathology Research Farm on the campus of Michigan State University in Lansing, MI and used 'Straight-Eight' cucumber. In 2021, seeds were sown on 30 July into raised beds with rows placed on 1.83 m centers and plants spaced 30.5 centimeters apart. In 2022, seeds were sown on 26 July into raised beds with rows placed on 2.44 m centers and plants spaced 30.5 centimeters apart. The site was prepared by plowing on 5 (2021) and 20 May (2022) and discing on 17 and 20 May (2021) and 1 June (2022). Pre-plant fertilizer (nitrogen 113.4 kg/ha, potassium 125.1 kg/ha, sulfur 26.6 kg/ha, boron 2.24 kg/ha) was applied on 20 May in 2021. In 2022, pre-plant fertilizer (nitrogen 113.4 kg/ha and potassium 52.1 kg/ha) was applied on 1 June. Raised beds were formed, plastic mulch was laid, and drip tape was established for irrigation on 25 May and 9 July, in 2021 and 2022, respectively. After crop establishment, fertilization occurred weekly using urea ammonium nitrate (28% nitrogen) at a rate of 9.6 L/ha. Weeds were controlled via mechanical and hand weeding. Insects were controlled with an application of imidacloprid (Admire Pro at 767.3 mL/ha, Bayer CropScience, Research Triangle Park, NC) through the drip tape on 13 (2021) and 10 August (2022). Non-target diseases (Alternaria leaf spot, Alternaria leaf blight and powdery mildew) were controlled with applications of azoxystrobin (Quadris at 1.13 L/ha, Syngenta Crop Protection, Greensboro, NC) and quinoxyfen (Quintec at 438.5 mL/ha, Corteva AgriSciences, Indianapolis, IN) on 25 August and 1 September in 2021 and applications of azoxystrobin (Quadris at 1.13 L/ha, Syngenta Crop Protection, Greensboro, NC) on 15 and 26

August and cyflufenamid (Torino at 248.5 mL/ha, Gowan Company, Yuma, AZ) on 26 August in 2022. Treatments were arranged in a randomized complete block design (RCBD) with four replicates. Each treatment replicate consisted of a single 6-meter-long plot with a 1.5 meter buffer between treatments within a row.

Table 1: Fungicides evaluated for control of *Pseudoperonospora cubensis* in 2021 and 2022 at the Michigan State University Plant Pathology Research Farm.

			FRAC		
Active Ingredient	Product Name	Manufacturer	Chemical Group	Code	Rate/ha
Ametoctradin/Dimethomorph	Zampro	BASF	QoSI/Carbamate	45/40	1.02 L
Chlorothalonil	Bravo WeatherStik	Adama	Multisite	M5	2.34 L
Cyazofamid	Ranman	FMC Corp.	Quinone inside Inhibitor (QiI)	21	0.2 L
Dimethomorph	Forum	BASF	Carboxylic Acid Amides (CAA)	40	0.44 L
Ethaboxam	Elumin	Valent USA	Thiazole Carboxamides	22	0.58 L
Fluazinam	Omega/Orbus 4F*	Atticus LLC	Uncoupler of Oxidative Phosphorylation	29	1.75 L
Fluopicolide	Presidio	Valent USA	Benzamides	43	0.29 L
Mancozeb	Koverall	FMC Corp.	Multisite	M3	3.36 kg
Oxathiapiprolin/Chlorothalonil	Orondis Opti	Syngenta	OSBPI/Multisite	49/M5	2.92 L
Propamocarb	Previcur Flex	Bayer	Carbamates	28	1.4 L
Pyraclostrobin	Cabrio	BASF	Quinone outside Inhibitor (QoI)	11	0.84 kg
Zoxamide/Chlorothalonil	Zing!	Gowan	Thiazole Carboxamides/Multisite	22/M5	2.63 L
Zoxamide/Mancozeb	Gavel	Gowan	Thiazole Carboxamides/Multisite	22/M3	2.24 kg

***In 2021 Omega was used; in 2022 Orbus 4F was used**

Treatment initiation and application. In 2021, fungicides were applied on 6, 13, 20, 26 August and 3, 10 September in 2021 and 10, 16, 23, 30 August and 6, 13 September in 2022 (Table 1). Fungicides were applied using a CO_2 backpack boom sprayer (Bellspray R & D Sprayers, Opelousas, LA) with three XR8003 flat-fan nozzles spaced 45.7 cm apart and calibrated to deliver 467.6 liters/ha at 241.3 kPa.

Disease assessments. Leaf blight was evaluated using a visual assessment of foliage (%) with disease symptoms. Disease evaluation occurred on 23, 30 August, and 3, 8, 13, 16 September 2021, and 19, 24, 29 August and 5, 9, 16 September 2022. Disease severity was used to calculate the area under the disease progress curve (AUDPC) to assess disease progression for each treatment over time. Relative AUDPC (rAUDPC) was calculated to compare disease progression between treatments and years. The area under the disease progress curve (AUDPC) was

calculated based on methods developed by Shaner and Finney (1977). Using AUDPC data, the relative AUDPC (rAUDPC) was calculated in order to normalize AUDPC data in order to compare disease progress between years (Fry 1978). Treatments were compared based on AUDPC and rAUDPC values and the disease severity on the last assessment date.

Statistical analysis. Statistical analysis was performed using SAS software, Version 9.4 of the SAS System for Windows. Copyright © 2002-2012 by SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA. First, a global analysis of variance (ANOVA) F-test was performed to determine if significant differences in treatment means existed (using PROC MIXED). Fungicide treatments were considered fixed effects and replication (block) was considered as a random effect. For rAUDPC and disease severity data, year was added as a fixed factor in the model to analyze for differences between years and fungicide × year interaction. If differences in treatment means were present, all pair-wise comparisons were determined using Fisher's least significant difference (LSD) test. Levene's test was performed to analyze for any deviation from homogenous variance. If the p-value from the test was insignificant ($p>0.05$), homogenous variance was assumed. Normality of the data was assessed using the residual plots generated by the PLOTS= option in the PROC MIXED statement. If variance and normality assumptions were met, the original data were used and all pair-wise comparisons of treatment means were assessed using Fisher's LSD test. PROC GLIMMIX was used to determine mean separation letters. If the variance assumption was not met, either a square root or log transformation of the response variable was performed. Another Levene's test was performed on the transformed data to test whether the heterogenous variance was corrected by the transformation. If it was, the

transformed data were used and all pair-wise comparisons of the treatment means were performed using Fisher's LSD.

Results

Area under the disease progress curve. According to AUDPC data, all treatments resulted in significantly less disease than the untreated control in both years except for pyraclostrobin and fluopicolide (2022). OXTP/chlorothalonil was the most effective fungicide for both years. While propamocarb was more effective than the remaining fungicides, control was similar to cyazofamid and fluazinam (2021) and ethaboxam (2022). Application of ethaboxam resulted in significantly more disease than propamocarb, cyazofamid and fluazinam in 2021 but less disease than these same treatments in 2022, except for propamocarb. Cyazofamid and fluazinam were similar in 2021 and 2022. Applications of ametoctradin/dimethomorph resulted in an AUDPC that was similar to fluazinam in both years. According to the AUDPC data, ametoctradin/dimethomorph was similar to cyazofamid in 2022, but was less effective than cyazofamid in 2021. Zoxamide/mancozeb provided a similar level of disease control as mancozeb in both years. While treatments of either mancozeb or zoxamide/chlorothalonil were similar to chlorothalonil in 2021 they were more effective than chlorothalonil in 2022. Pyraclostrobin, fluopicolide, and dimethomorph were the least effective treatments in both years and resulted in more disease than all other fungicide treatments. Pyraclostrobin and fluopicolide were similar to each other in each year. Dimethomorph was similar to pyraclostrobin and fluopicolide in 2021 but was more effective than both in 2022 (Figures 1 and 2).

Relative area under the disease progress curve. According to relative AUDPC (rAUDPC) data, disease differed between years for propamocarb, ametoctradin/dimethomorph, ethaboxam, mancozeb, and fluopicolide but did not differ for all other treatments including the untreated

control. Disease was significantly higher in 2021 for propamocarb, ametoctradin/dimethomorph, ethaboxam, and mancozeb and significantly lower in 2021 for fluopicolide (Figure 3). In 2021, rAUDPC trends were similar to AUDPC trends except that the ametoctradin/dimethomorph treatment differed significantly from fluazinam according to the rAUDPC data but not according to the AUDPC data. Finally, mancozeb had less disease than chlorothalonil, but more disease than ethaboxam and ametoctradin/dimethomorph according to the rAUDPC data whereas according to AUDPC data it did not differ (Figures 1 and 3). In 2022, trends were also similar except that chlorothalonil was similar to zoxamide/chlorothalonil according to rAUDPC data whereas according to AUDPC data they were different. Additionally, according to rAUDPC data, dimethomorph was similar to fluopicolide whereas according to AUDPC data it was not (Figures 2 and 3).

Foliar disease severity. In 2021, disease progressed in the untreated control from 18.8% (23 August) to 87.5% (16 September) (see Appendix A, Figure 5). In 2022, disease progressed in the untreated control from 19.0% (19 August) to 81.8% (16 September) (see Appendix A, Figure 6). On the final assessment dates, the untreated control had significantly more disease than all treatments except pyraclostrobin and dimethomorph (both years) and fluopicolide (2022). There was significantly more disease in all treatments in 2021 compared to 2022 except for the untreated control, OXTP/chlorothalonil, fluopicolide, and pyraclostrobin which were similar. OXTP/chlorothalonil limited foliar disease better than all other treatments evaluated in both years with only trace amounts of disease observed at the end of each season. Propamocarb was more effective than the remaining fungicides in both years; however, it was similar to cyazofamid, ethaboxam, and fluazinam in 2022. Cyazofamid, ethaboxam, and fluazinam performed similarly in both years. In 2021, fluazinam was not different from

ametoctradin/dimethomorph and zoxamide/mancozeb; whereas, in 2022 it was more effective. Zoxamide/mancozeb was similar to mancozeb in 2021 but provided better control in 2022. Mancozeb and zoxamide/chlorothalonil were more effective than chlorothalonil. The least effective treatments were pyraclostrobin, fluopicolide, and dimethomorph. Pyraclostrobin and dimethomorph were similar to the untreated control in both years. Dimethomorph and fluopicolide were performed similarly in both years. Fluopicolide was more effective than the untreated control in 2021 but not in 2022. (Figure 4).

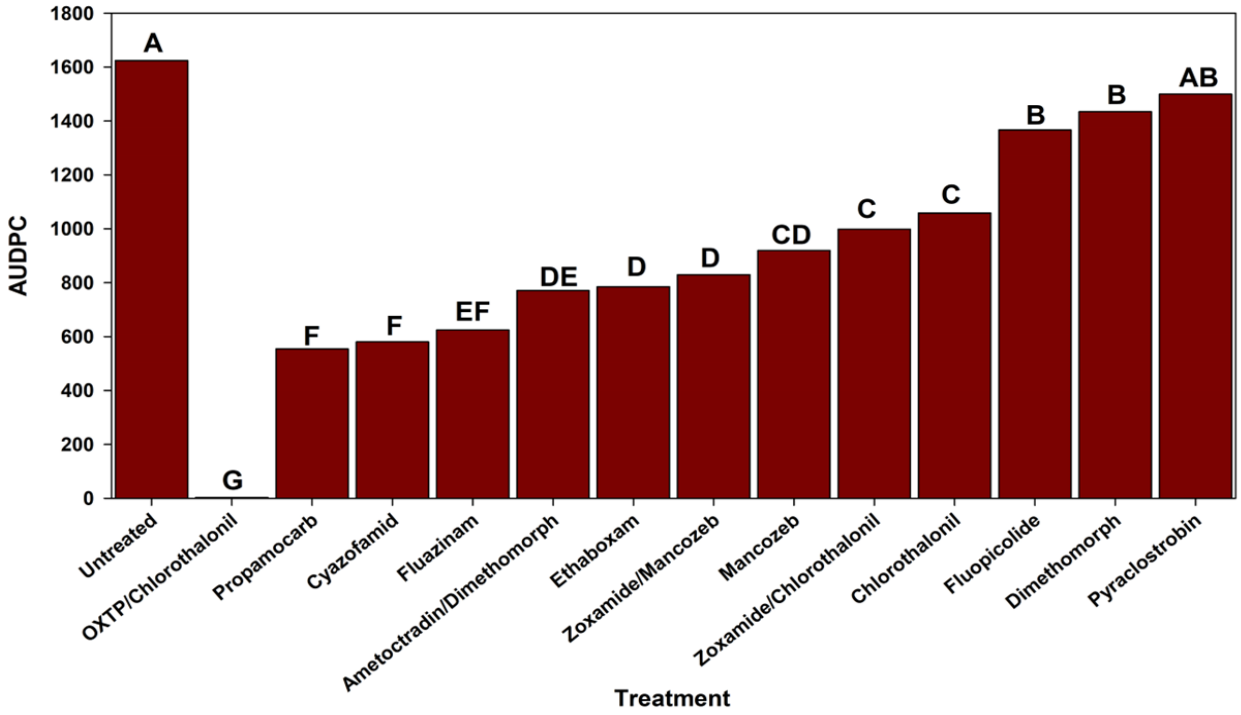

Figure 1: Area under the disease progress curve (AUDPC) for 'Straight-Eight' cucumbers sprayed with fungicides in 2021 to control *P. cubensis*.

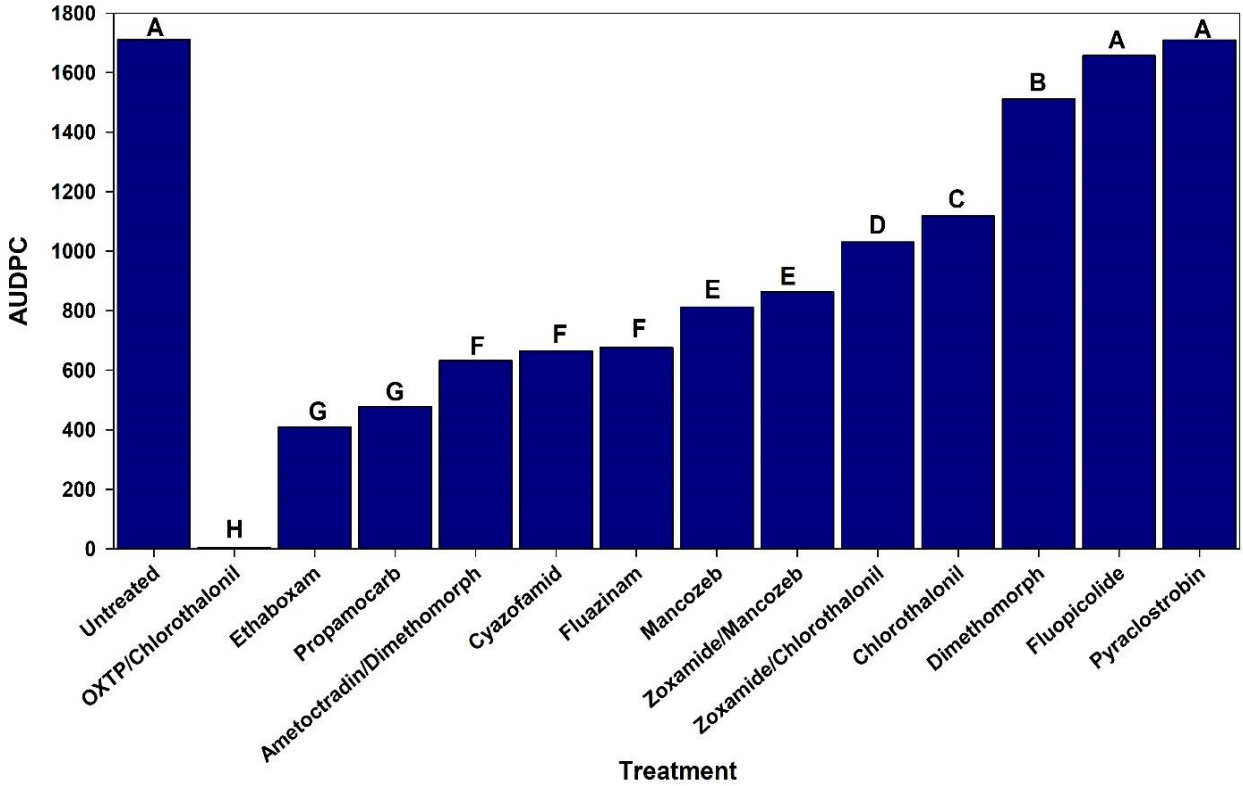

Figure 2: Area under the disease progress curve (AUDPC) for 'Straight-Eight' cucumbers sprayed with fungicides in 2022 to control *P. cubensis*.

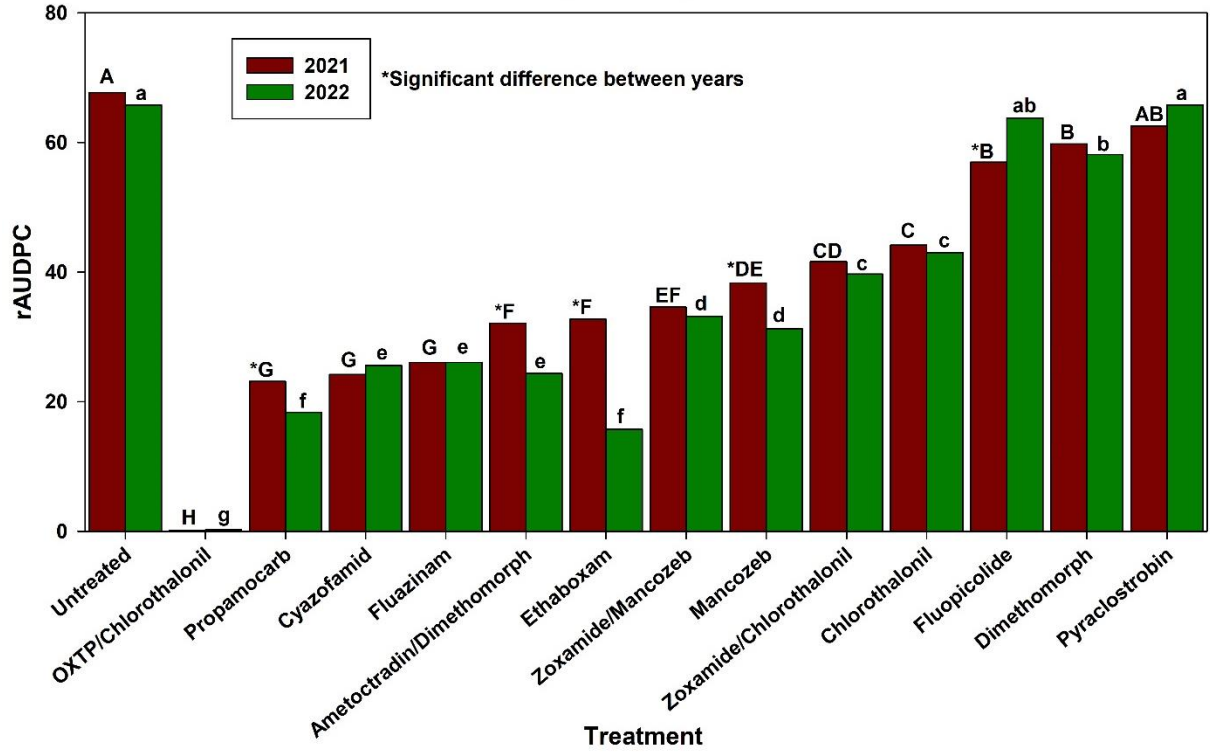

Figure 3: Relative area under the disease progress curve (rAUDPC) for 'Straight-Eight' cucumbers sprayed with fungicides in 2021 and 2022 to control *P. cubensis*.

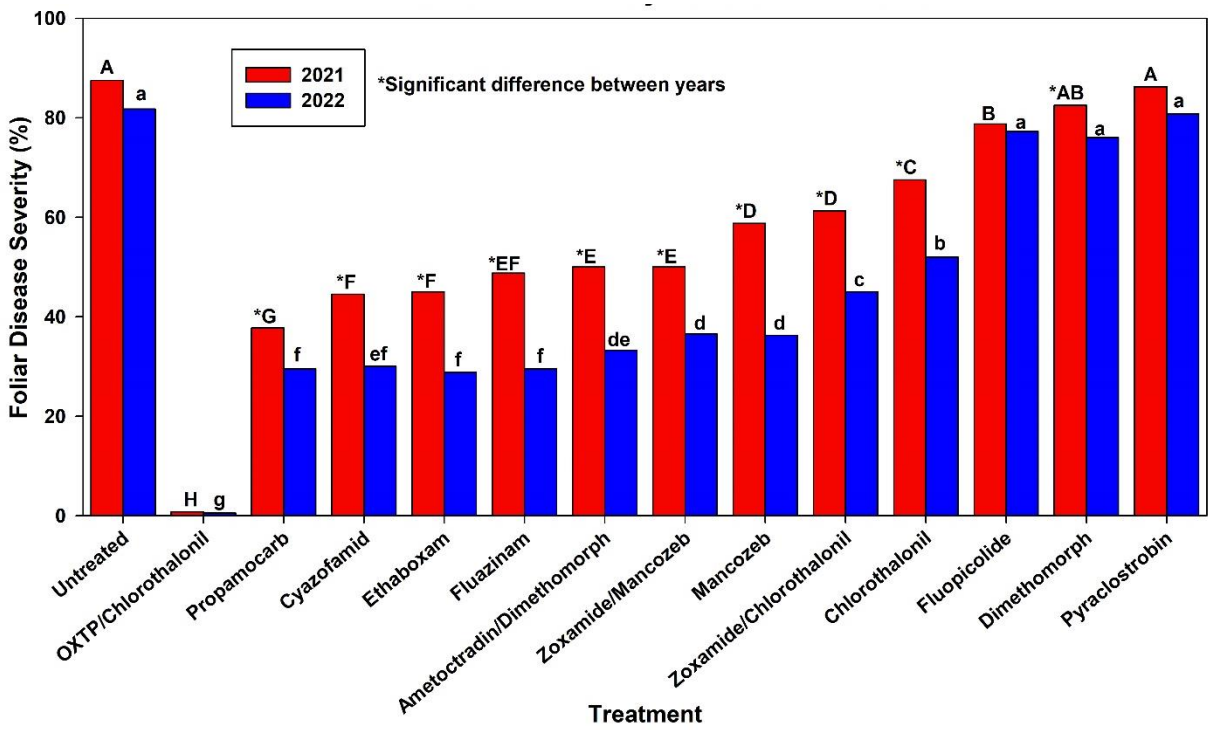

Figure 4: Foliar disease severity (%) at the last rating date on 'Straight-Eight' cucumbers sprayed with fungicides in 2021 and 2022 to control *P. cubensis*.

Discussion

Downy mildew is an annual threat to cucumber production in Michigan, reducing yield if efficacious fungicides are not applied in a timely way. Evaluating the efficacy of fungicides for control of CDM on pickling cucumber is needed to guide management strategies. We achieved high pathogen pressure for the duration of the field trial. Resistance screening using bioassays indicates pathogen fungicide sensitivity at a single point in time but may not provide an accurate picture of how a pathogen responds over the duration of a cropping period.

Our study found that pyraclostrobin, dimethomorph, and fluopicolide did not limit disease and was consistent with previous field trials (Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020) and reports of pathogen resistance (Blum et al. 2011; Ishii et al. 2001; Thomas et al. 2018). In contrast to previous field trials in Michigan conducted from 2015-2017 (Goldenhar and Hausbeck 2019), we found that propamocarb effectively limited disease in both years. This is consistent with Michigan field trial results from 2018 and 2019 (Hausbeck et al. 2019; Kenny et al. 2020). Thomas et al. (2018) found that propamocarb resistant isolates were widespread among U.S. states sampled (Alabama, Florida, Georgia, Illinois, Maryland, Michigan, North Carolina, Pennsylvania, and South Carolina) but only composed 26% of total isolates collected. Variability in the efficacy of propamocarb against *P. cubensis* on a yearly basis has been observed in Europe (Pavelková et al. 2014) and is most likely due to the pathogen population varying in sensitivity to the product.

Efficacy each year is most likely influenced by the sensitivity of the pathogen population(s) that enter Michigan as *P. cubensis* does not overwinter in the state and must be reintroduced each growing season (Ojiambo et al. 2015). The pathogen is known to overwinter in Florida and the coast of the Gulf of Mexico where cucurbits are grown year round (Quesada-

Ocampo et al. 2012). During the summer months, *P. cubensis* sporangia are transported to northern cucumber growing regions from the southeastern U.S. via wind currents (Ojiambo et al. 2015). Additionally, it has been noted that northern greenhouses that produce cucurbits year-round may also serve as the primary inoculum source for Michigan (Naegele et al. 2016). It is probable that the fungicide sensitivity of the *P. cubensis* populations causing disease in Michigan later in the season is influenced initially by the fungicides used in the southeastern U.S. or in northern production greenhouses. It is likely that the observed sensitivity in our field trial or a commercial field is a conglomerate of the population(s) of *P. cubensis* that become established in that given location as it has been shown that population diversity of *P. cubensis* can vary at the county or even field level (Naegele et al. 2016).

Overall, the most effective products were OXTP/chlorothalonil, propamocarb, cyazofamid, and fluazinam. Cyazofamid offers preventive activity against *P. cubensis* with moderate translaminar and curative activity while having stable residual activity and rain fastness (Mitani et al. 2002). There are no reports of resistance to cyazofamid in *P. cubensis*; however, resistance has been noted in isolates of *Phytophthora capsici* in Tennessee (Siegenthaler and Hansen 2021). Currently, cyazofamid is an important component of a fungicide program to control CDM based on our study and others. (Adams et al. 2014; D'Arcangelo et al. 2021; Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020). Fluazinam has protectant activity against both fungi and oomycetes with little systemic activity; however, it has excellent rain fastness and residual activity which are important traits for a protectant fungicide (Komyoji et al. 1995). Fluazinam has a low risk of pathogen resistance due to its broad, protectant activity (FRAC 2020) and there are no reports of *P. cubensis* resistance with only one

report of an oomycete (*Phytophthora infestans*) becoming resistant (Schepers et al. 2018). Fluazinam is a good option for *P. cubensis* control programs when used preventively.

Ametoctradin/dimethomorph and ethaboxam also effectively controlled CDM. Ametoctradin/dimethomorph is a premix of a FRAC group 45 A.I. (ametoctradin) and a FRAC group 40 A.I. (dimethomorph). Based on our results, the efficacy of this premix is dependent upon the efficacy of ametoctradin as dimethomorph used alone was not effective. Ethaboxam was registered for CDM in 2017 as a FRAC group 22 product, the same as zoxamide. Zoxamide is available as a premix with either chlorothalonil or mancozeb. In 2021, ethaboxam had significantly less disease than zoxamide premixed with chlorothalonil and a similar level of disease to zoxamide premixed with mancozeb. In 2022, ethaboxam had significantly less disease than the two premixes including zoxamide. This is the first report of ethaboxam showing improved efficacy compared to zoxamide under field conditions.

OXTP/chlorothalonil was the most effective fungicide in our trial. With the exception of 2018 (Hausbeck et al. 2019), this fungicide has been highly effective in Michigan field trials (Goldenhar and Hausbeck, 2019; Kenny et al. 2020). Additionally, it has been highly effective in field trials and bioassays in other states (D'Arcangelo et al. 2021; Jones et al. 2021). Cohen et al. (2015) found that OXTP/chlorothalonil was highly effective in controlling *P. cubensis* when used at low concentrations (0.0001 mg/L). After reports of reduced efficacy of OXTP in field trials in South Carolina and Georgia (Dutta 2021), Keinath (2022) investigated efficacy of OXTP against *P. cubensis* on different cucurbit hosts using a bioassay. He found that OXTP was still effective at all concentrations tested (74.8, 37.4, 18.7, 9.3, and 4.7 mg/L) including a disease severity <3% at 17.5 mg/L, the concentration of OXTP at the highest labelled field rate. However, he found that efficacy decreased when decreasing the concentration of OXTP. While

no resistance has been reported in *P. cubensis*, resistance has been reported in Tennessee in *Phytophthora capsici* (Siegenthaler and Hansen 2021). Thus, while we found OXTP to be highly effective in Michigan, the importance of OXTP as the most effective active ingredient against *P. cubensis* should not be taken for granted and continued monitoring of it and other fungicides on a yearly basis is critical to resistance management.

Overall, the two multisite fungicides (chlorothalonil and mancozeb) effectively controlled *P. cubensis* but were not as effective under high pathogen pressure compared to OXTP/chlorothalonil, propamocarb, cyazofamid, fluazinam, ametoctradin/dimethomorph, and ethaboxam. Neither chlorothalonil and mancozeb have systemic activity but inhibit growth of *P. cubensis* sporangia on the leaf surface (Schumann and D'Arcy 2010). Despite this limitation, mancozeb and chlorothalonil are important tank mix partners for higher risk site-specific active ingredients (Hobbelen et al. 2011) with proven efficacy.

P. cubensis has a high risk of developing fungicide resistance (FRAC 2019). Since few fungicides are relied on for control, there is considerable selection pressure for resistant pathogen isolates. To minimize the risk of resistance developing, alternating fungicides with differing modes of action is important. In this study, OXTP/chlorothalonil, propamocarb, cyazofamid, fluazinam, ethaboxam were effective. Mixing high-risk, site-specific products with multisite products including chlorothalonil or mancozeb is recommended.

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APPENDIX A

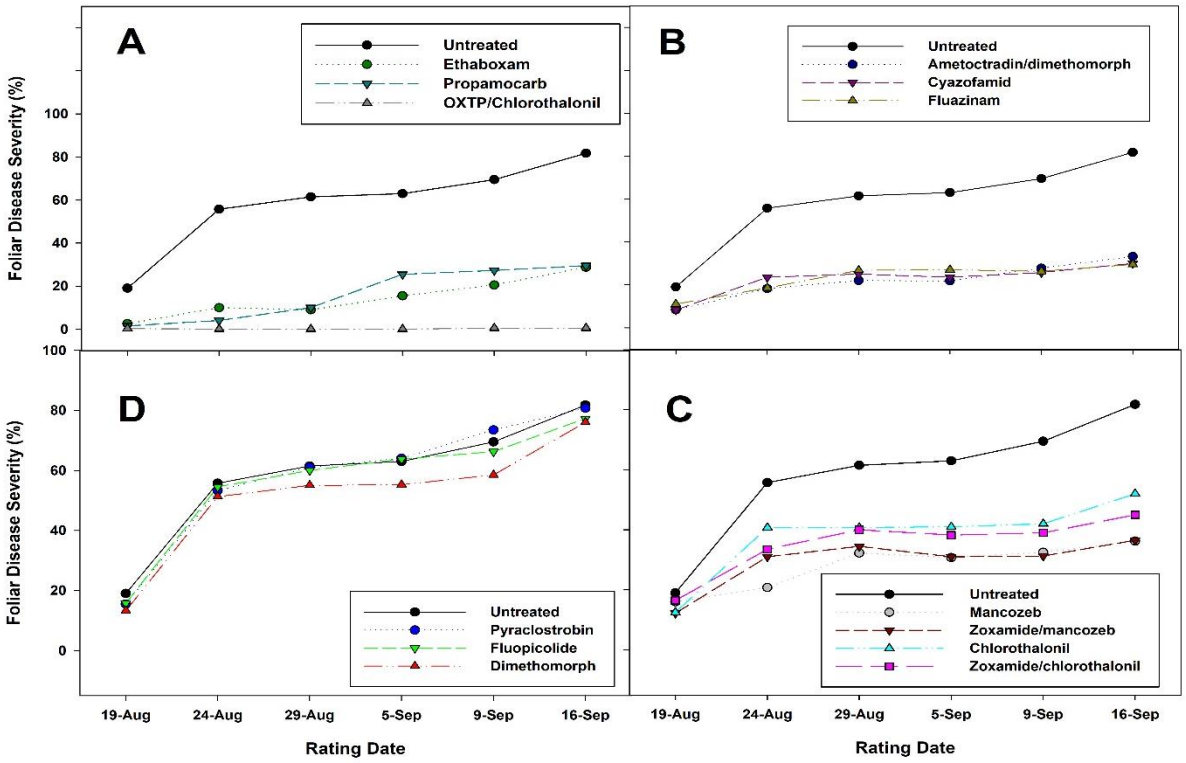

Figure 5, A-D: Disease progress for 'Straight-Eight' cucumbers sprayed with fungicides in 2021 to control *P. cubensis*.

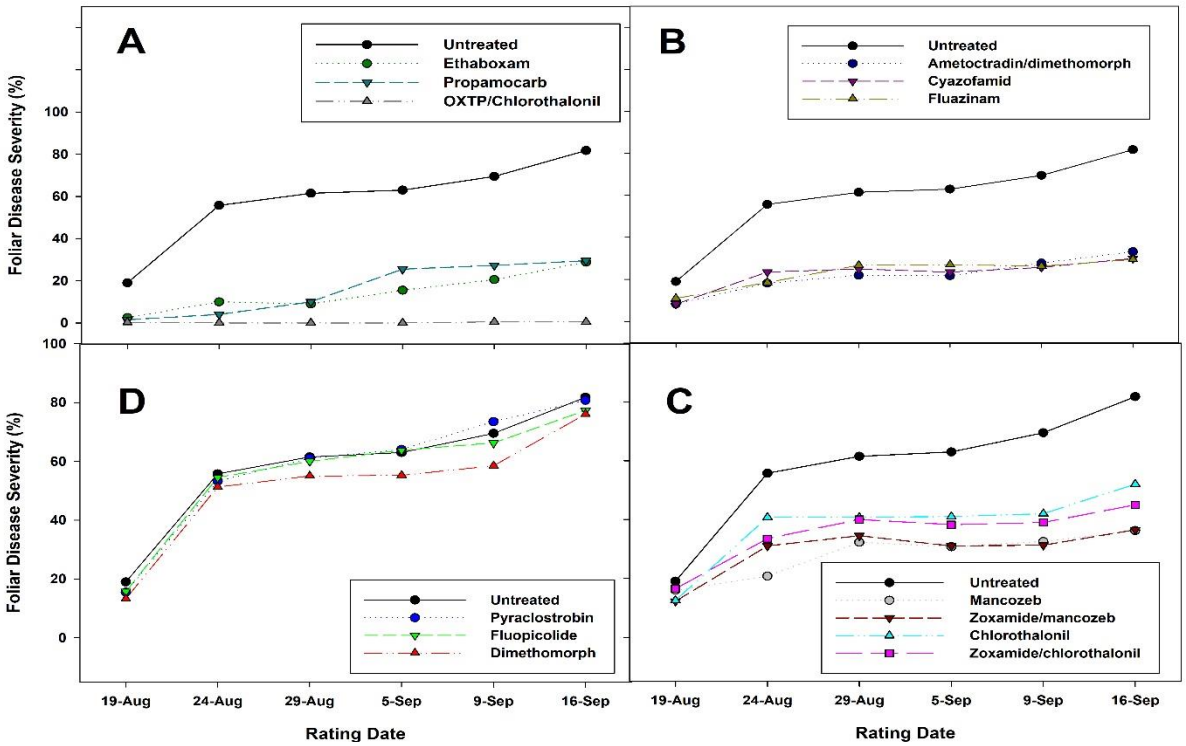

Figure 6, A-D: Disease progress for 'Straight-Eight' cucumbers sprayed with fungicides in 2022 to control *P. cubensis*.

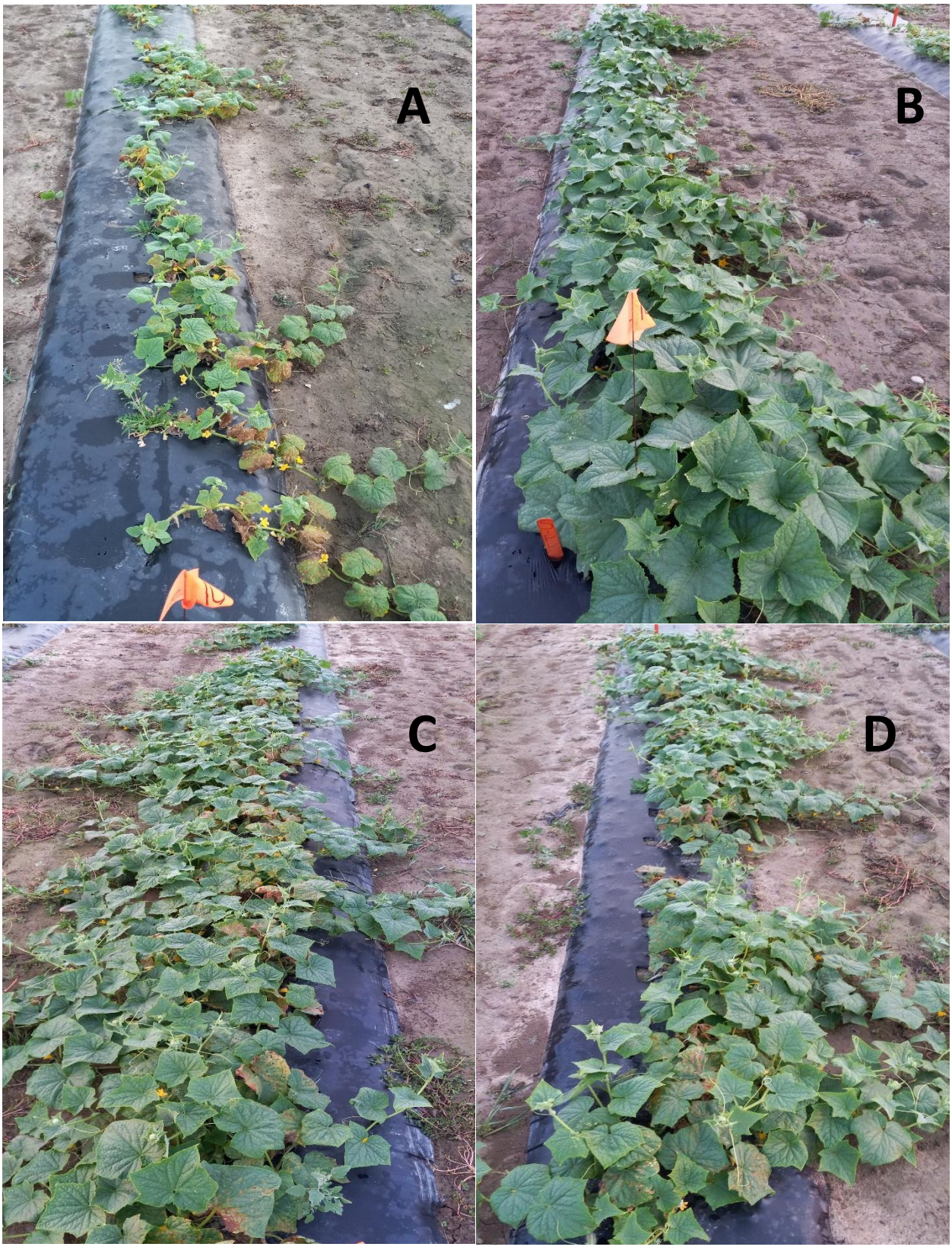

Figure 7: Cucumber plants sprayed with: A, No fungicide B, OXTP/chlorothalonil; C, Propamocarb; D, Cyazofamid to control *P. cubensis* in 2022.

CHAPTER 2. TESTING DISEASE FORECASTERS TO SCHEDULE FOLIAR FUNGICIDE APPLICATIONS FOR CONTROLLING *PSEUDOPERONOSPORA CUBENSIS* ON PICKLING CUCUMBER

Abstract

Cucumber production is important to Michigan's economy and was valued at more than $45 million in 2019. Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is an annual threat to Michigan's cucumber production and fungicides must be applied frequently to prevent major yield losses. Our objective was to evaluate the disease forecasting models, TOM-CAST, BLITE-CAST, and DM-CAST, for their application in scheduling fungicide applications for CDM control. Field trials were conducted in 2021 and 2022 at the Michigan State University Plant Pathology Research Farm in Lansing, MI. Each disease forecaster was evaluated at different spray thresholds and compared to an untreated control, a 7-day program, and a 10-day program. In 2021, all treatments received applications of cyazofamid (Ranman at 201 mL/ha, SummitAgro, Durham, NC) alternated with oxathiapiprolin/chlorothalonil (Orondis Opti at 2.9 L/ha, Syngenta Crop Protection, Greensboro, NC) alternated with ametoctradin/dimethomorph (Zampro at 1.02 L/ha, BASF Corporation, Research Triangle Park, NC) plus chlorothalonil (Bravo WeatherStik at 2.34 L/ha, ADAMA, Raleigh, NC). In 2022, the fungicide program was the same as 2021 with the following exception: cyazofamid was tank-mixed with chlorothalonil. Treatments were visually assessed for the foliar area (%) with CDM symptoms. Area under the disease progress curve was determined each year and relative AUDPC determined at the end of the second season. Results indicate that scheduling applications using DM-CAST or BLITE-CAST limited CDM and were similar to the 7-day program. The 7-day program was sprayed 7 and 6 times in 2021 and 2022, respectively. The BLITE-CAST 18 DSV and 15DSV treatments were sprayed 5 and 6 times each year, respectively. The DM-CAST 3/5 day treatment was sprayed 8 and 6 times in 2021 and 2022, respectively. The DM-CAST 5/7 day and DM-CAST 7/10 day treatments were sprayed 5 and 4 times, respectively, in 2022. This is

the first study to evaluate these disease forecasters for scheduling fungicide applications for CDM.

Introduction

Michigan is the leading producer of pickling cucumbers (*Cucumis sativus*) in the U.S. In 2019, more than 241K tons of pickling cucumbers were utilized in the state with an estimated value of $45.4 million USD (USDA, NASS 2020). Cucurbit downy mildew (CDM) caused by the obligate, biotrophic oomycete *Pseudoperonospora cubensis* is an annual threat to pickling cucumber production in Michigan (Cespedes-Sanchez et al. 2015). Prior to 2004, CDM on cucumber in the U.S. was predominately controlled via host resistance from plant introduction (PI) ascension 197087 integrated into commercial cultivars in the 1950s (Cohen et al. 2015). In 2004, cultivars with PI 197087 derived resistance were no longer resistant to *P. cubensis* populations, resulting in a CDM epidemic throughout the southeast U.S. and Atlantic coast; Michigan reported a significant outbreak in 2005 (Cohen et al. 2015). Since the breakdown in host resistance, frequent prophylactic fungicide applications are the primary means of controlling CDM (Goldenhar and Hausbeck 2019). Pickling cucumber cultivars grown in Michigan are susceptible or intermediately resistant to CDM (Call et al. 2012). *Cucumis sativus* and *Cucumis melo* are more susceptible to CDM than other cucurbit species (Cespedes-Sanchez et al. 2015).

Pseudoperonospora cubensis does not overwinter in Michigan or other regions with killing frost but arrives each growing season via prevailing wind currents from overwintering sites in the southeast U.S. or via release from greenhouses that grow cucurbits year round (Naegele et al. 2016; Quesada-Ocampo et al. 2012). Environmental conditions for *P. cubensis* reproduction and infection have been identified (Cohen and Eyal 1977; Cohen and Rotem 1969, 1971; Sun et al. 2017). Sporangia are produced on the lower leaf surface following a minimum of

six hours of leaf wetness (Cohen and Rotem 1969) and are dependent on the diurnal cycle. Sporangial production is amplified by increased day lengths (Cohen and Rotem 1971) with a minimum of six hours of darkness needed for sporangia to differentiate (Cohen and Eyal 1977). Sporulation occurs between 5 and 30° C; 20° C is optimal (Cohen and Rotem 1969, 1971). Sporangia are released during periods of decreasing relative humidity as the hygroscopic twisting of drying sporangiophores releases sporangia into the air (Lange et al. 1989). A sporangium may germinate after a minimum of 2 hours of leaf wetness at an optimum temperature of 20° C (Cohen 1977). While sporangia can germinate at temperatures of 5 to 25° C, longer leaf wetness periods (12 hours) are needed at 5° C (Cohen 1977). Sun et al. (2017) reported that *P. cubensis* sporangia germinate at temperatures up to 30 °C.

Limiting CDM requires timely fungicide applications (Goldenhar and Hausbeck 2019; Savory et al. 2011) that are estimated to cost $6 million annually (Goldenhar and Hausbeck 2019; Granke et al. 2014). In Michigan, it is recommended that fungicides be initiated immediately after an influx of sporangia into the production field is detected via spore traps (Granke et al. 2014; Granke and Hausbeck 2011). Due to the high cost of fungicides, Michigan growers are interested in scheduling fungicide applications when environmental conditions favor CDM (Hausbeck, *personal communication*) to reduce the number of applications required each season without compromising disease control and crop yield. Reducing the risk of pathogen resistance and the negative environmental impact associated with intensive fungicide programs are additional benefits of optimizing application schedules.

Disease forecasters guide the scheduling of fungicide applications based on the pathogen-host interaction and environmental conditions to determine the risk of an epidemic (Campbell and Madden 1990). While there is not a disease forecaster to schedule fungicides for CDM,

disease forecasting models are available for other pathosystems. TOM-CAST (TOMato disease foreCASTer) is a disease forecasting program originally developed to control Septoria leaf spot, early blight, and anthracnose fruit rot on tomatoes in Ontario, Canada (Pitblado 1992). TOM-CAST is a simplified version of the earlier forecasting system for *Alternaria solani* (FAST) developed by Madden et al. (1977) to control early blight on tomato (Pitblado 1992). TOM-CAST has proven effective against foliar blights in carrot caused by *Alternaria dauci* and *Cercospora carotae* and for purple spot on asparagus fern caused by *Stemphylium vesicarium* (Bounds et al. 2007; Meyer et al. 2000). The program uses the leaf wetness duration and the average temperature during the leaf wetness duration to calculate a daily Disease Severity Value (DSV) (Table 2) (Pitblado 1992). BLIGHT-CAST is a forecasting system developed to manage late blight of potato caused by *Phytophthora infestans* and uses hours of relative humidity (> 90%) and the mean temperature during this period to provide a daily DSV (Table 3) (Krause et al. 1975). DM-CAST (Downy Mildew foreCAST model) was developed to manage downy mildew on grapes caused by *Plasmopara viticola* and uses hourly temperature, relative humidity, and wetness period data to provide an hourly risk value of 0 to 100; higher values correspond to a greater severity during an infection period with a non-zero value corresponding to a predicted infection period (Park et al. 1997). Michigan pickling cucumber growers desire to schedule fungicide applications informed by the weather parameters favorable for CDM. The objective of this study was to evaluate the TOM-CAST, BLITE-CAST and DM-CAST forecasters to determine their applicability to schedule fungicide applications for CDM control in cucumbers.

Materials and Methods

Plot Establishment and Experimental Design. To ensure disease pressure, each year the trial was planted when CDM symptoms and pathogen signs were observed in an adjacent trap crop of

‘Straight-Eight’ cucumber. The trials were located at the Plant Pathology Research Farm on the campus of Michigan State University in Lansing, MI. The site was prepared by plowing on 5 (2021) and 20 May (2022) and discing on 17 and 20 May (2021) and 1 June (2022). Pre-plant fertilizer (nitrogen 113.4 kg/ha, potassium 125.1 kg/ha, sulfur 26.6 kg/ha, boron 2.24 kg/ha) was applied on 20 May in 2021. In 2022, pre-plant fertilizer (nitrogen 113.4 kg/ha and potassium 52.1 kg/ha) was applied on 1 June. Raised plant beds were formed, plastic mulch was laid, and drip tape established for irrigation on 25 May (2021) and 9 July (2022). ‘Straight-Eight’ cucumber seeds were sown on 30 July (2021) and 26 July (2022) with rows placed on 1.83 m (2021) and 2.44 m (2022) centers and plants spaced 30.5 cm apart. After crop establishment, fertilization occurred weekly each year using urea ammonium nitrate (28% nitrogen) at a rate of 9.6 L/ha. Weeds were managed mechanically and by hand weeding. Insects were controlled with an application of imidacloprid (Admire Pro at 767.3 mL/ha, Bayer CropScience, Research Triangle Park, NC) through the drip tape on 13 (2021) and 10 August (2022). Non-target diseases (Alternaria leaf spot, Alternaria leaf blight and powdery mildew) were controlled with applications of azoxystrobin (Quadris at 1.13 L/ha, Syngenta Crop Protection, Greensboro, NC) and quinoxyfen (Quintec at 438.5 mL/ha, Corteva AgriSciences, Indianapolis, IN) on 25 August and 1 September in 2021 and applications of azoxystrobin (Quadris at 1.13 L/ha, Syngenta Crop Protection, Greensboro, NC) on 15 and 26 August and cyflufenamid (Torino at 248.5 mL/ha, Gowan Company, Yuma, AZ) on 26 August in 2022. Treatments were arranged in a randomized complete block design (RCBD) with four replicates (blocks). Each treatment replicate consisted of a single 6-meter-long plot with a 1.5 meter buffer between treatments within a row.

Treatment initiation and application. Fungicide treatments were initiated once the first true leaves had expanded. Thereafter, each treatment received fungicide applications according to a

calendar schedule, a disease forecaster threshold, or received no fungicides (control) (Table 2). In 2021, the fungicide treatments included: cyazofamid (Ranman SC at 201 mL/ha, SummitAgro, Durham, NC) alternated with oxathiapiprolin/chlorothalonil (Orondis Opti SC at 2.9 L/ha, Syngenta Crop Protection, Greensboro, NC) alternated with ametoctradin/dimethomorph (Zampro SC at 1.02 L/ha, BASF Corporation, Research Triangle Park, NC) plus chlorothalonil (Bravo WeatherStik SC at 2.34 L/ha, ADAMA, Raleigh, NC). In 2022, the fungicide program was the same as 2021 with the following exception: the cyazofamid was tank-mixed with chlorothalonil (Bravo WeatherStik SC at 2.34 L/ha). Fungicides were applied using a CO_2 backpack boom sprayer (Bellspray R & D Sprayers, Opelousas, LA) with three XR8003 flat-fan nozzles spaced 45.7 cm apart and calibrated to deliver 467.6 liters/ha at 241.3 kPa. The 7-day treatment was sprayed seven (2021) or six (2022) times. Five applications were applied for the 10-day (2021), TOM-CAST 12DSV (2021), and BLITE-CAST 18DSV (2021, 2022) treatments. The TOM-CAST 15DSV (2021, 2022) and TOM-CAST 12 DSV (2022), and 10-day (2022) treatments were each sprayed four times. The BLITE-CAST 15DSV treatment was sprayed six times each year. The DM-CAST 3/5 day treatment was sprayed eight (2021) or six (2022) times (see Appendix B, Figures 16 and 17). In 2022, the DM-CAST 5/7 day and 7/10 treatments received 5 and 4 applications, respectively (see Appendix B, Figure 17).

Table 2: Forecasters and calendar programs used in 2021 and 2022 at the Michigan State University Plant Pathology Farm.

Timing Used	Threshold	Original Target Pathogen	Program Authority	Application Dates	
				2021	2022
Calendar	7-day	N/A	N/A	6[Z], 13[Y], 20[X], 26[Z] Aug; 3[Y], 10[X], 17[Z] Sep	10[Z], 16[Y], 23[X], 30[Z] Aug; 6[Y], 13[X] Sep
	10-day			6[Z], 16[Y], 25[X] Aug; 3[Z], 13[Y] Sep	10[Z], 19[Y], 29[X] Aug; 8[Z] Sep
TOM-CAST	15 DSV[a]	*Alternaria solani*	Madden et al. (1977) Pitblado (1992)	6[Z], 18[Y], 26[X] Aug; 9[Z] Sep	10[Z], 24[Y] Aug; 2[X], 13[Z] Sep
	12 DSV			6[Z], 16[Y], 23[X], 30[Z] Aug; 13[Y] Sep	10[Z], 22[Y], 30[X] Aug; 9[Z] Sep
BLITE-CAST	18 DSV	*Phytophthora infestans*	Krause et al. (1975)	6[Z], 20[Y], 25[X] Aug; 1[Z], 14[Y] Sep	10[Z], 18[Y], 25[X] Aug; 5[Z], 12[Y] Sep
	15 DSV			6[Z], 18[Y], 25[X], 30[Z] Aug; 7[Y], 14[X] Sep	10[Z], 15[Y], 23[X], 30[Z] Aug; 6[Y], 12[X] Sep
DM-CAST	RV[b] 3 3/5 day[c]	*Plasmopara viticola*	Park et al. (1997); Cornell University, UC-Davis, and University of Illinois spray recommedations	6[Z], 9[Y], 18[X], 21[Z], 25[Y] Aug; 5[X], 13[Z], 18[X] Sep	10[Z], 22[Y], 26[X], 29[Z] Aug; 3[Y], 12[X] Sep
	RV 3 5/7 day[d]			N/A	10[Z], 22[Y], 29[X] Aug; 3[Z], 12[X] Sep
	RV 3 7/10 day[e]			N/A	10[Z], 22[Y] Aug; 3[X], 12[Z] Sep

[a]Disease severity value
[b]Risk value
[c]Fungicides not applied when RV 3 observed within 3 and 5 days of cyazofamid or ametoctradin/dimethomorph and OXTP/chlorothalonil application, respectively
[d]Fungicides not applied when RV 3 observed within 5 and 7 days of cyazofamid or ametoctradin/dimethomorph and OXTP/chlorothalonil application, respectively
[e]Fungicides not applied when RV 3 observed within 7 and 10 days of cyazofamid or ametoctradin/dimethomorph and OXTP/chlorothalonil application, respectively
[Z]Application of cyazofamid (cyazofamid + chlorothalonil in 2022)
[Y]Application of OXTP/chlorothalonil
[X]Application of ametoctradin/dimethomorph + chlorothalonil

Weather data collection. A WatchDog® Wireless Weather Station (Spectrum Technologies Inc., 3600 Thayer Court, Aurora, IL) and a leaf wetness sensor (Spectrum Technologies Inc.) were mounted on a steel pole and placed in the corner of the research plots each year to collect temperature, relative humidity, rainfall, and leaf wetness data. The leaf wetness sensor was placed at canopy height facing north, per manufacturer recommendations. Weather data were

automatically collected and sent to a corresponding SpecConnect® account (Spectrum Technologies Inc.) at 15-minute intervals.

Forecasting software on SpecConnect®. Each disease model used in this study was purchased from Spectrum Technologies Inc. and used with a SpecConnect® account (Spectrum Technologies Inc.). The TOM-CAST and BLITE-CAST models implemented in our study were identical to the originally developed models. However, the DM-CAST model used was developed based on Cornell University, UC-Davis, and University of Illinois spray recommendations (SpecWare 9 Pro Software Guide). To determine the daily DSV for TOM-CAST and BLITE-CAST, disease models on the SpecConnect® website were run each morning for the accumulated DSVs. Once accumulated DSVs reached the target threshold, the corresponding treatments were sprayed and the accumulated DSVs were reset to zero. For the DM-CAST model, plots were sprayed when a daily risk value of 3 occurred, per the model recommendation. However, when a risk value of 3 occurred within 3, 5, or 7 days of a cyazofamid or ametoctradin/dimethomorph spray, plots were not sprayed and corresponded to the DM-CAST 3/5 day, 5/7 day, and 7/10 day treatments, respectively. Additionally, when a risk value of 3 occurred within 5, 7, and 10 days of an oxathiapiprolin/chlorothalonil spray for the DM-CAST 3/5 day, 5/7 day, and 7/10 day treatments, respectively, plots were not sprayed. This adjustment was made to accommodate residual fungicide activity (Shirley et al. 2022). Since oxathiapiprolin/chlorothalonil was more effective than cyazofamid or ametoctradin/dimethomorph in previous Michigan field trials, the spray interval following an oxathiapiprolin/chlorothalonil application was lengthened (Goldenhar and Hausbeck 2019; Hausbeck et al. 2019; Kenny et al. 2020).

Disease assessment. The foliage was visually assessed for disease symptoms (%) on 23 and 30 August, and 3, 8, 13, 16, 20 September 2021 and on 19, 24, and 29 August and 5, 9, 16, 20 September 2022. Disease data were used to calculate the area under the disease progress curve (AUDPC) to assess disease progression for each treatment over time. Relative AUDPC (rAUDPC) was calculated to compare disease progression among treatments and between years. The area under the disease progress curve (AUDPC) was calculated based on methods developed by Shaner and Finney (1977). Using AUDPC data, the relative AUDPC (rAUDPC) was calculated in order to normalize AUDPC data in order to compare disease progress between years (Fry 1978). Treatments were compared based on AUDPC and rAUDPC values and the disease (%) on the last assessment date.

Statistical analysis. Statistical analysis was performed using SAS software, Version 9.4. of the SAS System for Windows (SAS Institute, Cary, NC). First, a global analysis of variance (ANOVA) was performed for each trial to determine if significant differences in treatment means existed (PROC MIXED). Fungicide treatments were considered as fixed effects and replication (block) was considered as a random effect. For rAUDPC and disease severity data, year was added as a fixed factor in the model to analyze for differences between years and treatment × year interaction. Levene's test was performed to analyze for any deviation from homogenous variance. If the p-value from the test was insignificant ($p>0.05$), homogenous variance was assumed. Normality of the data was assessed using the residual plots generated in the PLOTS= option in the PROC MIXED statement. For all data analyzed, variance and normality assumptions were met and the original data were used and pairwise comparisons of treatment means were assessed using Fisher's least significant difference (LSD) test (PROC GLIMMIX).

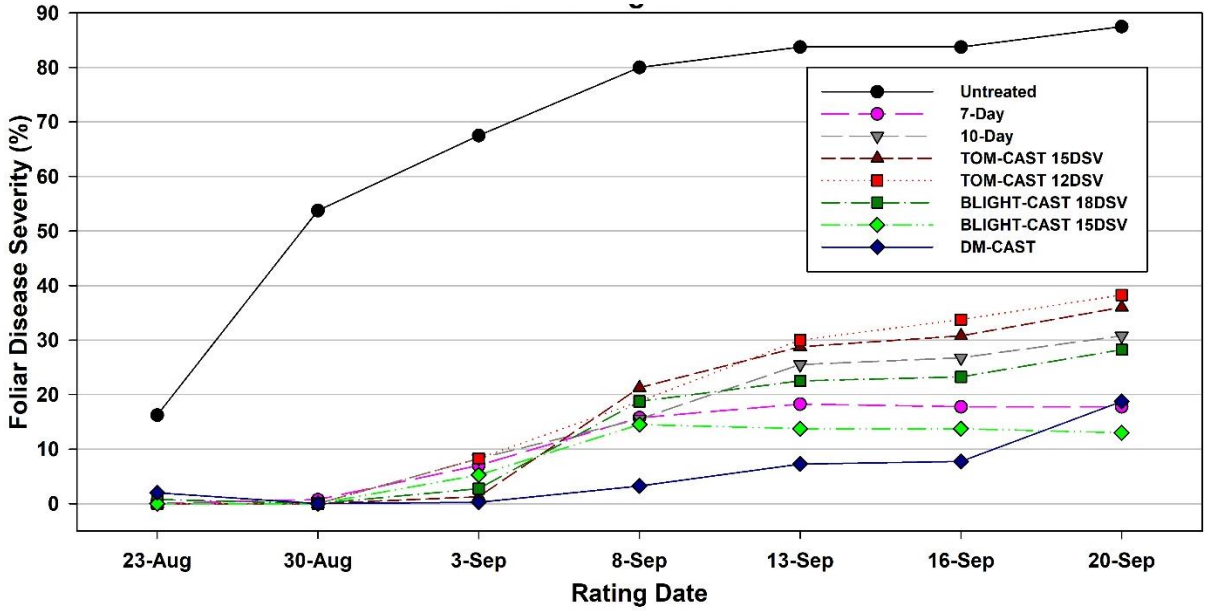

Figure 8: Disease progress on 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2021 for control of *P. cubensis*.

Foliar disease severity. Each year, all treatments reduced CDM disease compared to the control (Figures 8 and 9). The DM-CAST 3/5 day treatment (8 and 6 applications in 2021 and 2022, respectively) was one of the most effective treatments and was similar to the 7-day treatment (7 and 6 applications in 2021 and 2022, respectively) each year. In both years, BLITE-CAST

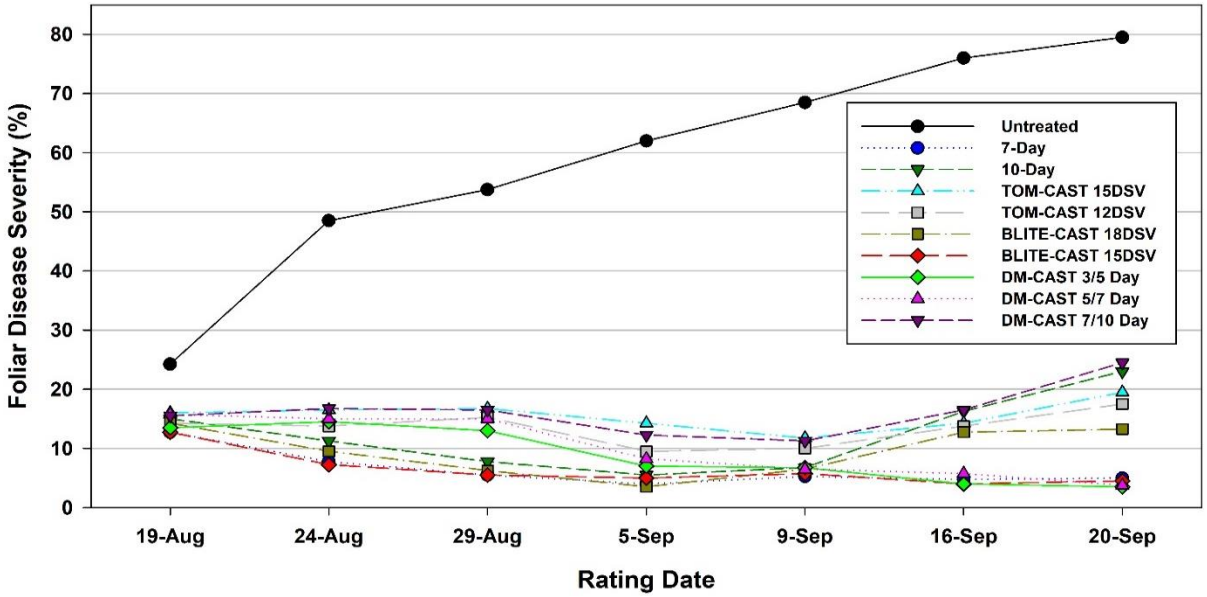

Figure 9: Disease progress on 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2022 for control of *P. cubensis*.

15DSV (6 applications) had the lowest disease severity, compared to all other treatments. In 2022, this treatment was similar to the 7-day, DM-CAST 3/5 day, and DM-CAST 5/7 day (5 applications) treatments. The 7-day treatment was more effective than the BLITE-CAST 18DSV (5 applications), 10-day (5 and 4 applications in 2021 and 2022, respectively), and both TOM-CAST treatments (4 applications in 2021; 4 and 5 applications in 2022 for 15DSV and 12DSV, respectively) each year. BLITE-CAST 18DSV was better than TOM-CAST regardless of DSV (2021, 2022) and the 10-day treatment (2022). Differences were not noted in either year between the TOM-CAST DSV levels tested. For the additional 2022 treatments (DM-CAST 5/7 day and DM-CAST 7/10 day), the DM-CAST 5/7 day treatment was similar to the DM-CAST 3/5 day treatments; DM-CAST 7/10 day (4 applications) had more CDM disease than all other treatments except the 10-day treatment (Figure 10, Appendix B Figures 16 and 17).

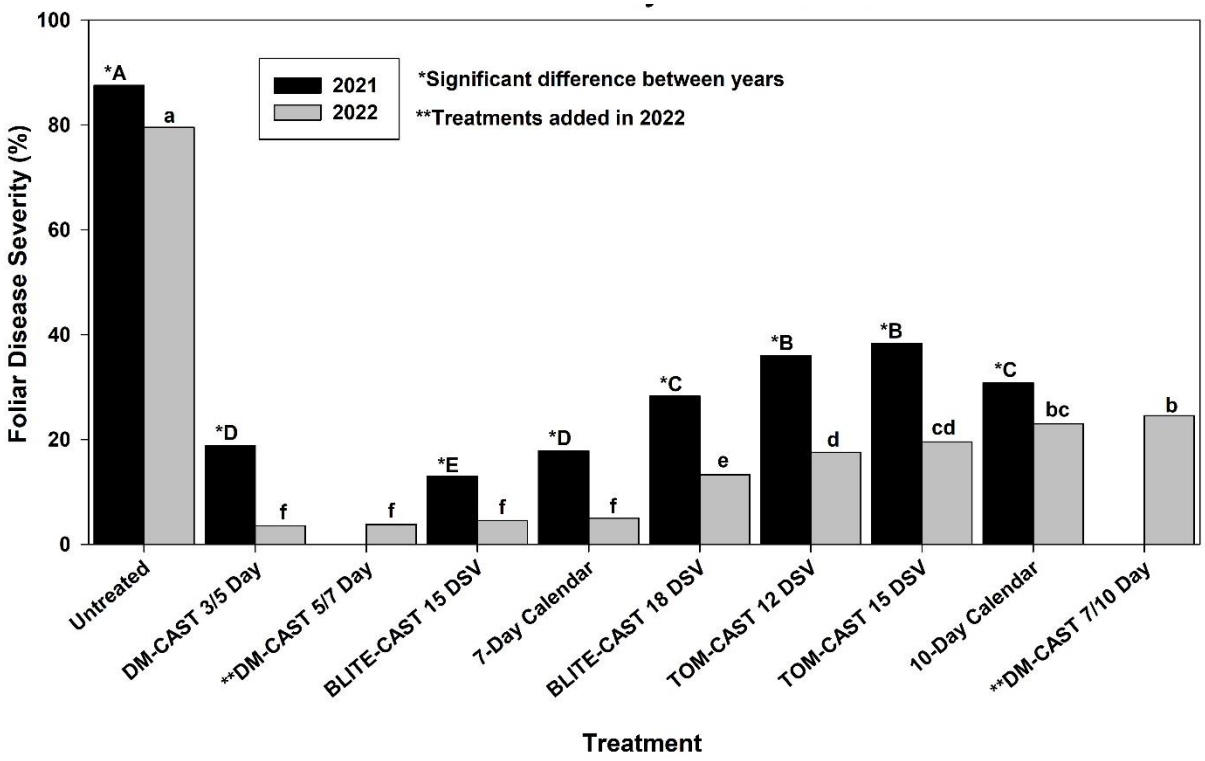

Figure 10: Percentage of foliar disease severity at the last rating date on 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2021 and 2022 for control of *P. cubensis*.

Area under the disease progress curve. According to the AUDPC data. all treatments limited CDM compared to the control each year. The AUDPC data indicated that the BLITE-CAST 15DSV treatment (6 applications) was among the most effective treatments each year and was as effective as the 7-day treatment (7 and 6 applications in 2021 and 2022, respectively). BLITE-CAST 15DSV also limited disease progression compared to the BLITE-CAST 18DSV (5 applications), 10-day (5 and 4 applications in 2021 and 2022, respectively), and both TOM-CAST (4 applications in 2021; 4 and 5 applications in 2022 for 15DSV and 12DSV, respectively) treatments each year. According to the AUDPC data, BLITE-CAST 18DSV limited CDM compared to TOM-CAST 12DSV (2021, 2022) and 15DSV (2022). The AUDPC data indicated that the TOM-CAST 15DSV had more CDM disease than DM-CAST 3/5 day, BLITE-CAST 15DSV, and the 7-day treatment each year (Figures 11 and 12). In 2021, the AUDPC data indicated that the DM-CAST 3/5 day treatment (8 and 6 applications in 2021 and 2022, respectively) was more effective than all others. The AUDPC values indicated that the BLITE-CAST 18DSV, and the 7-, and 10-day treatments were similar. TOM-CAST 12DSV had a significantly higher AUDPC value than all treatments except the 10-day treatment and TOM-CAST 15DSV (Figure 11). In 2022, the 7-day and BLITE-CAST 15DSV had similar AUDPC values and were lower than all other treatments. The AUDPC data indicated that BLITE-CAST 18DSV was similar to DM-CAST 3/5 day and 5/7 day (5 applications in 2022); the DM-CAST 3/5 day and 5/7 day treatments were similar to the 10-day treatment. The AUDPC data indicated that the 10-day treatment was more effective than both TOM-CAST treatments and the DM-CAST 7/10 day treatment (4 applications in 2022). According to AUDPC data, TOM-CAST 12DSV had significantly less disease than TOM-CAST 15DSV and DM-CAST 7/10; the TOM-CAST 15 DSV and DM-CAST 7/10 treatments had similar levels of CDM disease (Figure 12,

Appendix B Figures 16 and 17).

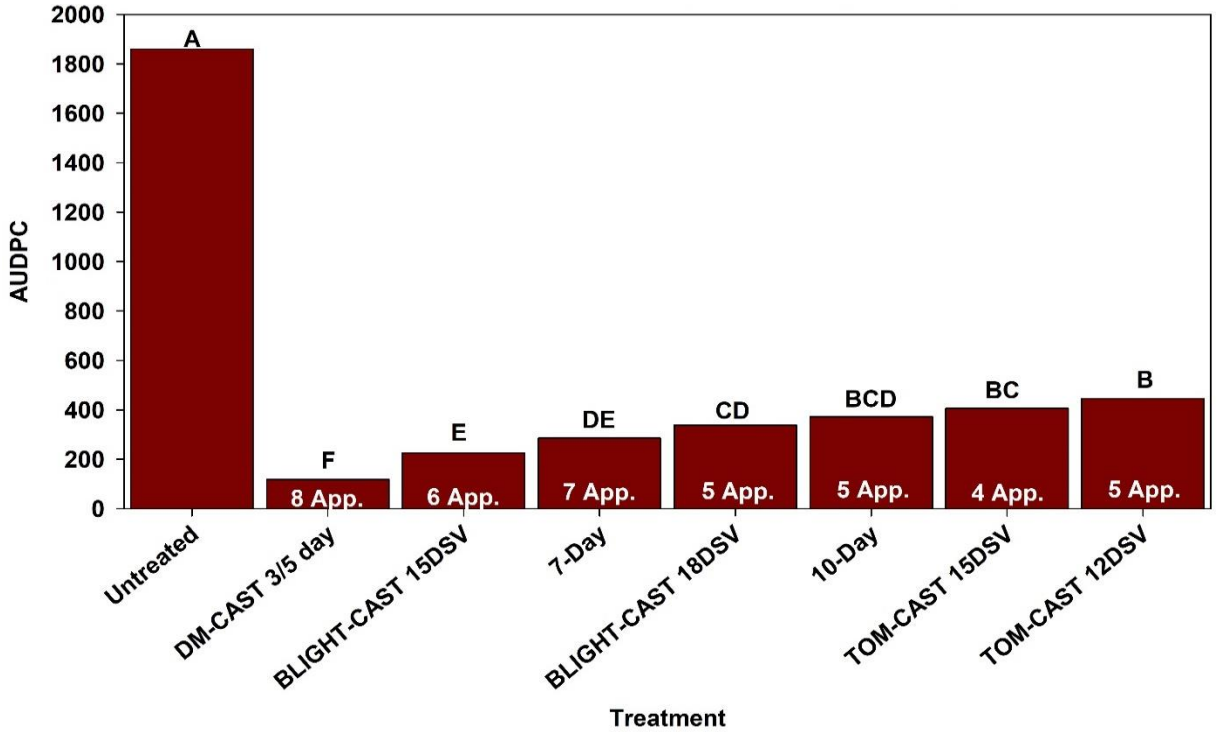

Figure 11: Area under the disease progress curve (AUDPC) on 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2021 for control of *P. cubensis*.

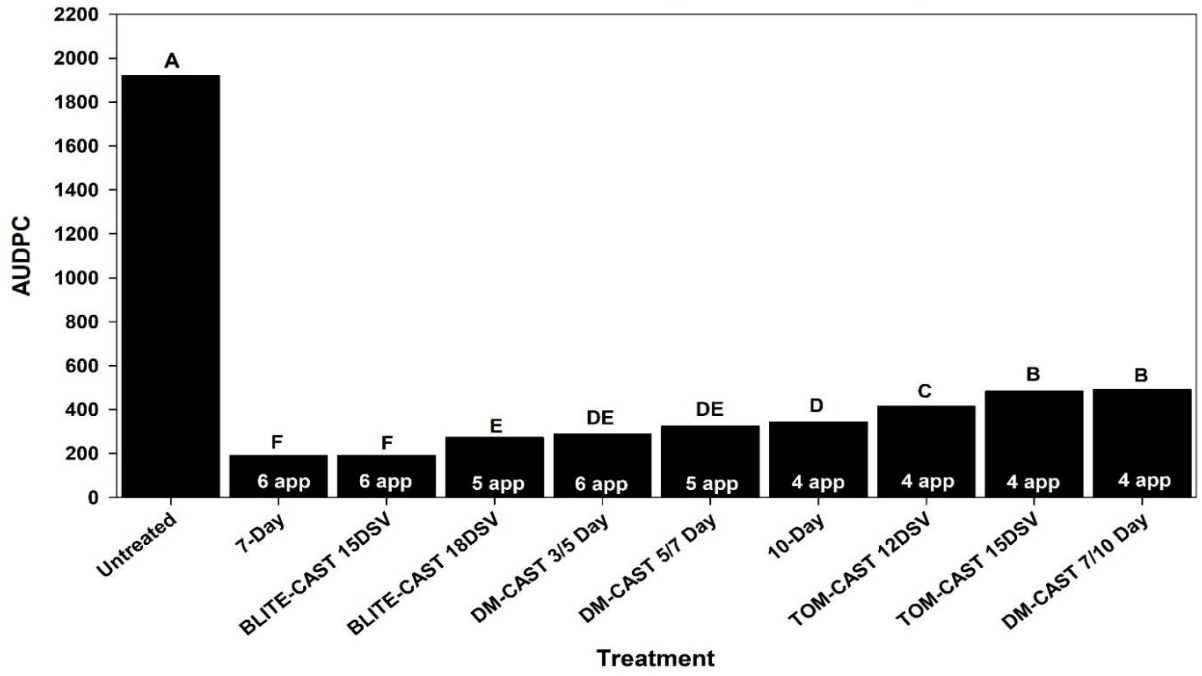

Figure 12: Area under the disease progress curve (AUDPC) on 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2022 for control of *P. cubensis*.

Relative area under the disease progress curve. According to rAUDPC data, there was significantly more disease in 2021 than 2022 except for the control and the BLITE-CAST 15DSV and TOM-CAST 15DSV treatments. The DM-CAST 3/5 day treatment had more disease in 2022 than 2021. While the rAUDPC and AUDPC data were similar, some differences were noted (Figure 13). According to the rAUDPC data, the BLITE-CAST 15DSV treatment was more effective in limiting CDM disease than the 7-day treatment in 2021, whereas the AUDPC data indicated that the treatments did not differ (Figures 11 and 13). According to the 2022 rAUDPC data, CDM disease was similar between the 10-day and the TOM-CAST 12DSV treatments but according to the AUDPC data, the 10-day treatment had significantly less disease (Figures 12 and 13).

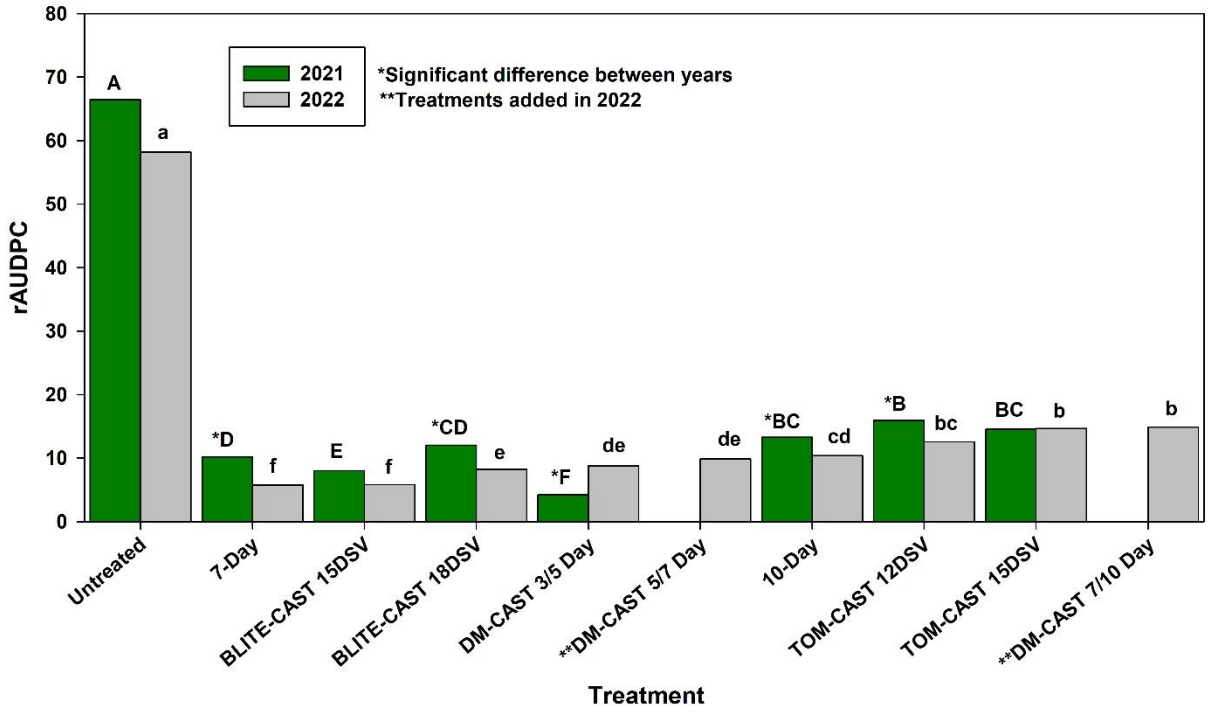

Figure 13: Relative area under the disease progress curve (rAUDPC) of 'Straight-Eight' cucumbers sprayed according to disease forecasters and calendar programs in 2021 and 2022 for control of *P. cubensis*.

Discussion

Popularly grown pickling cucumber cultivars in Michigan are either susceptible or moderately resistant to CDM (Call et al. 2012; Cespedes-Sanchez et al. 2015; Higgins et al. 2023). CDM affects cucumber leaves causing severe defoliation and yield reduction if not effectively managed (Cespedes-Sanchez et al. 2015). If a disease forecaster fails to accurately schedule fungicide applications, the pickling cucumber growers could face significant losses. Growers are typically risk averse and will often adopt a conservative disease management plan to protect potential yield (Campbell and Madden 1990). To control CDM, Michigan growers are encouraged to initiate a fungicide program once airborne *P. cubensis* sporangia are detected in key production areas as determined through a network of Burkard spore traps and disease scouting (Bello et al. 2021; Hausbeck 2022). Thereafter, Michigan growers apply fungicides every 7 to 10 days or more frequently, based on disease pressure and weather conditions (Granke et al. 2014). However, due to the high cost of fungicides, growers have expressed interest in scheduling fungicide applications after program initiation only when weather conditions favor CDM to reduce the total number of applications (Hausbeck, *personal communication*). Reducing the number of fungicide applications reduces input costs and the negative environmental impact of applying unnecessary fungicides, and may also reduce the risk of fungicide resistance developing in the pathogen population (Corkley et al. 2022). To facilitate grower adoption, it's important that potential disease forecasters limit CDM to a level similar to the standard 7-day program. We evaluated three disease forecasting programs for their effectiveness in scheduling fungicide applications for CDM control once an influx of sporangia were detected. Results of our study show that the BLITE-CAST and DM-CAST forecasters limited CDM progression and performed similarly to a 7-day program.

In our study, the BLITE-CAST 15DSV treatment was similar to the 7-day treatment according to AUDPC data in both years with fewer (2021) or the same number of applications (2022). AUDPC data indicate that the BLITE-CAST 18DSV treatment was similar to the 7-day treatment in 2021 and required two fewer applications. While the 2022 AUDPC data indicated that BLITE-CAST 18DSV required one less spray than the 7-day treatment there was significantly more CDM disease. The amount of disease observed in 2022 for the BLITE-CAST 18DSV treatment was commercially acceptable with less than 20% foliar disease at the last assessment. The disease pressure in the 2022 trial was high (<75%) in the control at the final assessment which is more severe than what a commercial grower would typically experience (Hausbeck, *personal communication*). In 2021, the DM-CAST (3/5 day) model required one more application but controlled disease better than the 7-day treatment based on the AUDPC data whereas in 2022 it had the same number of applications and significantly more disease. In 2022, two treatments were added to reduce the number of applications required using the DM-CAST model, a DM-CAST 5/7 day and a DM-CAST 7/10 day treatment. The DM-CAST 7/10 day treatment reduced the number of applications compared to the 7-day treatment (4 to 6) but had significantly more disease. The DM-CAST 5/7 day treatment had one less spray than the 7-day treatment but displayed significantly more disease according to the AUDPC data. However, this treatment had less disease than the DM-CAST 7/10 day treatment and a similar level of disease to the DM-CAST 3/5 day treatment. Similar to the BLITE-CAST 18DSV treatment, the amount of disease (<10%) was commercially acceptable. The TOM-CAST model, developed for use on fungal pathogens, was not as effective in limiting CDM as the other two models included in our study. The application of the TOM-CAST model for use in a CDM pathosystem may be limited because this forecaster was developed for *Alternaria solani*, *Septoria lycopersici*, and

Colletotrichum coccodes. These pathogens have an optimum temperature for sporulation and infection between 20 and 25°C (Dilliard 1989; Douglas 1972; Elmer and Ferrandino 1995) whereas *P. cubensis* has an optimal temperature for sporulation and infection of 15-20°C (Cohen and Rotem 1969, 1971; Sun et al. 2017).

Applications can be scheduled according to environmental conditions, as in our study and others (Bounds et al. 2007; Meyer et al. 2000; Raposo 1993; Ribeiro Ávila et al. 2020) or based on aerial spore loads determined using molecular diagnostic techniques (Dhar et al. 2020; Thiessen et al. 2016). Dhar et al. (2020) used aerial spore load as measured by qPCR coupled to impaction spore traps to schedule fungicide application intervals for lettuce downy mildew caused by *Bremia lactucae*. When an untreated control was compared to a calendar based standard and an advisory based spray (indicated by an aerial spore load), the advisory based spray system controlled disease better than the control but had significantly more disease than the standard. Thiessen et al. (2016) used impaction spore traps coupled to loop-mediated isothermal amplification (LAMP) to detect aerial spore loads of the grape powdery mildew pathogen *Erysiphe necator* and schedule fungicide applications. Disease incidence was similar when spraying according to the advisory based schedule and fungicide applications were reduced compared to the industry standard, calendar schedule. Due to the costs of implementing molecular diagnostic techniques across the large pickling cucumber acres, basing spray schedules on aerial spore loads may not be efficient for CDM control in Michigan. Using environmental conditions to schedule fungicide applications once airborne *P. cubensis* sporangia have been detected may be more efficient for Michigan's pickling cucumber growers. DM-CAST and BLITE-CAST have not been previously evaluated for control of *P. cubensis* on cucumber and our study indicates that they may be helpful tools to schedule fungicide

applications for CDM control in Michigan. Additional experiments may be needed to evaluate whether higher DSV thresholds (18DSV versus 15DSV) may be used when disease pressure is less than what was observed in our trials.

FUTURE WORK

Michigan pickling cucumber growers rely on university research and resulting recommendations to manage CDM. We evaluated currently labelled fungicides in order to make accurate management recommendations. Each year, fungicides were evaluated for an entire cucumber cropping period under high disease pressure. We were able to identify multiple fungicides that performed well under these conditions. While this information is valuable for developing recommendations, it does not explain in what ways *P. cubensis* populations may vary in fungicide sensitivity during one growing season. For this reason, future work may be done using a subset of the fungicides tested in this study in subsequent bioassays to determine how *P. cubensis* fungicide sensitivity changes during one growing season in Michigan.

Additionally, we were able to identify two disease forecasters that were able to successfully schedule fungicide applications under high disease pressure. However, we would not expect growers to experience the same high disease pressure that we experienced in our field trials. For this reason, we believe that evaluating BLITE-CAST with a greater DSV threshold (18 DSV or more) under conditions that more closely mimic a commercial grower's field would lead to fewer fungicide applications than what we observed in our study while maintaining disease control compared to a 7-day program. We could achieve this by planting earlier and beginning fungicide applications when sporangia are first detected in the state, which is typically what growers do, versus when sporangia are first detected on site, which is what we did for our study. Furthermore, we evaluated BLITE-CAST using a susceptible cultivar (Straight-Eight) when there are intermediately resistant cultivars available. Pairing BLITE-CAST with such cultivars may also allow for fewer fungicide applications.

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APPENDIX B

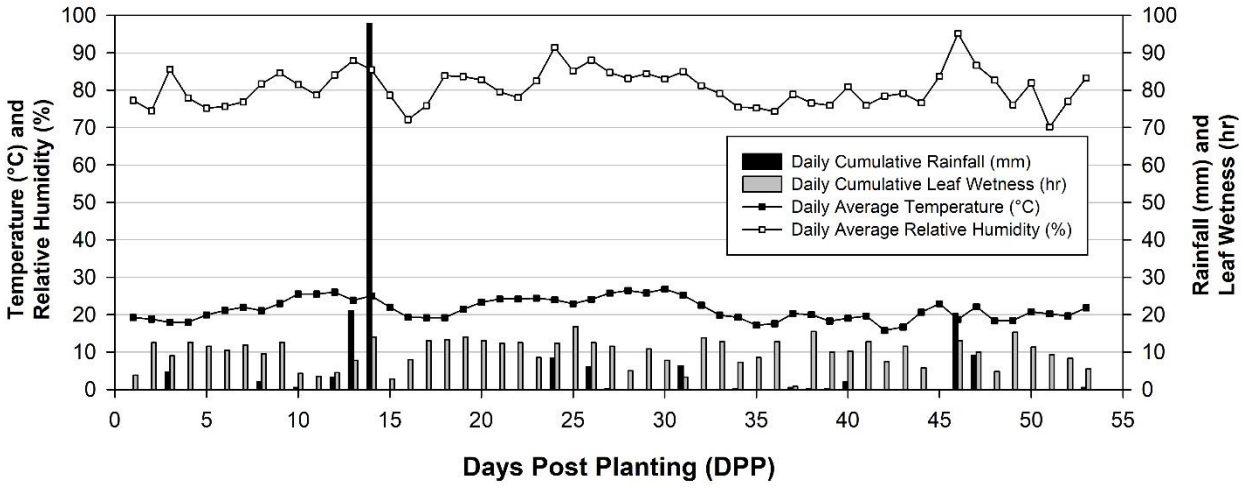

Figure 14: Full season weather data for 2021.

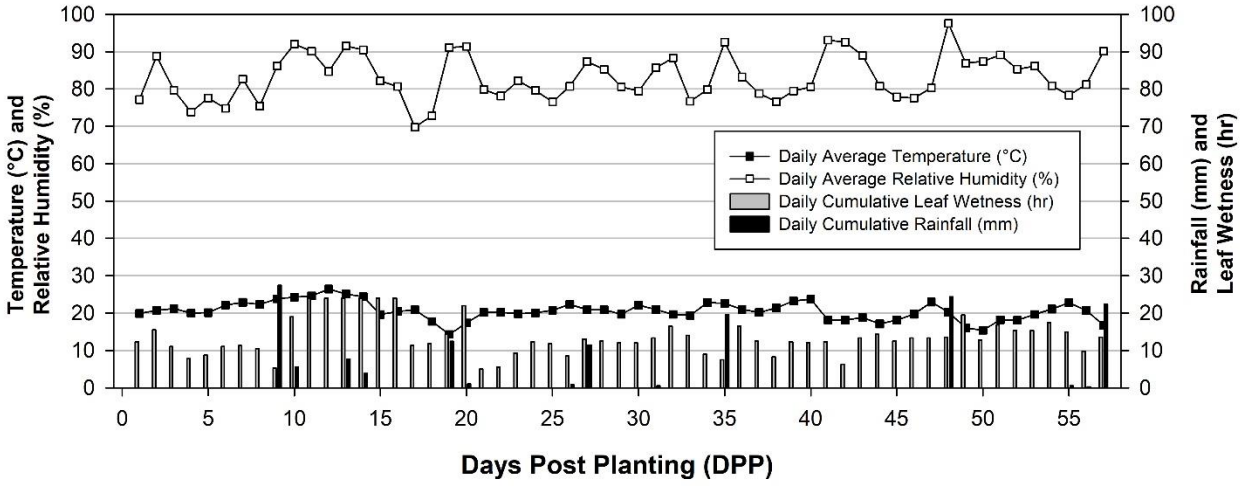

Figure 15: Full season weather data for 2022.

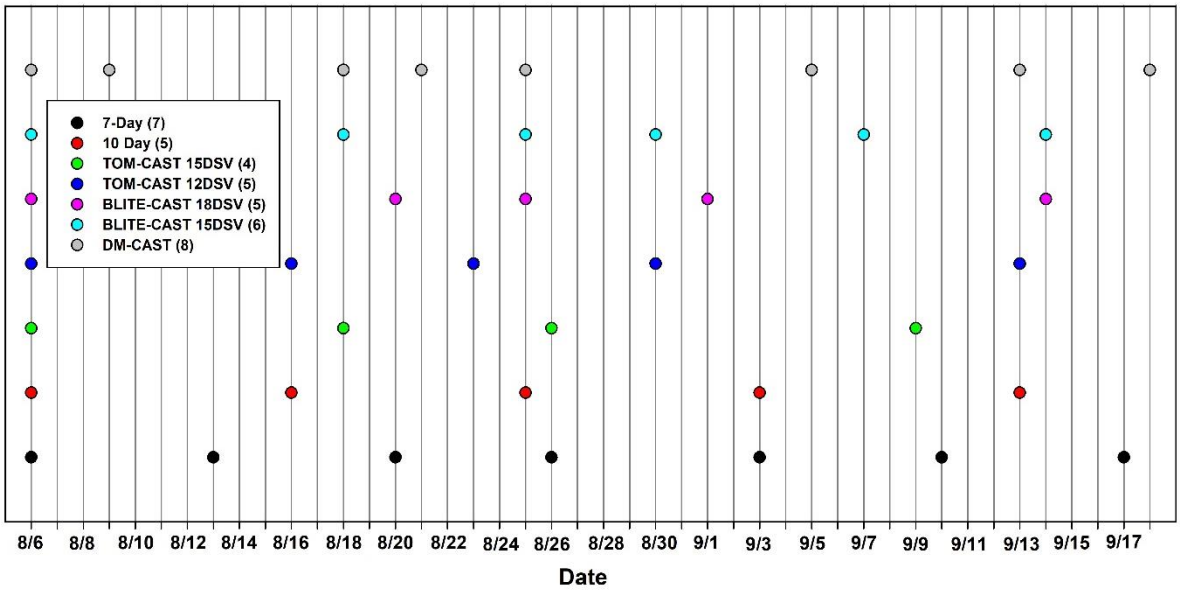

Figure 16: Fungicide application dates, 2021.

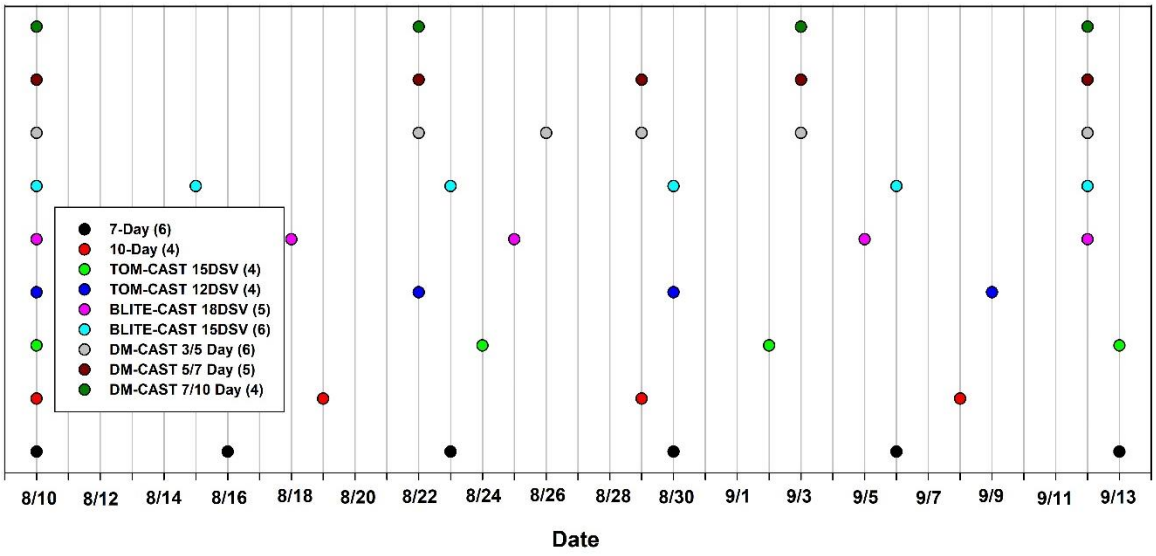

Figure 17: Fungicide application dates, 2022.

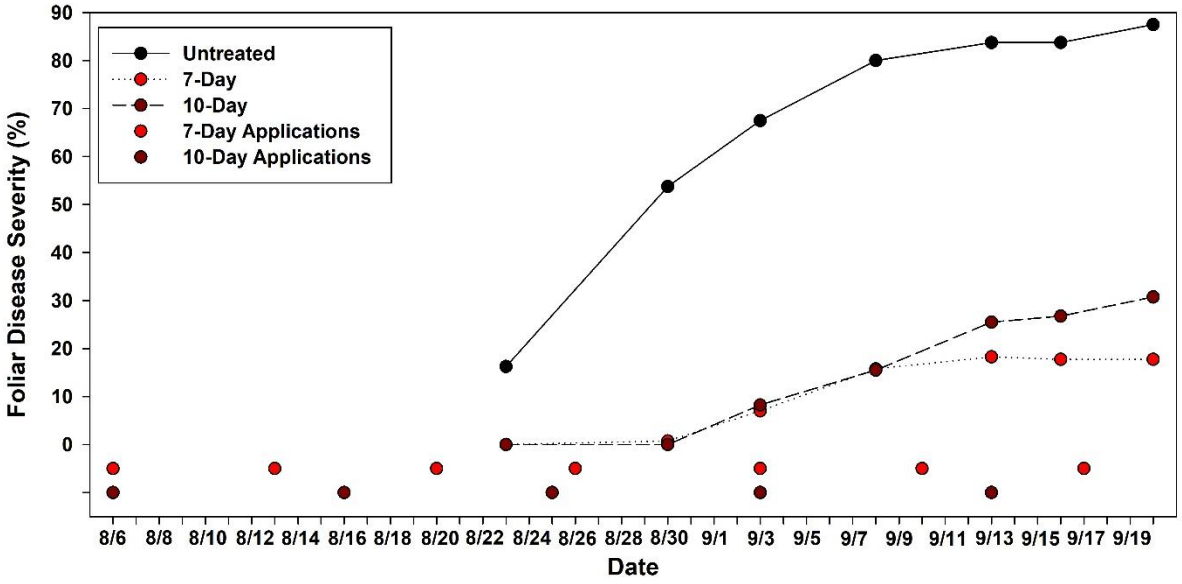

Figure 18: Disease progress for untreated, 7-Day, and 10-Day treatments with application dates, 2021.

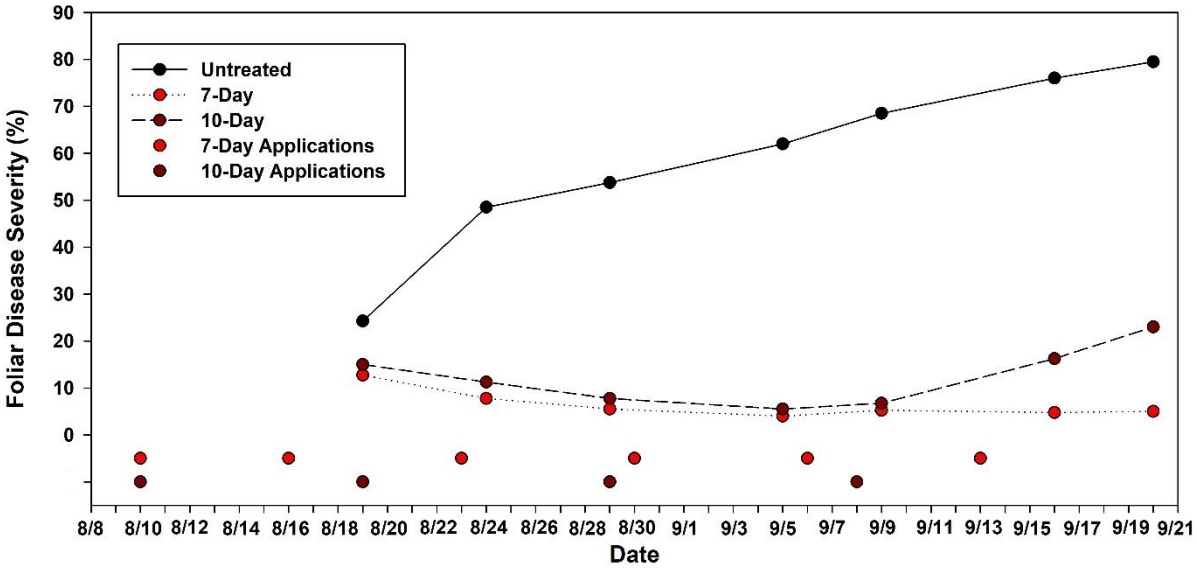

Figure 19: Disease progress for untreated, 7-Day, and 10-Day treatments with application dates, 2022.

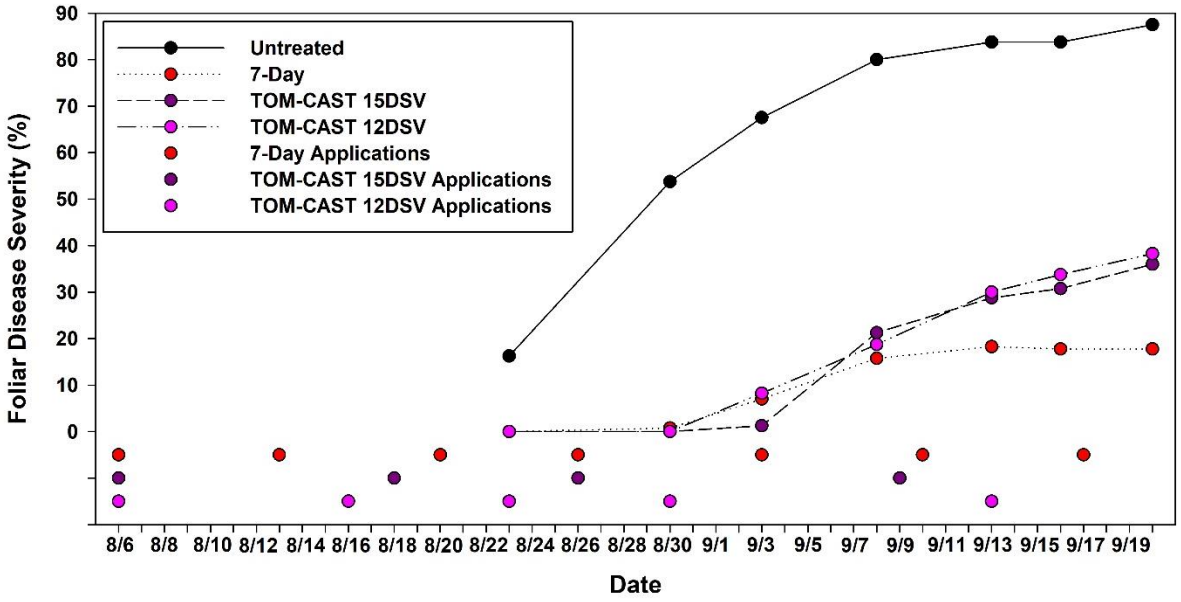

Figure 20: Disease progress for untreated, 7-Day, TOM-CAST 15DSV and TOM-CAST 12DSV treatments with application dates, 2021.

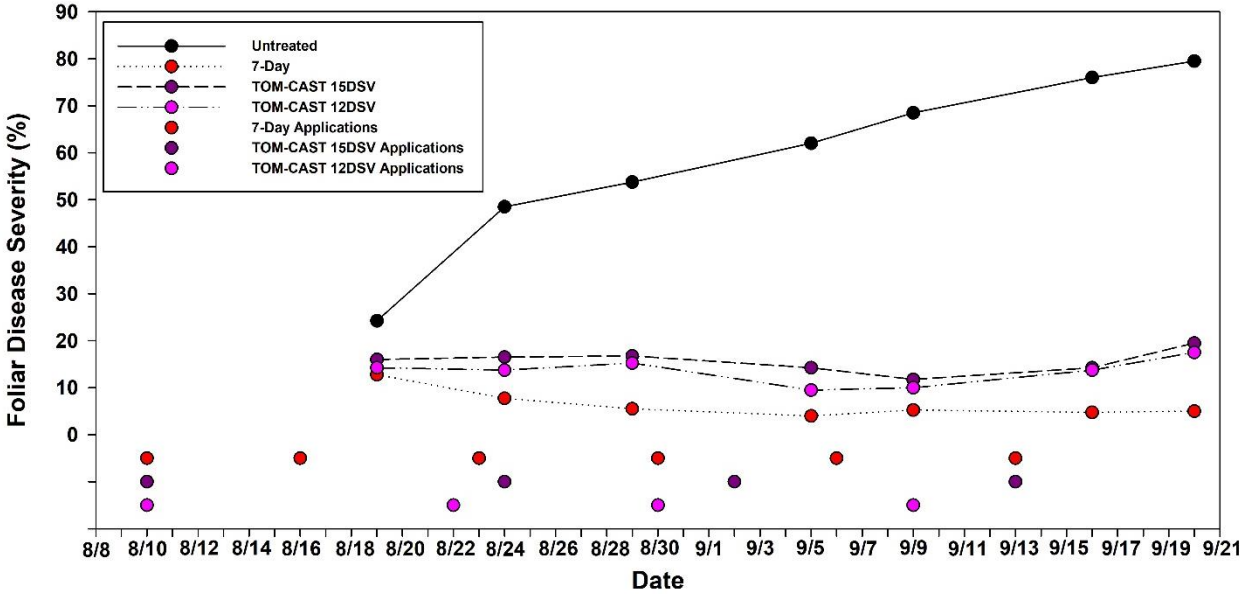

Figure 21: Disease progress for untreated, 7-Day, TOM-CAST 15DSV, and TOM-CAST 12DSV treatments with application dates, 2022.

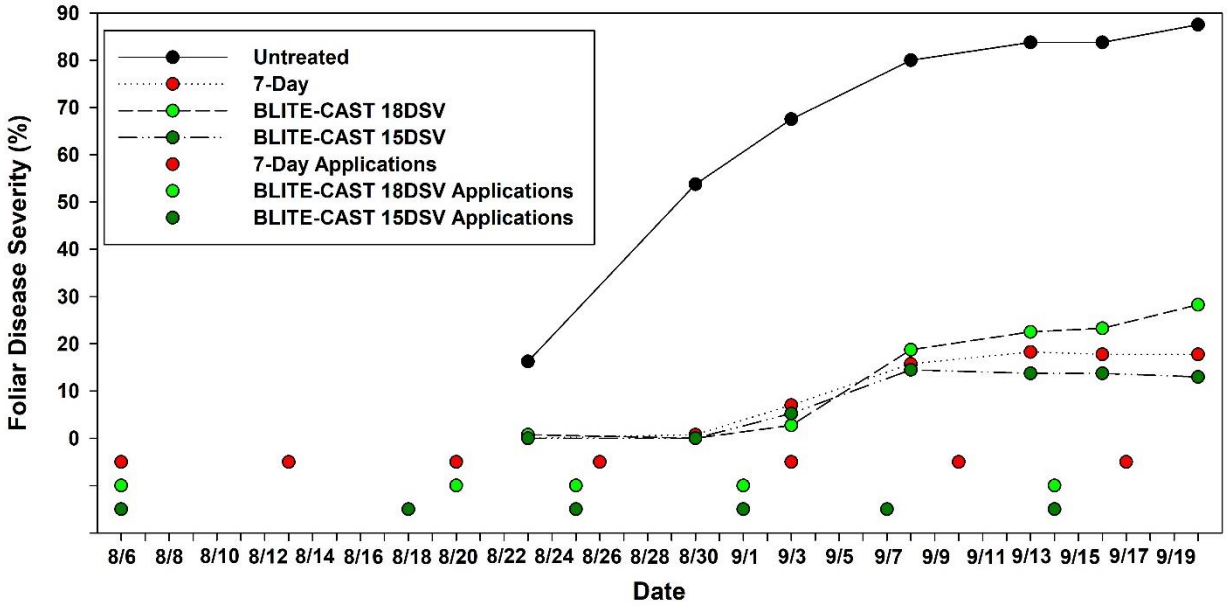

Figure 22: Disease progress for untreated, 7-Day, BLITE-CAST 18DSV, and BLITE-CAST 15DSV treatments with application dates, 2021.

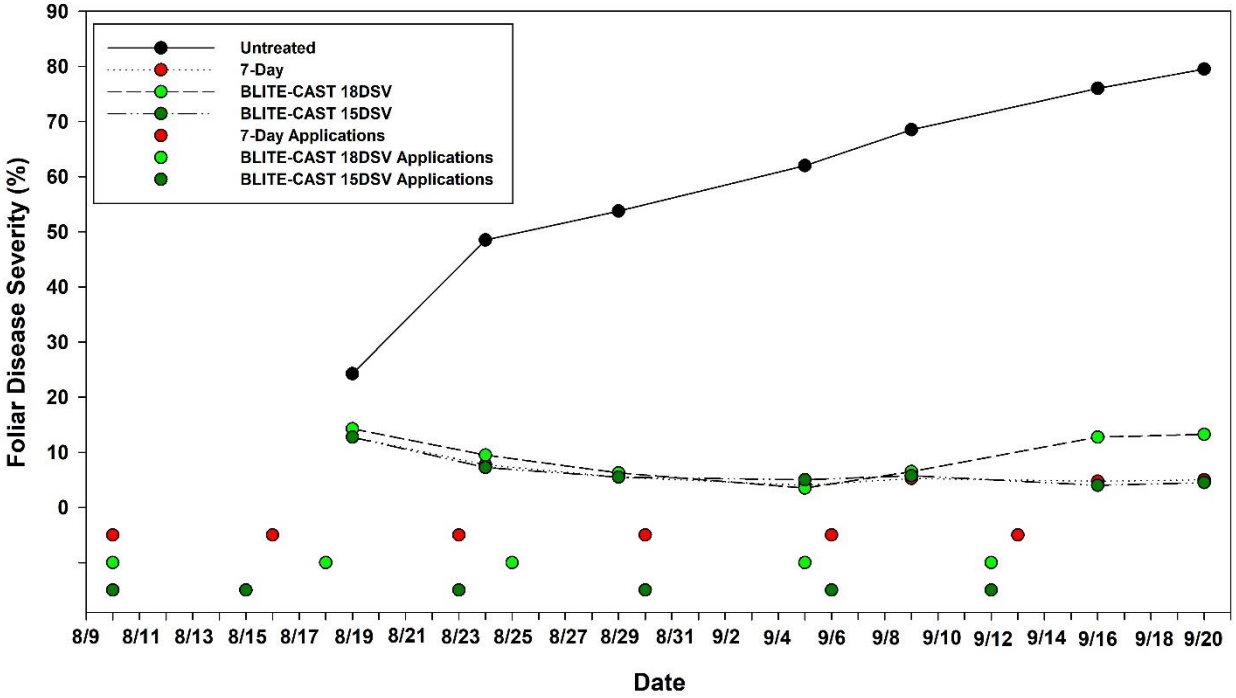

Figure 23: Disease progress for untreated, 7-Day, BLITE-CAST 18DSV, and BLITE-CAST 15DSV treatments with application dates, 2022.

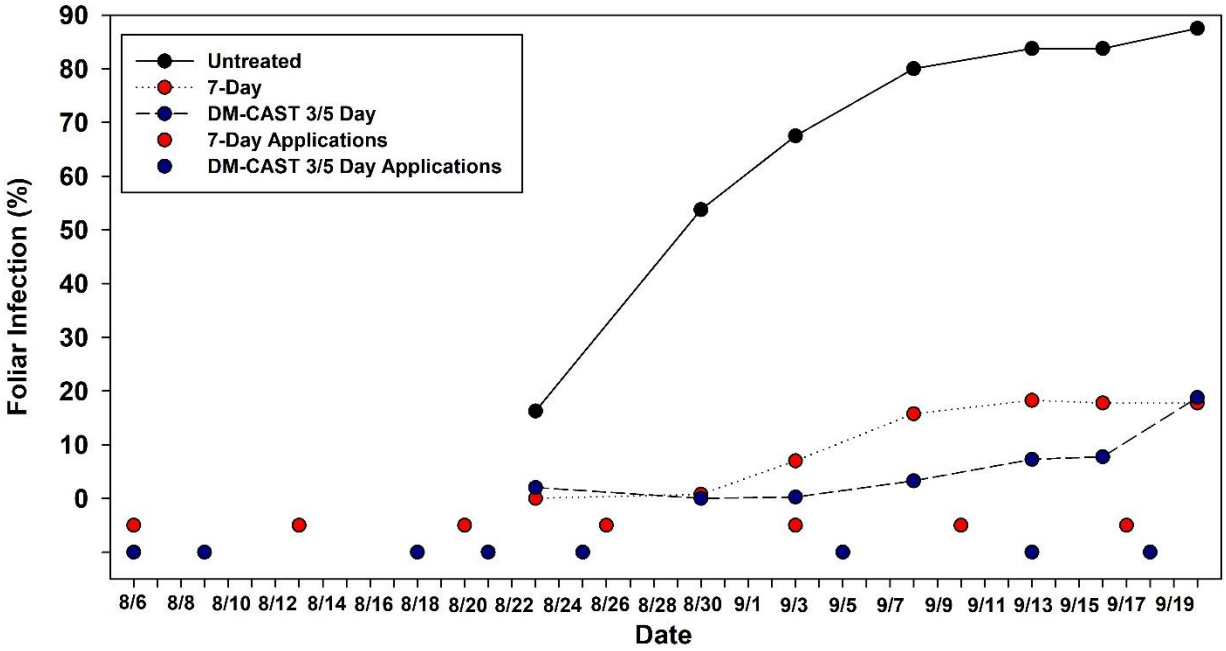

Figure 24: Disease progress for untreated, 7-Day and DM-CAST 3/5 day treatments with application dates, 2021.

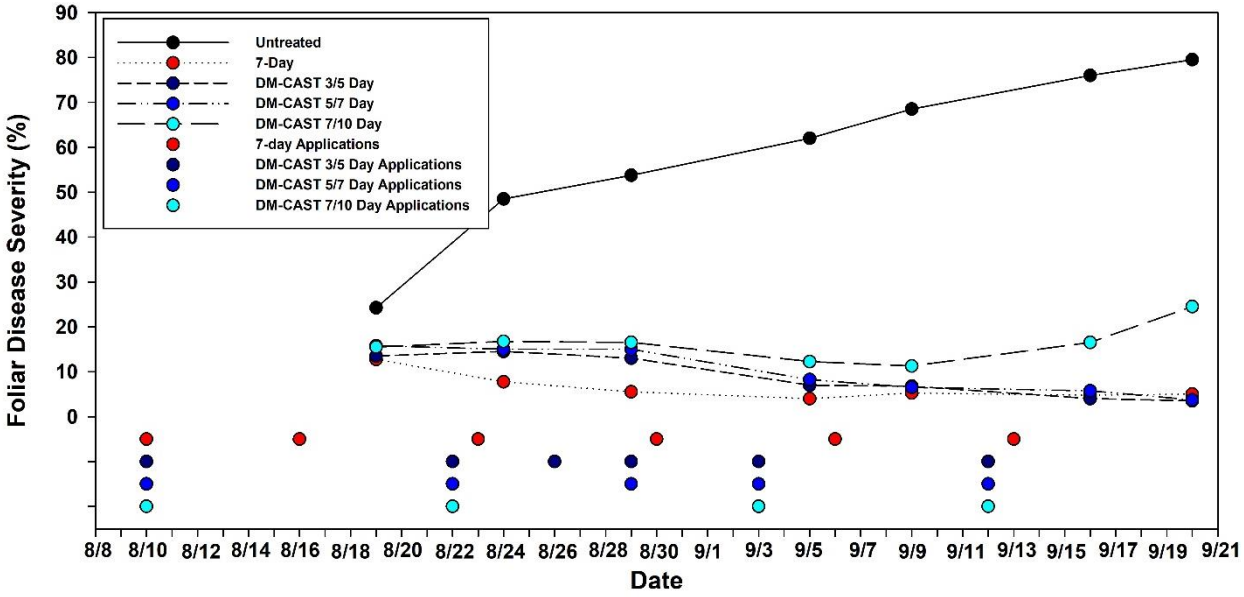

Figure 25: Disease progress for untreated, 7-Day, DM-CAST 3/5 day, DM-CAST 5/7 day, and DM-CAST 7/10 day treatments with application dates, 2022.