CONTROLLING DOWNY MILDEW INCITED BY *PSEUDOPERONOSPORA CUBENSIS* ON PICKLING CUCUMBERS WITH FUNGICIDES

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ABSTRACT

CONTROLLING DOWNY MILDEW INCITED BY *PSEUDOPERONOSPORA CUBENSIS* ON PICKLING CUCUMBERS WITH FUNGICIDES

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Pseudoperonospora cubensis, the causal agent of cucurbit downy mildew (CDM), is a destructive disease of cucumber resulting in blighting of the foliage. *P. cubensis* is a high-risk pathogen for developing resistance to fungicides. Field studies were conducted in 2019 and 2020 to test 14 fungicides when applied weekly for their ability to limit CDM. In both years, oxathiapiprolin + chlorothalonil held disease to ≤1% foliar symptoms at the end of the monitoring periods. In 2019, cyazofamid was similar to oxathiapiprolin + chlorothalonil on the last monitoring date with CDM symptoms observed on 10% of the foliage. In 2020, treatment with oxathiapiprolin + chlorothalonil resulted in less disease than all other treatments. In 2019, treatments including ametoctradin + dimethomorph (premix), fluazinam, propamocarb and zoxamide + chlorothalonil (premix) limited disease to ≤ 20.25% and provided control similar to cyazofamid. Results from trials comparing treatment programs consisting of tank mixes and alternating fungicides indicated that chlorothalonil alone or as part of a program with other fungicides but did not include oxathiopiprolin were the least effective in limiting CDM. The timing of fungicides is also a factor in reducing disease. A second set of trials conducted in 2019 and 2020 had 5 treatments: Untreated, 5-day, 7-day, BLITECAST, and TOMCAST. In both years of the study, the calendar sprays were the most effective. In 2019, the 5-day calendar spray was the most effective, but not different from BLITECAST, holding disease pressure to 30%. In 2020, the 7-day calendar treatment was the best treatment, holding disease to 31.3%, and no other treatment was similar.

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INTRODUCTION

Cucurbit downy mildew (CDM) disease is caused by *Pseudoperonospora cubensis* (Rostowzew, 1903, Berkeley & Curtis, 1868. Summarized in Colucci and Holmes, 2010). Forty plant species across 20 genera within the Cucurbitaceae family may serve as hosts for the pathogen (Palti & Cohen, 1980). First discovered in Cuba in 1868, the pathogen was first thought to be a new species of *Peronospora* (Berkeley and Curtis, 1868 Summarized in Colucci and Holmes, 2010). In 1903, it was discovered that *P. cubensis* sporangia were different from *Peronospora* spp and the pathogen was reclassified to *P. cubensis* (Rostowzew, 1903 Summarized in Colucci and Holmes, 2010).

The pathogen is a significant risk to cucumbers in Michigan (Cespedes-Sanchez et al., 2015) and other U.S. cucumber growing regions (Quesada-Ocampo et al., 2012). In 2004, when the pathogen re-emerged, cucumber growers in the southeast U.S. lost approximately 40% of their yield (Colucci et al., 2006). Cucurbit growers in Michigan spend an estimated $8 million on fungicides; fungicide resistant *P. cubensis* isolates have been detected (Hausbeck, 2006).

Cucumber: Cucumbers (*Cucumis sativus* L.) are a high-value vegetable crop in the U.S. They are grown as a dual purpose crop used for fresh market (slicers) or processing (pickling). Pickling is believed to have started in Mesopotamia and is defined as a process of preserving perishable items in vinegar, brine, or a similar solution (Terebelski & Ralph, 2003). Michigan is the number one producer of processing cucumbers, contributing $50 million to the state's economy; cucumbers for the fresh market contribute $15 million (Development, 2018). In 2019, Michigan grew 40,000 acres of cucumbers, comprising 27% of the national market. Following Michigan in production acres is Florida which produces cucumbers on 23,000 acres (NASS, 2019). According to the U.S. Department of Agriculture, the average American consumes

approximately 9 pounds of pickles per year, thus making domestic cucumber production important for consumers (Development, 2018).

In commercial processing cucumber regions of Michigan, cucumber seeds are planted into a fresh seed bed at an average of 65,000 seeds per acre (Talley, 2010). Slicing cucumbers are seeded into a raised bed covered in black plastic mulch with drip irrigation at 7,000 seeds per acre (Schrader et al., 2002). Cucumbers prefer light-textured, well-drained soils. Most cucumbers require pollination by honey bees; one hive per two acres is recommended (Talley, 2010). Bees are not needed for parthenocarpic cucumbers (Pike & Peterson, 1969). Historically all cucumbers were hand harvested, and still are in many regions of the U.S. (Sánchez, 2018, Holmes, 2005). In 1964, Michigan State University developed a machine for a one time harvest and ten years later 90% of Michigan's cucumber acres were machine harvested (Cargill, 1975).

CUCUMBER DISEASES

Growers of cucumbers must manage many diseases. Since 2004, cucurbit downy mildew has re-emerged as the most economically important pathogen of cucumbers (Quesada-Ocampo et al., 2012). Prior to that, *Phytophthora capsici,* a soil borne pathogen causing fruit rot and plant death, had been considered the most destructive pathogen (Gevens, 2005). Other notable pathogens include *Pseudomonas syringae* pv *lachrymans* (incites angular leaf spot), and *Colleotrichum orbiculare* (incites anthracnose), *Sphaerotheca fuliginea* (incites powdery mildew), *Didymella byroniae* (incites gummy stem blight), *Phomopsis cucurbitae* (incites Phomopsis blackrot), and *Corynespora cassiicola* (incites target leaf spot) (Keinath et al., 2017).

TAXONOMY

The Cucurbitaceae family has 118 genera with 825 species; 20 genera and more than 50 species are downy mildew hosts (Thomas et al., 2017). Cucurbit downy mildew is the most destructive disease of cucurbitaceous crops worldwide (Ojiambo et al., 2010).

P. cubensis is an obligate biotrophic oomycete from the kingdom Chromista, subdivision Peronosporomycotina, class Peronosporomycetes, order Peronosporales (Voglmayr, 2008). Oomycetes are water molds and more closely related to algae than fungi (Baldauf et al., 2000). Their cell walls are a mix of cellulosic compounds and glycans instead of chitin found in fungi, and the nuclei are diploid rather than haploid (Baldauf et al., 2000).

The *Pseudoperonospora* genus contains five species: *P. cubensis, P. humuli, P. cannabina, P. urticae,* and *P. celtidis* (Choi et al., 2005). *P. humuli*, the downy mildew of hops, is known as sister species to *P. cubensis* because they are morphologically similar (Mitchell et al., 2009). These two pathogens can be found in spore traps in areas where both hosts are grown requiring the use of PCR as a means to distinguish between them (Bello et al., 2021).

The sexual reproduction of *P. cubensis* is mostly unknown as it has not been observed in nature, but there are two mating types, A1 and A2 (Thomas et al., 2017). In lab studies, when both mating types were present on the same leaf an oospore formed; this has not been observed in the field (Thomas et al., 2017). Cohen et al. (2015) tested oospore production on both melon and cucumber leaves. The appearance of oospores occurred within 5-7 or 7-10 days on melon and cucumber leaves, respectively (Cohen et al., 2015). The oospores are yellow or hyaline, and 22-42 μm in length (Babadoost, 2016).

The asexual stage of the pathogen including sporangiophores, sporangia, and zoospores is observed in the field. The sporangiophores are branched, producing sporangia that are grayish-

purple in color and lemon-shaped (Palti, 1980). The sporangia contain approximately 2-15 motile zoospores with two flagella (Palti & Cohen, 1980). The zoospores are highly infective, but not long lived, surviving for 1 to 16 days (Cohen & Rotem, 1971). This obligate biotroph keeps the cells surrounding it alive as long as possible (Lebeda & Schwinn, 1994). To do this, *P. cubensis* forms a haustorium to penetrate the cell without breaking the cell membrane allowing the pathogen to feed without disrupting normal plant cell systems (Fraymouth, 1956).

SIGNS AND SYMPTOMS

On cucumber, *P. cubensis* forms angular lesions defined by the leaf veins (Zitter et al., 1996). The chlorotic lesions appear on the adaxial side of the leaf. The abaxial side of the leaf can appear purple or gray, indicating that sporangiophores have erupted through stomata and are producing sporangia (Babadoost, 2016). Signs and symptoms appear within 3-12 days following infection (Babadoost, 2016). On Squash, pumpkin, and cantaloupe the lesions are not angular or contained within the leaf veins.

Before the discovery of clades, *P. cubensis* was known to cause hypersensitive response on muskmelon (Cohen et al., 1989). Further studies found the clades of P. cubensis have specific hosts, clade 1 infecting watermelon, squash, and pumpkin whereas clade 2 infects cucumber and muskmelon (Summers et al., 2015). The current knowledge of clades, it is possible that the hypersensitive response occurred with clade 1 which does not readily infect muskmelon (Rahman et al., 2021).

DISEASE CYCLE

Under saturated environmental conditions, the sporangium releases zoospores that may encyst on the leaf surface. Once encysted, the zoospore forms a germ tube penetrating the leaf tissue via a stomata (Iwata, 1949) and a haustorium forms within the cells. Once the pathogen becomes established, sporangiophores are formed on the abaxial side of the leaf bearing the sporangia. The sporangia are released from the sporangiophores via a hygroscopic twisting motion (Lange et al., 1989), and are picked up by air currents and disseminated to nearby hosts (Savory et al., 2011, Lange et al., 1989). *P. cubensis* is polycyclic and has a short disease cycle (Babadoost, 2016).

Ojiambo and Kang (2013) theorized that *P. cubensis* sporangia can travel 1000 km each season with green bridging to infect hosts across several states. Other scientists have proposed that greenhouses in the northern United States and Canada may provide overwintering sites for the pathogen (Naegele et al., 2014). Sporangia may also move via machines and human contact (Zitter et al., 1996).

The sexual stage, oospores, of *P. cubensis* is rare, but it can occur in warm regions (Palti & Cohen, 1980). Studies suggest that if oospores are produced they could be an important source of overwintering inoculum for northern areas (Holmes et al., 2015).

ENVIRONMENTAL CONDITIONS

Temperature and leaf wetness are important environmental conditions for infection and sporulation of *P. cubensis* (Cohen, 1977). The minimum temperature for infection is 1-9°C, while the maximum is 27-32°C (Cohen, 1977). The optimal temperature for infection is considered to be 15°C (Savory, et al., 2010). Conducive daytime temperatures can range from

25-30°C, as long as the nighttime temperatures drop to 10-15°C (Lebeda & Cohen, 2010). The optimal temperature range for *P. cubensis* may be affected by the duration of leaf wetness (Cohen, 1977). For instance, the optimal leaf wetness period is six hours, but the pathogen can infect after 2 hours if the temperature is 20˚C or higher (Cohen, 1977, Lebeda & Cohen, 2010). At the optimal time period of six hours the temperatures can be lower (10-20˚C), but as the air cools (5-10˚C) infection becomes dependent on the amount of inoculum due to the reduced germination (Cohen, 1977). Sporulation can occur when temperatures range from 9-30˚C (Lebeda & Cohen, 2010). Temperatures of 10-20˚C resulted in delayed disease symptoms, however, the sporangia survived for a relatively long period of time, resulting in a higher sporangial count compared to when temperatures were higher than 20˚C (Rotem et al., 1978).

Light can also affect sporulation. The greatest amount of sporulation occurs during the nighttime and followed by long days. Long days extend the duration of sporulation by slowing down lesion development (Palti & Cohen, 1980). Spore production can be reduced by 70-90% if light is introduced for 10 minutes per hour (Rotem et al., 1978).

Leaf wetness is necessary for infection and can be a limiting factor in sporulation if the duration of leaf wetness is not adequate (Cohen, 1977, Lebeda & Cohen, 2010). This is due to a reduction in viability of the sporangia if there are intermittent periods of wetting and drying. Wetting periods shorter than two hours in duration are especially limiting (Palti & Cohen, 1980). Sporulation is optimized with 6 to 9 hours of leaf wetness. In one wetting period occurring during the nighttime, 10^5-10^6 sporangia/cm^2 can be produced (Rotem et al., 1978).

Relative humidity is also important for sporulation of *P. cubensis*. Relative humidity greater than 90% is required for sporulation (Palti & Cohen, 1980, Rotem et al., 1978). A high level of relative humidity, however, limits the time period that the sporangia are viable (Rotem et

al., 1978). The reduction of humidity in the air causes the sporangiophores to twist and this twisting movement allows the release of the sporangia from the sporangiophore (Lange et al., 1989). For each generation of sporangia the amount of inoculum increases (Rotem et al., 1978). Inoculum reaches its peak when 70% of the leaf is infected (Rotem et al., 1978). The sporangia erupt from the undersides of the leaves at high concentrations (Rotem et al., 1978).

OTHER DOWNY MILDEW PATHOGENS OF SIGNIFICANCE

Downy mildew affects many different hosts and is incited by pathogens representing different genera and species. The genus *Peronospora* includes approximately 400 species, many of them are plant pathogens (Constantinescu, 1991). Species within the *Pseudoperonospora* and *Peronospora* genera differ in the way that their sporangia germinate. Sporangia of *Pseudoperonospora* spp. germinate via a cytoplasmic cleavage resulting in zoospores. Whereas, *Peronospora* spp. sporangia germinate directly from the germ tube (Palti & Cohen, 1980, Rostowzew, 1903).

Several species within the genus *Peronospora* negatively impact economically important host crops including onion (*P. destructor)*, snapdragon (*P. antirrhini*), rose (*P. sparsa*), and basil (*P. belbahrii*) (Byrne et al., 2005). Fungicides are the primary control strategy for the downy mildew affecting these crops (Anonymous, 2016). Infection of onion by *P. destructor* is optimized with 2-3 hours of leaf wetness at 6-10ºC. Sporulation of *P. destructor* occurs when relative humidity is greater than 95%, and the previous day temperatures are less than 24ºC (Hildebrand, 1982). The *P. antirrhini* can become systematic, killing the terminal buds of snapdragon causing undesirable branching (Byrne et al., 2005). *P. antirrhini* is likely to sporulate when leaf wetness is greater than six hours (Byrne et al., 2005); the optimal temperature is 13ºC (Yarwood, 1947). Temperature does not have a large effect on infection unless they exceed 30ºC,

or are less than 10ºC (Byrne et al., 2005). On roses, optimal infection by *P. sparsa* occurs during temperatures ranging from 15-20ºC; 85-100% relative humidity is also required (Achar, 1997). The optimal temperature for sporulation is 18ºC with relative humidity of 85% (Filgueira D & Zambrano, 2014). On basil, *P. belbahrii* infection is optimized at 20ºC with relative humidity of 90% or higher. Sporulation of this pathogen occurs at 21-23ºC under high relative humidity (Djalali Farahani-Kofoet et al., 2012).

HOST RANGE

Early reports of the downy mildews claimed that their pathogens had narrow host ranges, limited to single species (Crute, 1981). *P. cubensis* is one of the few of the downy mildew species can infect distantly related host species (Choi et al., 2005). *P. cubensis* can infect 60 cucurbit species including: cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*), cantaloupe (*Cucumis melo*), watermelon (*Citrullus lanatus*), and pumpkin (*Cucurbita pepo*) (Lebeda & Widrlechner, 2003, Runge & Thines, 2009). Initial studies noted that there were six physiological races of *P. cubensis* that are pathogenic to the cucurbit species (Thomas et al., 1987). These races were discovered in Japan (Races 1, 2), Israel (Races 3, 6), and the U.S. (Races 4, 5) (Thomas et al., 1987, Cohen et al., 2003). All races were pathogenic on cucumber and muskmelon, and showed differences in pathogenicity on squash, pumpkin, and watermelon (Savory et al., 2011). Runge et al. (2011) discovered that there was a monophyletic group with two lineages. The first lineage showed two clades (1 and 2) of *P. cubensis*, and the second lineage comprising the sister species, *P. humuli*. Both *P. cubensis* clades have a broad host range. The pathogen is especially virulent on cucumber varieties used for pickling (Cespedes-Sanchez et al., 2015). *P. cubensis* isolates were found to differ genetically based on host and location

(Quesada-Ocampo et al., 2012). Summers et al. (2015) determined that the hosts could be separated into groups. The first group included squash, pumpkin, and watermelon. The second group included cucumber and cantaloupe. Wallace et al. (2020) found that wild type calabash (*Lagenaria siceraria*) was infected by clade 2 similar to cucumber and cantaloupe. Wild type bitter melon (*Momordica charantia*) was most affected by clade 1. Clade 1 also affects squash, watermelon, and pumpkin. Buffalo gourd (*Cucurbita foetidissima*) was affected similarly by both clades (Wallace et al. 2020). The wildtypes separate similarly to the hosts. Wallace et al (2020) also showed that the population of each clade fluctuates in North Carolina by season and location. Bello (Rodriguez, 2020), found that Michigan has predominately clade 2 population.

MANAGEMENT STRATEGIES

HOST RESISTANCE: Resistance to downy mildew was bred into cucumber cultivars in the United States during the 1950s (Holmes et al., 2015). This genetic resistance to *P. cubensis* lasted until 2004 and required only minimal fungicide use (Holmes et al., 2015). The resistant cucumber line, PI 197087, provided complete resistance to downy mildew with the *dm1* gene. This gene was naturally occurring in cucumber, but was recessive (Shimizu et al., 1963). It is believed that a new pathogenic isolate was introduced into the United States resulting in the downy mildew outbreak in 2004 (Thomas et al., 2017). This presumed introduction of a new isolate into the U.S. was responsible for the losses experienced during the 2004 and 2005 growing seasons when 80-100% of the cucumber crop was destroyed in production regions along the east coast (2004) and Michigan (2005) (Thomas et al., 2017). Runge et al (2011) found clade 1 prior to 2004 whereas clade 2 was found after 2004. Call et al. (2013) studied the cultivars with the *dm1* gene versus cultivars without the resistant gene during 2008 to 2009 in North Carolina

and Michigan. It was found that cultivar lines associated with PI 197087 had the highest disease resistance, but weekly applications of fungicide were needed to protect the yield. Since 2004, efforts to breed commercially acceptable cucumber cultivars with genetic resistance to *P. cubensis* has had only moderate success (Call et al., 2012).

FUNGICIDES: In the absence of genetic resistance, effective fungicides are crucial (Savory, et al. 2010). While many fungicides are labeled for control of *P. cubensis*, the pathogen is resistant to mefenoxam, strobilurins, fluopicolide, and propamocarb (Keinath et al., 2019, Holmes et al., 2015). Resistance to fungicides with a single mode of action (MOA) can develop quickly in *P. cubensis* populations (Miao et al., 2018) leading to reduced efficacy of many fungicides (McGrath, 2001). The single-site inhibitor fungicides include the phenylamides (PA), quinone outside inhibitors (QoI), carboxylic acid amides (CAA). Multi-site inhibitors include: dithiocarbamates, phthalimides, chlorothalonil, copper and sulfur formations, cymoxanil, fosetyl-Al, fluazinam, quinone insides inhibitors (QiI) and quinone outside Stigmatellin inhibitor (QoSI) (Holmes et al., 2015, Niks).

The PAs inhibit ribosomal RNA synthesis in oomycetes (Fisher & Hayes, 1982), and have long-lasting preventive activity, high mobility, and curative potential. Two-years after the introduction of the PAs, resistance was detected in *P. cubensis* in Israel, however, it is still used in the United States (Reuveni et al., 1980). The Fungicide Resistance Action Committee (FRAC) recommends that mefenoxam be used no more than twice during a growing season, and that it be applied preventively prior to disease detection (Cohen et al., 2015).

QoI obstructs mitochondrial respiration by interrupting the electron transport in cytochrome b. This is accomplished by the binding to the Qo site on the outer side of the mitochondria (Ishii et al., 2007, Gisi & Lebeda, 2002). As of 2014, it was no longer

recommended by FRAC to use QoI mode of action fungicides as a singular product (Cohen et al., 2015). QoSI is a mode of action that affects the cytochrome bc1 complex and is not cross resistant with QiIs, or QoIs. The difference between a QoI and QoSI is the attachment location. QoSI attaches to the mitochondria at the stigmatellin binding site (Gisi & Lebeda, 2002).

Recently, Oxathiapiprolin has been changed from FRAC code group U15 to group 49 This change is due to the discovery of the site of action, known as the oxysterol binding protein homologue inhibition (OSBPI). It is a systemic fungicide that protects the plant as it grows (Pasteris et al., 2016).

CAA fungicides are in the FRAC code group 40, and interfere with cell wall deposition and cellulous biosynthesis (Gisi et al., 2019). The CAA fungicide dimethomorph, developed in the 1980s, is registered for use on *P. cubensis,* and limits the germination of the cystospores, sporangia, and growth of the germ tubes and mycelium (Gisi et al., 2019).

The fungicides may harm the environment by affecting beneficial soil microbes (Santísima-Trinidad et al., 2018). Fungicides recommended for downy mildew affect honey bee populations (Christen et al., 2019). While chlorothalonil, azoxystrobin, and folpet all affected honey bees, azoxystrobin and folpet were significantly less harmful than chlorothalonil (Christen et al., 2019).

SPORE TRAPPING: There are different systems that can be used as a tool to monitor the CDM epidemics such as the CDM ipmPIPE. The impPIPE relies on scouting reports that are entered into the website (Ojiambo et al., 2011) and can guide growers as to when an initial fungicide spray should be made (Neufeld et al., 2018). Spore trapping can be used to monitor airborne sporangial concentrations as a tool to determine when fungicide sprays should be initiated. Two different spore trapping machines can be used including the Burkard volumetric

spore trap and the impaction trap (Frenz, 1999). The Burkard volumetric trap samples the air by drawing air into the machine via an intake orifice whereby particles present in the air are impacted onto melinex tape mounted on a reel and covered in a silicone adhesive (Frenz, 1999). The tape may be divided so as to examine one-half of the tape using light microscopy, and the other half may be used in a PCR reaction (Frenz, 1999, Bello et al., 2021). The impaction trap includes two plastic rods coated in a silicone grease that rotate (Frenz, 1999). The rods are then removed and may be examined using light microscopy or evaluated using PCR (Bello et al., 2021). In Michigan, the Burkard spore trap can be used to alert growers of airborne sporangia (Granke et al., 2014, Hausbeck, 2020). Using PCR distinguishes between *P. cubensis* and *P. humulii*, and between the two clades of *P. cubensis* (Bello et al., 2021) providing growers with the most accurate information available.

FORECASTING: Forecasting predicts when a pathogen will likely cause an economic impact by using weather conditions, data, and epidemiology of the pathogen (Hardwick, 2006). There has been success in the forecast modeling of *P. destructor*. There are programs using models to predict sporulation 17 out of 24 nights (De Visser, 1998), and models used to predict future sporulation periods (Friedrich et al., 2003).

There is not a forecasting system for downy mildew of cucurbits. With the intensive use of fungicides, a forecasting method could help determine spray intervals (Holmes et al., 2015). Currently, the sprays following the initiation of a fungicide program are calendar based. Growers may spray every 7 days, but the intervals between sprays can range from 5 to 14 days. This method is not only expensive, but also not environmentally efficient. Thus, a daily infection risk model is very important to develop in the near future, because it is known that the Eastern United States will get CDM, but the uncertainty lies in the timing of sporangia production (Neufeld et

al., 2018). Fungicides applied to cucumbers to prevent the infection and spread of the disease cost growers $6 million dollars annually (Granke et al., 2014).

A well-developed forecasting system is for the prediction of downy mildew of onions. There are many different forecasting systems for onion downy mildew, DOWNCAST, MILIONCAST 1, MILIONCAST 2, and many others. DOWNCAST was the first forecasting system to be developed for onion downy mildew which reduced fungicide application by 40% (Gilles et al., 2004). DOWNCAST was based on environmental conditions required for sporulation based on data from Canada, however, it does not predict the amount of sporangia produced in the sporulation period (Gilles and Kennedy, 2004; Gilles et al, 2004). Later ONIMIL, was developed based on the original DOWNCAST, this forecaster gives a quantitative prediction for sporulation, however, this was not what was needed by the onion growers because knowing how much sporulation occurs is not as important as knowing when it will occur. MILIONCAST was developed after DOWNCAST. MILIONCAST 1 was developed with the thought that sporulation starts at the onset of darkness, taking into account the relationship between sporulation and temperature and humidity; yet it does not show the relation between temperature and humidity (Gilles et al., 2004). MILIONCAST 2 was then developed with the thought of sporulation occurring when the humidity is at 92% or above (Gilles et al., 2004).

Disease severity values (DSV) are used in many of the forecasting models. Each of the models have a different way to calculate the DSV based on the pathogen the model is forecasting. A DSV is assigned each day based on weather conditions. The more favorable the weather, the greater the accumulation of DSVs (Hardwick, 2006).

The late blight forecaster, BLITECAST, works to forecast the infection of *Phytophthora infestans*, the potato late blight pathogen (Krause, et al., 1975). The pathogen thrives in humidity

greater than 90%, and sporulation optimal temperatures fall between 18-22˚C (Schumann, 2005). It is a forecasting system that is the product of combining two older forecasters with a partial modification (Krause, et al., 1975). The forecasting system works by using the mean temperatures during periods of relative humidity greater than 90%, which then calculates a severity value for that day based on the weather parameters (Wallin, 1962). Once an accumulation of 18 severity values occurred, a spray was triggered (Wallin, 1962). A study performed in 1993 by Raposo et al concluded that BLITECAST was not a better tool to use than a spray program with fixed intervals (Raposo et al., 1993).

Table 0.1. The BLITECAST DSV Chart that uses the average temperature during hours of relative humidity greater than 90%[1]

	Hours of Relative humidity (>90%)						
Average Temp (˚C)	0-9	10-12	13-15	16-18	19-21	22-24	25+
7-12	0	0	0	1	2	3	4
6-15	0	0	1	2	3	4	4
15-26	0	1	2	3	4	4	4
More than 26	No severity values accumulated						

[1]This table was adapted from Wallin (1962).

The forecasting model for tomato early blight, TOMCAST, is based on the duration of leaf wetness and the temperature during the leaf wetness duration in a 24 hour period (Pitblado, 1992). Early blight is caused by the fungus *Alternaria soloni* which thrives in warm (24-29˚C), humid conditions (Kemmitt, 2013). The model was developed by Pitblado in 1975; it is a simplified version of the FAST (Forecasting *Alternaria solani* on Tomatoes) forecasting model (Pitblado, 1992). TOMCAST assigns a DSV for the day based on whether the weather conditions

are favorable or unfavorable for the day, 0 being unfavorable and 4 being highly favorable (Pitblado, 1992). It is currently used as a tool to manage early blight and Septoria leaf spot (*Septoria lycopersici)*. Optimal conditions are 25˚C and 48 hours of leaf wetness for Septoria leaf spot (Meyer et al., 2000). Meyer et al. found in their studies that TOMCAST could reduce fungicide applications up to 60% without compromising asparagus fern health when compared to a weekly spray schedule (2000). It is also helpful for managing anthracnose incited by *Colletotrichum coccodes*; the optimal conditions are 12-35 ˚C and wet weather (Bolkan, 1994). *Alternaria dauci* and *Cercospora carotae* are two carrot pathogens that TOMCAST has also been successful in reducing the amount of fungicide applications in a field season (Dorman et al., 2009)

Table 0.2. The TOMCAST DSV chart that uses the average temperature during the hours of leaf wetness[1]

Mean Temp of day (˚C)	Hours of Leaf Wetness				
	0	1	2	3	4
13-17	0-6	7-15	16-20	21+	
18-20	0-3	4-8	9-15	16-22	23+
21-25	0-2	3-5	6-12	13-20	21+
26-29	0-3	4-8	9-15	16-22	23+

[1]This table is adapted from Pitblado (1992).

CHAPTER 1: FIELD EVALUATION OF FUNGICIDES FOR CONTROL OF *PSEUDOPERONOSPORA CUBENSIS* ON CUCUMBER

ABSTRACT

Pseudoperonospora cubensis, the causal agent of cucurbit downy mildew (CDM), is a destructive disease of cucumber resulting in blighting of the foliage and reduction of yield and fruit quality. In 2004, isolates of *P. cubensis* overcame the genetic resistance in pickling cucumber varieties leaving growers to rely on the intensive use of fungicide to protect their crop. *P. cubensis* is a high-risk pathogen for developing resistance to fungicides. Field studies were conducted in 2019 and 2020 to test 14 fungicides when applied weekly for their ability to limit CDM. In both years, oxathiapiprolin + chlorothalonil held disease to ≤1% foliar symptoms at the end of the monitoring periods. In 2019, cyazofamid was similar to oxathiapiprolin + chlorothalonil on the last monitoring date with CDM symptoms observed on 10% of the foliage. In 2020, treatment with oxathiapiprolin + chlorothalonil resulted in less disease than all other treatments. In 2019, treatments including ametoctradin + dimethomorph (premix), fluazinam, propamocarb and zoxamide + chlorothalonil (premix) limited disease to ≤ 20.25% and provided control similar to cyazofamid. Fluazinam was effective throughout both growing seasons with 12.5% symptomatic foliage on the last observations date. The newest fungicide, ethaboxam, was effective throughout the 2019 season with 22.5% symptomatic foliage on the last observation date. However, in 2020, plots treated with ethaboxam had less disease than the untreated control on the first two observations dates but were similar to the untreated plots on 23 and 30 August with 46.3% disease at the end of the trial. Results from trials comparing treatment programs consisting of tank mixes and alternating fungicides indicated that chlorothalonil alone or as part of a program with other fungicides that did not include oxathiopiprolin were the least effective in limiting CDM.

INTRODUCTION

Cucumbers (*Cucumis sativus* L.) are grown as a dual purpose crop used for fresh market (slicers) or processing (pickling). Michigan is the number one producer of processing cucumbers, with 40,000 acres of cucumbers, comprising 27% of the national market. Ranking second in processing cucumbers, Florida produces cucumbers on 23,000 acres (NASS, 2019). Growers of cucumbers must manage both foliar and fruit rot diseases (Zitter et al., 1996). Since 2004, cucurbit downy mildew (CDM) has re-emerged as the most economically important pathogen of cucumbers (Quesada-Ocampo et al., 2012). CDM is caused by *Pseudoperonospora cubensis* (Rostowzew, 1903, Berkeley & Curtis, 1868. Summarized in Colucci and Holmes, 2010). Forty plant species across 20 genera within the Cucurbitaceae family may serve as hosts for the pathogen (Palti & Cohen, 1980). *Pseudoperonospora cubensis* is a significant risk to the cucumber industry in Michigan (Cespedes-Sanchez et al., 2015). In 2004, when the pathogen re-emerged, cucumber growers in the southeast U.S. lost approximately 40% of their yield (Colucci et al., 2006). Cucurbit growers in Michigan spend an estimated $6 million annually on fungicides; fungicide resistant *P. cubensis* isolates have been detected (Granke et al., 2014).

On cucumber, infection by *P. cubensis* results in angular lesions defined by the leaf veins (Zitter et al., 1996). The chlorotic lesions appear on the adaxial side of the leaf in a mosaic pattern similar to angular leaf spot. The abaxial side of the leaf can appear purple or gray, indicating that sporangiophores have erupted through stomata and are producing sporangia (Babadoost, 2016). Signs and symptoms appear within 3 to 12 days following pathogen infection (Babadoost, 2016). The sporangia are released from the sporangiophores via a hygroscopic twisting motion (Lange et al., 1989) and are picked up by air currents and disseminated to nearby hosts (Savory et al., 2011, Lange et al., 1989). *P. cubensis* is polycyclic and has a short disease

cycle (Babadoost, 2016). Under saturated environmental conditions, the sporangium releases zoospores that may encyst on the leaf surface. Once encysted, the zoospore forms a germ tube penetrating the leaf tissue via a stomata (Iwata, 1949) and a haustorium forms within the cells.

Ojiambo and Kang (2013) theorized that *P. cubensis* sporangia can travel 1000 km each season to infect hosts across several states with green bridging. Other scientists have proposed that cucumber production greenhouses in the northern U.S. and Canada may provide overwintering sites for the pathogen (Naegele et al., 2014). Sporangia may also move via machines and human contact (Zitter et al., 1996). Resistance to *P. cubensis* was bred into cucumber cultivars in the U.S. during the 1950s (ref) and lasted until 2004; only minimal fungicide use was required (Holmes et al., 2015). The resistant cucumber line, PI 197087, was completely resistant to CDM as a result of the *dm1* gene. While this gene was naturally occurring in cucumber, it was recessive (Shimizu et al., 1963). The *P. cubensis* isolate resulting in the CDM outbreak in 2004 is presumed to be an introduction of a new isolate into the U.S.. The losses experienced during the 2004 and 2005 growing seasons include destruction of 80-100% of the cucumber crop in production regions in the eastern U.S. (2004) and Michigan (2005)(Thomas et al., 2017). Runge et al (2011) found clade 1 prior to 2004 whereas clade 2 was found after 2004. Call et al. (2013) studied the cultivars with the *dm1* gene versus cultivars without the resistant gene during 2008 to 2009 in North Carolina and Michigan. It was found that cultivar lines associated with PI 197087 had the highest disease resistance, but weekly applications of fungicide were needed to protect the yield. Since 2004, efforts to breed commercially acceptable cucumber cultivars with genetic resistance to *P. cubensis* has had only moderate success (Call et al., 2012). The objectives of this research is to evaluate single fungicide products for field efficacy because of the likelihood of resistance to the fungicide the

pathogen develops. The final objective includes evaluating the fungicide products in a program. The programs rotate active ingredients to reduce the likelihood of resistance.

MATERIALS AND METHODS

In 2019, two fungicide trials were established at the Michigan State University Plant Pathology Farm in Lansing, MI, in a field of Capac loam soil previously planted to cucumber. The first trial consisted of 14 treatments applied singly and included: untreated control, ametoctradin + dimethomorph (Zampro, 1.01 liter (L)/hectare (ha), BASF Cooperation, Research Triangle Park, NC), chlorothalonil (Bravo WeatherStik, 2.34 L/ha, Syngenta Crop Protection, Greensboro, NC), cyazofamid (Ranman, 0.20 L/ha, Summit Agro, Durham, NC), dimethomorph (Forum, 0.44 L/ha, BASF Cooperation, Research Triangle Park, NC), ethaboxam (Elumin, 0.60 L/ha, Valent USA, Walnut Creek, CA), fluazinam (Omega, 1.75 L/ha, Syngenta Crop Protection, Greensboro, NC), fluopicolide (Presidio, 0.30 L/ha, Valent USA, Walnut Creek, CA), mancozeb (Koverall, 3.36 kg/ha, FMC Cooperation, Philadelphia, PA), mancozeb + zoxamide (Gavel, 2.25 kg/ha, Gowan Company, Yuma, AZ), oxathiapiprolin + chlorothalonil (Orondis Opti, 0.35 L/ha, Syngenta Crop Protection, Greensboro, NC), propamocarb (Previcur Flex, 0.10 L/ha, Bayer Crop Science, Research Triangle Park, NC), pyraclostrobin (Cabrio, 0.45 kg, BASF Cooperation, Research Triangle Park, NC), zoxamide + chlorothalonil (Zing!, 2.64 L, Gowan Company, Yuma, AZ) (Table 1.1).

The second trial consisted of fungicide applied in an overall program with other fungicides and had nine treatments including: 1) untreated control, 2) chlorothalonil, 3) dimethomorph + ametoctradin (premix) + chlorothalonil alternated with cyazofamid + chlorothalonil, 4) chlorothalonil alternated with cyazofamid + chlorothalonil alternated with

ethaboxam + chlorothalonil alternated with oxathiapiprolin + chlorothalonil (premix) alternated with dimethomorph + ametoctradin (premix) + chlorothalonil, 5) cyazofamid + chlorothalonil alternated with oxathiapiprolin + chlorothalonil alternated with dimethomorph + ametoctradin (premix) + chlorothalonil, 6) oxathiapiprolin + chlorothalonil (premix) alternated with ethaboxam + chlorothalonil alternated with cyazofamid + chlorothalonil alternated with dimethomorph + ametoctradin (premix) + chlorothalonil, 7) oxathiapiprolin + chlorothalonil (premix) alternated with ethaboxam + chlorothalonil alternated with cyazofamid + chlorothalonil alternated with dimethomorp + ametoctradin (premix) + chlorothalonil on a 10-day spray schedule, 8) cyazofamid + chlorothalonil alternated with propamocarb + chlorothalonil alternated with oxathiapiprolin + chlorothalonil (premix) alternated with dimethomorph + ametoctradin (premix) + chlorothalonil, 9) cyazofamid + chlorothalonil alternated with ethaboxam + chlorothalonil alternated with oxathiapiprolin + chlorothalonil (premix) alternated with dimethomorph + ametoctradin (premix) + chlorothalonil (Table 1.4).

Each field was prepared by plowing and disking on 21 June and disking on 7 July for weed management and seed bed preperation; urea preplant fertilizer (112 kg/ha) was also applied on 7 July. Raised beds were formed, black plastic was laid, and drip tape was established for irrigation on 8 July. Weeds were controlled with an application of ethalfuralin (Curbit 3.17 L/ha, Loveland Products, Inc, Greenly, CO), clomazone (Command 3ME 1.16 L/ha, FMC Corporation, Philadelphia, PA), and S-metolachlor (Dual II Magnum 0.94 L/ha, Syngenta Crop Protection, Greensboro, NC) on 17 July prior to planting. Cucumber ‘Vlaspik’ seeds were sown on 29 July and spaced 30.5 cm. apart in rows that were spaced on 1.7 m centers. Treatments were arranged in a completely randomized block design with four replicates. Each treatment replicate consisted of a single 6.1 m row plot with a 0.9 m buffer between treatments within the row. The

trial was fertilized throughout the growing season with weekly applications of 20-20-20 via drip tape at 5.6 kg/ha. Four weekly spray treatments were applied on 22 and 29 August; 5 and 12 September using a CO_2 backpack sprayer and a broadcast boom equipped with three XR8003 flat-fan nozzles spaced 45.7 cm. apart, calibrated at 50 psi and delivering 467.6 L/ha. Foliage was evaluated for CDM symptoms on 6, 12, 19, and 27 September.

In 2020, the two trials previously described were established at the Kenny Brothers' Farm in Merrill, MI, in a field of Parkhill loam soil previously planted to sugar beet. Fertilizer and insecticide were applied at planting on 17 July. Fertilizer was applied 5.1cm deep and 5.1 cm away from the seed bed. The fertilizer applied was Urea ammonium nitrate (28-0-0; 68.3 L/ha), ammonium thiosulfate (28.06 L/ha), and ammonium polyphosphate (10-34-0; 46.7 L/ha), and the insecticide was bifenthrin (Sniper 0.30 L/ha, Loveland Products, Inc, Greenly, CO). The field was prepared by plowing and disking on 17 July. Weeds were controlled with an application of Curbit EC (3.17 L/ha), Command 360 ME (1.16 L/ha) on 17 July prior to emergence. Cucumber 'Vlaspik' seed were sown on 17 July and spaced 10.2 cm. apart in rows that were spaced on 50.8 cm centers. Treatments were arranged in a completely randomized block design with four replicates. Each treatment replicate consisted of a single 6.1 m row plot with a 1.5 m buffer between treatments within the row. Three weekly spray treatments were applied on 10, 17, and 24 August using a CO_2 backpack sprayer and a broadcast boom equipped with one XR8003 flat-fan nozzle calibrated at 35 psi and delivering 467.6 L/ha. Foliage was evaluated for symptoms of CDM on 12, 17, 23 and 30 August. The data that was collected was a visual foliar rating on a scale of 0-100% symptomatic foliage.

The data were analyzed for the area under the disease progress curve (AUDPC) and repeated measures for both years using SAS (SAS Institute, Cary, NC). AUDPC was calculated

by using the equation AUDPC = Σ [((Yi + Yi-1) (Xi – Xi-1))/2] where Y_i= foliar necrosis and X_i= time (Shaner & Finney, 1977). An ANOVA F-Test was calculated to determine if the treatments had significant differences. The foliar assessments were repeated measures over time. The analysis of repeated measures shows the fixed factors as treatments, and the random factor was replications. Treatments were separated using Fischer's least significant differences ($p< 0.05$). Treatments were sliced to determine the mean separation of letters.

RESULTS

Single product trial: Disease progressed in the untreated control with >88% and >50% of the foliage in the plot with CDM symptoms at the end of monitoring period for 2019 and 2020, respectively (Tables 1.2 and 1.3). In both years, oxathiapiprolin + chlorothalonil held disease to ≤1% foliar symptoms at the end of the monitoring periods. In 2019, cyazofamid was similar to oxathiapiprolin + chlorothalonil on the last monitoring date (27 September) with CDM symptoms observed on 10% of the foliage (Figure 1). In 2020, treatment with oxathiapiprolin + chlorothalonil resulted in less disease than all other treatments (Figure 2). In 2019, treatments including ametoctradin + dimethomorph (premix), fluazinam, propamocarb and zoxamide + chlorothalonil (premix) limited disease to ≤ 20.25% and provided control similar to cyazofamid. Fluazinam was effective throughout both growing seasons with 12.5% symptomatic foliage on the last observations date. The newest fungicide, ethaboxam, was effective throughout the 2019 season with 22.5% symptomatic foliage on the last observation date. However, in 2020, plots treated with ethaboxam had less disease than the untreated control on the first two observations dates but were similar to the untreated plots on 23 and 30 August with 46.3% disease at the end of the trial.

Applications of the two protectant fungicides of mancozeb and chlorothalonil were similar through the monitoring period in 2019 for most of the assessment dates; they were similar in 2020 for all assessment dates. When the products differed in 2019, mancozeb was more effective that chlorothalonil. Mancozeb was similar to mancozeb + zoxamide in both years with only one exception (12 September 2019). In 2019, treatments of chlorothalonil were less effective than zoxamide + chlorothalonil for observations from 10 to 27 September; there were no differences between these treatments in 2020. Disease incidence in the plots treated with dimethomorph or pyraclostrobin was >88% (2019) and ≥47.5% (2020) on the last assessment date. In 2019, the fungicide treatments of dimethomorph or pyraclostrobin were similar to the untreated control for each evaluation date throughout the monitoring period; fluopicolide was similar to the untreated control from 6 to 16 September. In 2020, the treatments of pyraclostrobin, fluopicolide, and dimethomorph were similar to the untreated control throughout the rating period with one exception.

According to the AUDPC data, oxathiopiprolin + chlorothalonil had the least amount of disease in both years; propamocarb was similar to oxathiapiprolin + chlorothalonil in 2019 but not in 2020. While plots treated with cyazofamid were similar to those treated with propamocarb according to the 2019 AUDPC data, they had more disease than the plots treated with oxathiapiprolin + chlorothalonil. Plots treated with chlorothalonil, mancozeb, mancozeb + zoxamide, ethaboxam, were similar according to AUDPC data and less effective than cyazofamid. In 2020, only ethaboxam was less effective than cyazofamid according to AUDPC data. In 2019, the AUDPC data indicated that pyraclostrobin and dimethomorph treatments were similar to the untreated control whereas in 2020 plots treated with dimethomorph, ethaboxam, fluopicolide, or pyraclostrobin were similar to the untreated control.

Program trial: For both years, all fungicide treatment programs had less disease at the final observation date than the untreated control (≥45%) and according to the AUDPC data (Tables 1.5 and 1.6). While treatment with chlorothalonil was better than the untreated control at the final observation date and according to AUDPC data, all other fungicide treatment programs were more effective. In 2020, the program that included oxathiapiprolin + chlorothanil as the first spray with subsequent sprays at 7-day intervals was more effective than most other treatment programs included in the study. According to the AUDPC data, the alternating treatment program that excluded oxathiapiprolin + chlorothalonil was among the least effective fungicide programs in each year although it performed better than chlorothalonil alone. In 2019, all fungicide programs with the exception of chlorothalonil were similar for foliar symptoms at the last rating date. According to the 2019 AUDPC data, the programs that were initiated with applications of cyazofamid + chlorothalonil were more effective than all others with the exception of the treatment program initiated with an application of oxathiopiprolin + chlorothalonil (7-day spray program).

DISCUSSION

The goal of this study was met by the evaluation of single fungicide products and products in a program. In the previous years of this study oxathiopiprolin + chlorothalonil did not perform in the same way it did in this study (Goldenhar et al., 2018). We believe that the inoculum source in these years were highly virulent, and the sources in which we received our sources in the years of our study were not. In 2019, we believe the source of the inoculum came from the southern US because of the effectiveness of propamocarb. In 2020, we believe the

inoculum source was from the southern US at first, but then changed to a source where propamocarb is not an effective fungicide.

Fungicide recommendations are made yearly to Michigan growers of pickling cucumbers based on replicated field trials. Since the reemergence of the pathogen in the state in 2005, some fungicides have become ineffective as the pathogen developed resistance to them. To test the efficacy of specific fungicides to control CDM, products were tested alone and as part of an overall treatment regime including tank mixes that were alternated. The field trials were replicated over two years (2019, 2020). In 2019, the trials were located at the research plant pathology farm and disease assessment occurred over 21 days. In 2020, the trial was established at a grower cooperator's farm and was subjected to all commercial practices; disease assessment occurred over an 18-day period. Cultural growing practices differed between the two years and locations resulting in visibly different growth of the cucumber plants. For instance, the 2019 field plot at the MSU research farm was frequently irrigated and fertigated whereas the 2020 field plot established with the grower cooperator relied on rainfall. As a result, the 2019 field plot exhibited lush growth that formed a canopy which may have contributed to the overall higher disease pressure observed that year. It's also possible that an extended observation period in 2020 would have revealed an increase in disease.

Despite the differences in disease pressure observed across the two years, the results for many of the fungicides tested as single products were consistent and support the recommendations that have been made to growers. Most notably, these recommendations include alternating among the following fungicides: oxathiapiprolin + chlorothalonil, cyazofamid, ametoctradin + dimethomorph, and fluazinam and tank mixing single active ingredient fungicides with either chlorothalonil or mancozeb. Conversely, excluding the fungicides

fluopicolide, dimethomorph, and pyraclostrobin from fungicide recommendations for Michigan cucumber growers is warranted based on the results of this study. Ethaboxam is a relatively new fungicide labeled for use against CDM. The results from each year indicated that while ethaboxam was effective in 2019, it did not provide adequate protection in 2020. Similarly, propamocarb effectively limited disease in 2019 but not in 2020. This discrepancy between years could be due to a change in the pathogen population. Fluctuation in efficacy for propamocarb has been noted previously (Goldenhar et al., 2018). In addition to the change of the field sites from 2019 to 2020, there were fewer applications of fungicides made for the study that included alternating fungicides.

In the absence of genetic resistance in pickling cucumber cultivars, effective fungicides are crucial (Savory, et al. 2010). While many fungicides are labeled for control of CDM, the pathogen is resistant to mefenoxam, the strobilurins, fluopicolide, and propamocarb (Keinath et al., 2019, Holmes et al., 2015). Resistance to fungicides with a single mode of action can develop quickly in *P. cubensis* populations (Miao et al., 2018) leading to reduced efficacy of many fungicides (McGrath, 2001). The single-site inhibitor fungicides include the phenylamides, quinone outside inhibitors (QoI), and carboxylic acid amides (CAA). Multi-site inhibitors include: dithiocarbamates, phthalimides, chlorothalonil, copper and sulfur formations, cymoxanil, fosetyl-Al, fluazinam, quinone insides inhibitors (QiI) and quinone outside Stigmatellin inhibitor (QoSI) (Holmes et al., 2015, Niks).

The phenylamides including mefenoxam and metalaxyl inhibit ribosomal RNA synthesis in oomycetes (Fisher & Hayes, 1982) and have long-lasting preventive activity, high mobility, and curative potential. Two years after the introduction of the PAs, resistance was detected in *P. cubensis* in Israel (Reuveni et al., 1980). The Fungicide Resistance Action Committee (FRAC)

recommends that it be used no more than twice during a growing season, and that it be applied preventively prior to disease detection (Cohen et al., 2015). Neither mefenoxam or metalaxyl are used to control CDM in Michigan as these fungicides proved to be ineffective when the pathogen reemerged in the state in 2005 (Holmes et al., 2015) and were not included in this study.

Quinone outside inhibitors obstructs mitochondrial respiration by interrupting the electron transport in cytochrome b; the fungicide that is still used in cucumber with this mode of action is pyraclostrobin. This is accomplished by the binding to the Qo site on the outer side of the mitochondria (Ishii et al., 2007, Gisi & Lebeda, 2002). As of 2014, it was no longer recommended by FRAC to use QoI mode of action fungicides as a singular product (Cohen et al., 2015). Quinone outside inhibitor, stigmatellin binding type is a mode of action that affects the cytochrome bc1 complex and is not cross resistant with quinone inside inhibitors like Ranman, or QoIs. The difference between a QoI and QoSI is the attachment location. QoSI attaches to the mitochondria at the stigmatellin binding site; ametoctradin has this mode of action (Gisi & Lebeda, 2002).

Recently, oxathiapiprolin has been changed from FRAC code group U15 to group 49. This chemical group is known as the oxysterol binding protein homologue inhibition (OSBPI). Oxathiapiprolin is a systemic fungicide that protects the plant as it grows (Pasteris et al., 2016).

CAA fungicides are in the FRAC code group 40, and interfere with cell wall deposition and cellulous biosynthesis (Gisi et al., 2019). The CAA fungicide, dimethomorph, was developed in the 1980s and is registered for use on *P. cubensis*. This fungicides limits the germination of the cystospores, sporangia, and growth of the germ tubes and mycelium (Gisi et al., 2019).

In both years, fungicide programs that included oxathiapiprolin + chlorothalonil were more effective in limiting CDM than those programs that did not.

Table 1.1. A collective list of fungicides used on all trials

Active Ingredient	Product	Registrant[w]	FRAC[x]		Rate/ha	PHI (days)
			Chemical Group[y]	Code[z]		
Ametoctradin + Dimethomorph	Zampro SC	BASF	QoSI/CAA	45/40	1.0 L	0
Chlorothalonil	Bravo WeatherStik SC	Syngenta	M	M5	2.3 L	0
Cyazofamid	Ranman SC	Summit Agro	Qil	21	0.2 L	0
Dimethomorph	Forum SC	BASF	CAA	40	0.4 L	0
Ethaboxam	Elumin SC	Valent	Thiazole carboxamide	22	0.6 L	2
Fluazinam	Omega SC	Syngenta	Phenyl-pyridinamine	29	1.8 L	30
Fluopicolide	Presidio SC	Valent	Benzamide	43	0.3 L	2
Mancozeb	Koverall DG	FMC	M	M3	3.4 kg	5
Mancozeb + Zoxamide	Gavel DF	Gowan	M/Benzamide	M3/22	2.3 kg	5
Oxathiapiprolin + Chlorothalonil	Orondis Opti SC	Syngenta	OSBPI/M	49/M5	0.4 L	0
Propamocarb	Previcur Flex SL	Bayer	Carbamate	28	0.1 L	2
Pyraclostrobin	Cabrio EG	BASF	QoI	11	0.5 kg	0
Zoxamide + Chlorothalonil	Zing! SC	Gowan	Benzamide/M	22/M5	2.6 L	0

[w]FMC Corporation (Philadelphia, PA), BASF Cooperation (Research Triangle Park, NC), Valent USA (Walnut Creek, CA), Syngenta Crop Protection (Greensboro, NC), Bayer Crop Science (Research Triangle Park, NC), Gowan Company (Yuma, AZ), Summit Agro USA (Durham, NC).

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

[y]CAA= carboxylic amino acid; M= multisite inhibitor; OSBPI= oxysterol-binding protein homolog inhibitor; Qil= quinone inside inhibitor; OoI= quinone outside inhibitor; QoSI= quinone outside inhibitor, stigmatellin binding type.

[z]Codes given to fungicides based on their activity on pathogens

Table 1.2. Singular fungicide treatments rating for foliar infection symptoms on 'Vlaspik' cucumbers from 0-100% applied every 7 days located on the MSU Plant Pathology Farm in 2019

	2019						
	Foliar Infection (%)						AUDPC
Treatment	6-Sep	10-Sep	12-Sep	16-Sep	19-Sep	27-Sep	
Untreated	7.5 e	12.5 c	48.8 d	37.5 e	48.8 h	88.8 g	973.8 h
Oxathiapiprolin/Chlorothalonil	0.0 a	0.0 a	0.0 a	0.2 a	0.0 a	0.0 a	4.9 a
Propamocarb	0.0 a	0.0 a	0.3 a	0.0 a	0.0 a	20.3 b-d	85.4 ab
Cyazofamid	0.3 a	0.8 ab	7.0 ab	6.5 bc	10.5 b	10.0 ab	147.6 bc
Fluazinam	0.8 ab	0.0 a	8.8 ab	11.3 bc	15.0 c	12.5 bc	203.6 cd
Ametoctradin/Dimethomorph	0.8 ab	1.8 ab	12.0 b	8.5 bc	12.5 bc	16.3 bc	211.9 cd
Ethaboxam	1.0 ab	1.0 ab	10.0 ab	13.8 c	13.0 bc	22.5 c-e	249.1 d
Zoxamide/Chlorothalonil	2.0 a-c	4.8 b	12.5 b	13.8 c	16.3 cd	16.3 bc	263.5 d
Mancozeb/Zoxamide	1.5 a-c	4.0 b	13.8 b	16.3 c	20.0 de	28.8 de	344.3 e
Mancozeb	3.5 b-d	2.3 ab	26.3 c	16.3 c	22.5 ef	32.5 e	414.5 ef
Chlorothalonil	4.0 cd	9.5 c	26.3 c	23.8 d	25.0 f	31.3 e	472.0 f
Fluopicolide	7.3 e	14.0 c	47.5 d	42.5 e	42.5 g	62.5 f	851.6 g
Dimethomorph	6.3 de	11.8 c	50.0 d	42.5 e	50.0 h	88.8 g	998.4 h
Pyraclostrobin	8.8 e	10.0 c	53.8 d	42.5 e	48.8 h	88.8 g	1003.1 h

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

[y]Based on a visual estimation of the percentage of foliage infected.

Table 1.3. Singular fungicide treatments rating for foliar infection symptoms on 'Vlaspik' cucumbers from 0-100% applied every 7 days located on a grower cooperator commercial field in 2020

	2020[y]				
	Foliar Infection (%)[z]				AUDPC
Treatment	12-Aug	17-Aug	23-Aug	30-Aug	
Untreated	7.0 c	25.0 g	47.5 e	51.3 e	643.1 d
Oxathiapiprolin/Chlorothalonil	0.5 a	0.5 a	0.0 a	1.0 a	7.5 a
Fluazinam	5.5 c	6.8 cd	11.0 b	12.5 b	166.1 b
Mancozeb/Zoxamide	7.0 c	8.0 de	10.0 b	13.8 bc	174.6 b
Ametoctradin/Dimethomorph	7.0 c	9.8 de	11.8 b	15.0 bc	200.0 b
Cyazofamid	5.0 bc	3.0 c	7.0 b	11.3 b	202.8 b
Mancozeb	7.0 c	7.8 d	15.0 bc	15.0 bc	210.1 b
Chlorothalonil	7.0 c	8.3 de	15.0 bc	16.3 bc	217.3 b
Zoxamide/Chlorothalonil	6.5 c	8.8 de	15.0 bc	16.3 bc	218.8 b
Propamocarb	1.5 b	6.8 cd	37.5 cd	45.0 d	353.3 c
Ethaboxam	0.3 a	1.5 b	33.8 de	46.3 de	391.4 c
pyraclostrobin	6.0 c	13.0 ef	47.5 e	50.0 e	570.3 d
Fluopicolide	7.0 c	20.0 fg	47.5 e	50.0 e	611.3 d
Dimethomorph	7.0 c	22.5 g	47.5 e	47.5 de	616.3 d

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

[z]Based on a visual estimation of the percentage of foliage infected.

Table 1.4. A list of the fungicide programs used for the program trials at the MSU Plant Pathology Farm in 2019, and the grower cooperator commercial field in 2020

	Applications		Last Fungicides applied	
Treatments	**2019**	**2020**	**2019**	**2020**
Untreated				
Program 1: Chlorothalonil	**4**	**3**	Chlorothalonil	Chlorothalonil
Program 2: Dimethomorph + Ametoctradin + Chlorothalonil *alt* Cyazofamid + Chlorothalonil	**4**	**3**	Cyazofamid + Chlorothalonil	Dimethomorph + Ametoctradin + Chlorothalonil
Program 3: Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**4**	**3**	Dimethomorph + Ametoctradin + Chlorothalonil	Oxathiapiprolin + Chlorothalonil
Program 4: Cyazofamid + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**4**	**3**	Cyazofamid + Chlorothalonil	Dimethomorph + Ametoctradin + Chlorothalonil
Program 5: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**4**	**3**	Dimethomorph + Ametoctradin + Chlorothalonil	Cyazofamid + Chlorothalonil
Program 6[z]: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**3**	**2**	Cyazofamid + Chlorothalonil	Ethaboxam + Chlorothalonil
Program 7: Cyazofamid + Chlorothalonil *alt* Propamocarb + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**4**	**3**	Dimethomorph + Ametoctradin + Chlorothalonil	Oxathiapiprolin + Chlorothalonil
Program 8: Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	**4**	**3**	Dimethomorph + Ametoctradin + Chlorothalonil	Oxathiapiprolin + Chlorothalonil

[z]This program is on a 10-day spray schedule to compare with 7-day spray schedules

Table 1.5. Foliar infection ratings from 0-100% on 'Vlaspik' cucumbers on the MSU Plant Pathology Farm in 2019

Treatments applied at 7-day intervals except where indicated	2019[y]					
	Foliar Infection (%)[z]					AUDPC
	6-Sep	9-Sep	12-Sep	16-Sep	19-Sep	
Untreated control	6.3 c	6.0 c	57.5 d	46.3 c	45.0 c	458.0 e
Program 7: Cyazofamid + Chlorothalonil *alt* Propamocarb + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.3 a	0.0 a	0.0 a	0.0 a	0.0 a	0.4 a
Program 4: Cyazofamid + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.0 a	0.0 a	0.0 a	0.0 a	0.5 a	0.8 a
Program 8: Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.0 a	0.5 ab	0.0 a	0.3 a	0.0 a	2.4 ab
Program 5: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.0 a	0.0 a	2.0 ab	2.0 a	4.0 a	20.0 b
Program 6[z]: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.0 a	0.0 a	3.0 ab	1.3 a	4.0 a	20.9 b
Program 3: Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	0.5 a	2.0 b	4.0 ab	3.0 a	5.5 a	39.5 c
Program 2: Dimethomorph + Ametoctradin + Chlorothalonil *alt* Cyazofamid + Chlorothalonil	0.5 a	1.8 ab	7.8 b	4.8 a	8.8 a	62.9 c
Program 1: Chlorothalonil	3.0 b	4.3 c	21.8 c	14.5 b	26.3 b	183.5 d

[x]Program 6 is the same program as 5, but is on a 10-day spray schedule

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

[z]Based on a visual estimation of the percentage of foliage infected.

Table 1.6. Foliar infection ratings from 0-100% on 'Vlaspik' cucumbers in a grower cooperator commercial field in 2020

Treatment applied at 7-day intervals except where indicated	2020[y]				
	Foliar Infection (%)[z]				AUDPC
	12-Aug	17-Aug	23-Aug	30-Aug	
Untreated control	7.0 a	25.0 b	50.0 f	48.8 f	650.6 e
Program 5: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	21.3 ab	10.0 a	7.0 a	249.0 a
Program 4: Cyazofamid + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	18.8 a	20.0 bc	12.5 b	294.4 ab
Program 8: Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	23.8 ab	17.5 c	11.3 ab	301.3 ab
Program 7: Cyazofamid + Chlorothalonil *alt* Propamocarb + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	23.8 ab	18.8 c	12.5 b	313.8 b
Program 3: Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Oxathiapiprolin + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	22.5 ab	17.5 c	17.5 c	316.3 b
Program 6[x]: Oxathiapiprolin + Chlorothalonil *alt* Ethaboxam + Chlorothalonil *alt* Cyazofamid + Chlorothalonil *alt* Dimethomorph + Ametoctradin + Chlorothalonil	7.0 a	20.0 ab	23.8 b	15.0 bc	334.4 b
Program 2: Dimethomorph + Ametoctradin + Chlorothalonil *alt* Cyazofamid + Chlorothalonil	7.0 a	25.0 b	32.5 d	23.8 d	449.4 c
Program 1: Chlorothalonil	7.0 a	22.5 ab	45.0 e	33.8 e	551.9 d

[x]Program 6 is on a 10-day spray schedule

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

[z]Based on a visual estimation of the percentage of foliage infected.

CHAPTER 2: COMPARING FUNGICIDE APPLICATION INTERVALS TO MANAGE DOWNY MILDEW ON CUCUMBER

ABSTRACT

Cucurbit downy mildew (CDM), caused by *P. cubensis*, is currently managed through the intensive use of fungicides. Cucumbers are especially susceptible to CDM. The cost of control and the development of pathogen resistance to key fungicides necessitates the advancement of strategies including the timing of sprays. The objective of this study was to compare fungicide application intervals to limit CDM in pickling cucumbers. A field trial was conducted at the Michigan State University Plant Pathology farm (2019) and replicated in a commercial field located in Merrill, MI (2020). The trial had five treatments with different intervals replicated four times in a RCBD. The fungicide program used for all treatments was cyazofamid + chlorothalonil alternated with oxathiapiprolin premixed with chlorothalonil alternated with ametoctradin premixed with dimethomorph + chlorothalonil. The fungicide treatment program was applied according to the following intervals: 1) untreated control, 2) 5 day, 3) 7 day, 4) TOMCAST disease severity value 15 (DSV) , and 5) BLITECAST severity value 18. The treatments in 2019 held disease to $\leq$ 47.5% while the untreated was at 88.8%. BLITECAST on the last rating date had the lowest percentage (28.8%), but the 5-day calendar was lower over the course of the season making the AUDPC lower at 182.5 where BLITECAST was at 346.1 at the end of the season. In 2020, the 7-day, 5-day, and TOMCAST kept disease $\leq$ 48.8%, BLITECAST kept disease to 77.5%, and the untreated rose up to 97.3% diseased at the end of the season.

INTRODUCTION

Cucumbers (*Cucumis sativus* L.) are a high-value vegetable crop in the U.S. They are grown as a dual purpose crop used for fresh market (slicers) or processing (pickling). Michigan

is the number one producer of processing cucumbers, contributing $50 million to the state's economy; cucumbers for the fresh market contribute $15 million (MDARD, 2018). In 2019, Michigan grew 40,000 acres of cucumbers, comprising 27% of the national market. Florida produces cucumbers on 23,000 acres (NASS, 2019).

Since 2004, downy mildew caused by *Pseudoperonospora cubensis* has re-emerged as the most economically important pathogen of cucumbers (Quesada-Ocampo et al., 2012) and is the most destructive disease of cucurbitaceous crops worldwide (Ojiambo et al., 2010). The Cucurbitaceae family has 118 genera with 825 species; 20 genera and more than 50 species are downy mildew hosts (Thomas et al., 2017). On cucumber, *P. cubensis* forms angular lesions defined by the leaf veins (Zitter et al., 1996). The chlorotic lesions appear on the adaxial side of the leaf in a mosaic pattern. The abaxial side of the leaf can appear purple or gray, indicating that sporangiophores have erupted through stomata and are producing sporangia (Babadoost, 2016). The sporangia are released from the sporangiophores via a hygroscopic twisting motion (Lange et al., 1989), and are picked up by air currents and disseminated to nearby hosts (Savory et al., 2011, Lange et al., 1989). Under high humidity environmental conditions, the sporangium releases zoospores that may encyst on the leaf surface. Once encysted, the zoospore forms a germ tube penetrating the leaf tissue via a stomata (Iwata, 1949) and a haustorium forms within the cells. *P. cubensis* is polycyclic and has a short disease cycle with signs and symptoms appearing within 3-12 days following infection (Babadoost, 2016).

P. cubensis is especially virulent on cucumber varieties used for pickling (Cespedes-Sanchez et al., 2015). Thomas et al. (1987) identified six physiological races of *P. cubensis* pathogenic to cucurbits. More recently, Quesada-Ocampo et al. (2012) determined that *P. cubensis* isolates differed based on host and location. Runge et al. (2011) discovered that there

was a monophyletic group with two lineages. The first lineage showed two clades (1 and 2) of *P. cubensis*, and the second lineage comprising the sister species, *P. humuli*. Summers et al. (2015) separated the pathogen into two groups including squash, pumpkin, and watermelon (group 1) and cucumber and cantaloupe (group 2). Wallace et al. (2020) found that wild type calabash (*Lagenaria siceraria*) was infected by clade 2 similar to cucumber and cantaloupe. The wild type bitter melon (*Momordica charantia*) was primarily infected by clade 1 along with squash, watermelon, and pumpkin. Buffalo gourd (*Cucurbita foetidissima*) was infected similarly by both clades (Wallace et al. (2020). In North Carolina, the population of each clade fluctuates according to season and location (Wallace et al., 2020). In Michigan, predominately clade 2 has been detected (Rodriguez, 2020).

In the 1950s, cucumber cultivars in the U.S. were bred to be resistant to downy mildew and this genetic resistance lasted until 2004 (Holmes et al., 2015). Resistance was conferred by the PI 197087 cucumber line; the *dm1* gene provided complete resistance (Shimizu et al., 1963). While the gene was naturally occurring in cucumber, it was recessive (Shimizu et al., 1963). It is believed that a new pathogenic *P. cubensis* isolate was introduced into the U.S. resulting in a CDM outbreak during the 2004 and 2005 growing seasons. Approximately 80-100% of the cucumber crop was destroyed in production regions along the southeast coast including Florida, Georgia, North and South Carolina (2004) and Michigan (2005) (Thomas et al., 2017). Runge et al. (2011) found clade 1 prior to 2004; clade 2 was detected after 2004. Since 2004, efforts to breed commercially acceptable cucumber cultivars with genetic resistance to *P. cubensis* has had only moderate success. Call et al. (2013) studied the cultivars with and without the *dm1* gene during 2008 to 2009 in North Carolina and Michigan. Cultivar lines associated with PI 197087 had the highest disease resistance but weekly fungicide sprays were needed to protect the yield.

In the absence of genetic resistance, fungicides effective against CDM are crucial (Savory, et al. 2010). While several fungicides are labeled for control of *P. cubensis*, the pathogen is resistant to mefenoxam, the strobilurins, fluopicolide, and propamocarb (Keinath et al., 2019, Holmes et al., 2015). Resistance to fungicides with a single mode of action (MOA) can develop quickly in *P. cubensis* populations (Miao et al., 2018) leading to reduced efficacy (McGrath, 2001). Fungicides recommended for CDM may negatively affect honey bee populations (Christen et al., 2019). Chlorothalonil was significantly more harmful than other fungicides tested (Christen et al., 2019) and is considered a staple of CDM management programs in Michigan.

Timing fungicides according to a forecaster could help determine spray intervals (Holmes et al., 2015). Currently, growers apply fungicide sprays every 5-14 days depending on pathogen pressure and general weather conditions. The forecasting model for early blight (*Alternaria solani*) on tomato, TOMCAST, is based on the duration of leaf wetness and the temperature during the leaf wetness period (Pitblado, 1992) and is a simplified version of the FAST forecasting model (Pitblado, 1992). The TOMCAST model assigns a disease severity value (DSV) of 0 (unfavorable) to 4 (highly favorable) for each 24-hr period (Pitblado, 1992). The forecaster, BLITECAST, was developed for *Phytophthora infestans*, the potato late blight pathogen (Krause, et al., 1975) and uses the mean temperatures during periods of relative humidity greater than 90%, to determine a severity value for each day. A spray is triggered after an accumulation of 18 disease severity values.

The objective of this study was to determine the efficacy of a fungicide program for CDM on pickling cucumbers when applied according to intervals of 5 or 7 days, or applied according to TOMCAST DSV 15 or BLITECAST DSV 18.

MATERIALS AND METHODS

In 2019, the trial was established at the Michigan State University Plant Pathology Farm in Lansing, MI, in Capac loam soil previously planted to cucumbers. The field was plowed and disced on 21 June. Fertilizer (urea 112 kg/ha) was incorporated on 7 July. On 8 July, raised plant beds were formed, spaced 1.7 meters apart and covered with black plastic mulch. Drip tape was used for irrigation and fertilization. Preemergence herbicides were applied on 17 July and included ethalfuralin (Curbit 3.17 L/ha, Loveland Products, Inc, Greenly, CO), clomazone (Command 3ME 1.16 L/ha, FMC Corporation, Philadelphia, PA) and S-metolachlor (Dual II Magnum 0.94 L/ha, Syngenta Crop Protection, Greensboro, NC). 'Vlaspik' seed was planted on 29 July. The treatments were arranged in a completely randomized block design with four replications. Each treatment replicate was 6.1 meters with a 0.9 meter buffer between treatments within the row. The trial was fertilized weekly with 20-20-20 (5.6 kg/ha) applied through the drip tape. The fungicide program used was cyazofamid (Ranman 0.50 L/ha, Summit Agro, Durham, NC) + chlorothalonil (Bravo WeatherStik 5.77 L/ha, ADAMA, Raleigh, NC) alternated with oxathiapiprolin + chlorothalonil (Orondis Opti 7.22 L/ha, Syngenta Crop Protection, Greensboro, NC) alternated with ametoctradin + dimethomorph (Zampro 2.52 L/ha, BASF Corporation, Research Triangle Park, NC) + chlorothalonil. Each fungicide was applied at a rate of 467.6 L/ha with a CO_2 backpack sprayer and a three nozzle broadcast boom calibrated to 50 PSI. The nozzles used were XR8003 flat-fans spaced at 45.7 cm apart.

The fungicides were applied according to intervals of 5 or 7 days, or applied according to TOMCAST DSV 15 or BLITECAST DSV 18. The TOMCAST and BLITECAST forecasting models used SpecWare 9 Pro Software; weather data was collected by a Watchdog Micro-station (Spectrum Technologies, Aurora, Illinois). The TOMCAST program called for fungicide

applications on 22 August, 11 September, and 1 October. The BLITECAST program prompted fungicide applications on 22 August, and 4, 16, 23 September. The 5-day program had eight applications from 22 August to 1 October, and the 7-day program had six applications from 22 August to 26 September. Due to weather conditions, calendar-based sprays in 2019 were applied every 5-7 (5-day program) or 7-10 days (7-day program). Fruit were harvested on 20, 23, 26 September and 4 October from the middle 10 feet of each treatment replicate when fruit were approximately 31.75 millimeters (mm) in diameter but no larger than 50.8 mm.

In 2020, the trial was established with a grower cooperator in Merrill, MI, in a field of Parkhill loam soil previously planted to sugar beet. The field was prepared by plowing and disking. Fertilizer and insecticide were applied at planting on 17 July. Fertilizer included 28% urea ammonium nitrate (168.3 L/ha), thiosulfate (28.06 L/ha), and 10-34-0 (46.76 L/ha) and the insecticide was bifenthrin (Sniper 0.30 L/ha, Loveland Products, Inc, Greenly, CO). Weeds were controlled with ethalfuralin (Curbit 3.17 L/ha, Loveland Products, Inc, Greenly, CO) and clomazone (Command 3ME 1.16 L/ha, FMC Corporation, Philadelphia, PA) applied on 17 July prior to emergence. 'Vlaspik' seed was sown, spaced 101.6 mm apart in rows that were spaced on 508 mm centers. Treatments were arranged in a completely randomized block design with four replicates. Each treatment replicate consisted of a single 6.1 m row plot with a 1.5 m buffer between treatments within the row. Spray treatments were initiated on 31 July. The 5-day program included sprays on 31 July, 3, 10, 14, 19, 24, 27, 31August, and 5 September. The 7-day program included sprays on 31 July, 7, 14, 21, 27 Aug, and 5 Sep. The TOMCAST program was sprayed on 31 July, 14 August and 5 September. The BLIGHTCAST program was sprayed on 31 July, August 7 and 5 September. A CO_2 backpack sprayer and a broadcast boom equipped with one XR8003 flat-fan nozzle calibrated at 35 psi and delivering 467.6 L/ha was used. Foliage was

visually assessed for disease symptoms on 12, 17, 23 and 30 August using a continuous scale from 0 to 100% of the total amount of tissue within the treatment row exhibiting symptoms. Fruit that were 31.75-50.8 mm in diameter were harvested from the inner 3 m of each treatment replicate on 1 and 11 September. Statistics were run each year using SAS (SAS Institute, Cary, NC). The data were analyzed for the area under the disease progress curve (AUDPC) and repeated measures for both years. AUDPC was calculated by using the equation AUDPC = Σ [((Yi + Yi-1) (Xi – Xi-1))/2] where Y_i= foliar necrosis and X_i= time (Shaner & Finney, 1977).

(Shaner & Finney, 1977). An ANOVA F-Test was calculated to determine if the treatments had significant differences. The foliar assessments were repeated measures over time. The analysis of repeated measures shows the fixed factors as treatments, and the random factor was replications. Treatments were separated using Fischer's least significant differences ($p< 0.05$). Treatments were sliced to determine the mean separation of letters.

RESULTS

In 2019, CDM symptoms were first observed in the research plots on 21 August. The first disease assessment occurred on 6 September and continued weekly until 6 October. Over the monitoring period, the development of CDM increased from 6.0% on 6 September to 88.8% on 6 October. From 19 to 27 September, CDM increased from 48.8% to 86.3% in the untreated control. All fungicide treatments had significantly less disease than the untreated control on each assessment date (Table 2.1). The 5-day application schedule was more effective than the other fungicide treatments on several observation dates including 12, 16, and 19 September. On the last assessment date (6 October) the fungicide treatments had disease ratings that were similar to each other. According to the AUDPC data, the 5-day treatment program had significantly less

disease than all other treatments except for BLITECAST (Table 2.1). The ADUPC data indicated that BLITECAST was similar to the 7-day treatment program. The 2019 harvest totals for the fungicide treatments were significantly greater than the untreated control with the exception of BLITECAST; the fungicides treatments were similar (Table 2.4).

In 2020, symptoms of CDM were observed in the research plot on 8 August. On the first disease assessment on 12 August, plants in the untreated control had disease symptoms covering 7% of the leaf tissue. At the end of the trial on 15 September, 97.25% of the foliage displayed symptoms. Between the observation dates of 30 August and 7 September, the foliar disease increased from 48.8% to 78.8%. The calendar program of 5- or 7-day and the TOM-CAST program were similar and more effective than BLITECAST program. The AUDPC data indicate that the TOMCAST and 5-day programs were similar and better than the BLITECAST program; the 7-day program had the least amount of disease (Table 2.2). The harvest totals for the 5-day and TOM-CAST programs were significantly greater than the untreated control. The BLITECAST and 7-day harvest totals were not significantly different from the other treatments (Table 2.4).

In 2019, the temperature high during the evaluation period was 32.2˚C occurring in week 1, and the temperature minimum was 4.4˚C during week 7. The average temperature over the course of the 7 weeks was 20.2˚C. Average relative humidity never dropped below 58.1%, and had a high of 84.8%. Rainfall totaled 148.5 mm over the course of the 7 week period with the highest total accruing in week 6. In 2020, the minimum temperature was 10.2˚C happening over two weeks in the evaluation period, weeks 2 and 6; the maximum temperature was 35.2˚C in week 3. The average temperature over the 6 weeks was 21.8˚C. The lowest average relative humidity was 67.5% during week 4, and the highest was in week 2 at 82.5%. Total rainfall over

the evaluation period was 120.5 mm, the most happened in week 2, and the least happening in week 4 (Table 2.3).

DISCUSSION

The objectives for this study was to evaluate forecasting models versus calendar based applications. This study allowed us to see that while the forecasting methods are not ready to be commercialized for growers, but there is a potential for a forecasting model to be successful in reducing the amount of disease without being harmful to the yield. Further research is needed to continue to discover when fungicide applications are the most necessary in a growing season.

The average U.S. consumer eats approximately 9 pounds of pickles each year, making domestic cucumber production important (MDARD, 2018). *P. cubensis* is a significant risk to cucumbers in Michigan (Cespedes-Sanchez et al., 2015) and other U.S. cucumber growing regions (Colucci et al., 2006). In 2004, when the pathogen re-emerged, cucumber growers in the southeast U.S. lost approximately 40% of their yield (Colucci et al., 2006). Cucurbit growers in Michigan spend an estimated $8 million on fungicides; fungicide resistant *P. cubensis* isolates have been detected (Hausbeck, 2006). Ojiambo and Kang (2013) theorized that *P. cubensis* sporangia can travel 1000 km each season to infect hosts across several states. Other scientists have proposed that greenhouses in the northern United States and Canada may provide overwintering sites for the pathogen (Naegele et al., 2014).

Michigan pickling cucumber producers desire a decision-making tool to guide the timing of fungicide applications in an overall effort to reduce the number of sprays needed to limit CDM. TOMCAST has been proven for use against fungal pathogens (Pitblado, 1992, Dorman et al., 2009, Meyer et al., 2000) whereas BLITECAST is used to manage an oomycete

plant pathogen (Krause et al., 1975). In both years of this study, the forecasting programs reduced the total number of fungicide applications. Early blight is caused by the fungus *Alternaria soloni* which thrives in warm (24-29˚C), humid conditions (Kemmitt, 2013). It is currently used as a tool to manage early blight and Septoria leaf spot (*Septoria lycopersici)*; optimal conditions are 25˚C and 48 hours of leaf wetness. TOMCAST was similar to the 7-day program in 2019 and similar to the 5-day program in 2020. In 2019, fungicide applications continued until October for some treatments, but not the TOMCAST program because DSVs did not accrue due to the low temperatures during the leaf wetness period.

Disease severity values (DSV) are used in the TOMCAST and BLITECAST models. Each model calculates DSVs using different parameters. Each forecasting model has a specified accumulations of DSVs before a fungicide application is made (Krause et al., 1975, Pitblado, 1992). Research is needed to determine the optimal number of DSVs to limit *P. cubensis.* A DSV is assigned each day based on weather conditions. The more favorable the weather, the greater the accumulation of DSVs (Hardwick, 2006).

According to the AUDPC data, the 7-day application program had significantly less disease than the 5-day program in 2020 but not 2019, indicating that the timing of applications is an important consideration. This finding shows that while in some years more applications are needed (2019), that as long as the spray is applied at the right time it has the ability to reduce the amount of sprays (2020).

Temperature and leaf wetness are important environmental conditions for infection and sporulation of *P. cubensis* (Cohen, 1977). The minimum/maximum temperature for infection is 1-9°C/27-32°C (Cohen, 1977). The optimal temperature for infection is 15°C (Savory, et al., 2010). Conducive daytime temperatures can range from 25-30°C, as long as the nighttime

temperatures drop to 10-15°C (Lebeda & Cohen, 2010). The optimal temperature range for *P. cubensis* may be affected by the duration of leaf wetness (Cohen, 1977). The optimal leaf wetness period is six hours, but the pathogen can infect after 2 hours if the temperature is 20˚C or higher (Cohen, 1977, Lebeda & Cohen, 2010). At the optimal time period of six hours the temperatures can be lower (10-20˚C), but as the air cools (5-10˚C) infection becomes dependent on inoculum amount (Cohen, 1977). Sporulation occurs when temperatures range from 9-30˚C (Lebeda & Cohen, 2010). Temperatures of 10-20˚C resulted in delayed disease symptoms, however, the sporangia survived for a relatively long period of time, resulting in a higher sporangial count compared to when temperatures were higher than 20˚C (Rotem et al., 1978). Leaf wetness is necessary for infection and can be a limiting factor in sporulation if the duration of leaf wetness is not adequate (Cohen, 1977, Lebeda & Cohen, 2010). This is due to a reduction in viability of the sporangia if there are intermittent periods of wetting and drying. Wetting periods shorter than two hours in duration are especially limiting (Palti & Cohen, 1980). Sporulation is optimized with 6 to 9 hours of leaf wetness. In a one wetting period occurring during the nighttime, 10^5-10^6 sporangia/cm^2 can be produced (Rotem et al., 1978).

Relative humidity greater than 90% is required for sporulation of *P. cubensis*. (Palti & Cohen, 1980, Rotem et al., 1978). High relative humidity, however, limits the duration that the sporangia are viable (Rotem et al., 1978). A decrease in relative humidity causes the sporangiophores to twist which allows the release of the mature sporangia (Lange et al., 1989). In lab studies, low inoculum doses lead to increased inoculum levels in the second generation (Cohen, 1977). Inoculum reaches its peak when 70% of the leaf is infected (Rotem et al., 1978). The sporangia erupt from the undersides of the leaves at high concentrations (Rotem et al., 1978).The fungicide program used was cyazofamid + chlorothalonil alternated with

oxathiapiprolin + chlorothalonil alternated with ametoctradin + dimethomorph + chlorothalonil. Recently, oxathiapiprolin has been changed from FRAC code group U15 to group 49. This chemical group are known as the oxysterol binding protein homologue inhibition (OSBPI). It is a systemic fungicide that protects the plant as it grows (Pasteris et al., 2016). CAA fungicides are in the FRAC code group 40, and interfere with cell wall deposition and cellulous biosynthesis (Gisi et al., 2019). The CAA fungicide dimethomorph, developed in the 1980s, is registered for use on *P. cubensis,* and limits the germination of the cystospores, sporangia, and growth of the germ tubes and mycelium (Gisi et al., 2019).

An increase in disease pressure occurred between September 19 and 27, 2019; the rainfall during that time period was 46 mm (Table 2.3). Minimum temperatures during that week never went below the *P. cubensis* threshold of 10˚C, and maximum temperatures were just above at 28.3˚C. The average temperature of the week was right at optimal at 18.4˚C and relative humidity at an average of 73.2. The increase in disease pressure was caused by all of these environmental conditions. In 2020, the increase in pressure occurred between August 17 and 23; the untreated control jumped from 20% to 50%. The week leading up to the increase and the week of, the rainfall totaled 41.7 mm. The average temperature in that time period was 22.8˚C with the minimum being 10.4˚C and the maximum being 35.2˚C. Average relative humidity was 73.8%. The average temperature being higher than 20˚C means the time in which the leaves are wet is shorter. The rainfall accumulation, high temperatures and relative humidity increased disease pressure for the 2020 season.

Table 2.1: Foliar infection rated 0-100% on 'Vlaspik' cucumbers at the MSU Plant Pathology Farm in 2019

Treatment[z]	Application no.	2019[x]								
		Foliar infection (%)[y]								
		6-Sep	9-Sep	12-Sep	16-Sep	19-Sep	27-Sep	30-Sep	6-Oct	AUDPC
Untreated control	--	6.0 c	7.5 b	52.5 c	48.8 d	48.8 d	86.3 c	88.8 d	88.8 c	1794.0 d
Ranman SC 2.75 fl oz + BWS 2 pt alternated with Orondis Opti SC 2.5 pt alternated with Zampro SC 14 fl oz + BWS 2 pt										
5- to 7-day intervals	8	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.5 a	17.5 ab	30.0 ab	182.5 a
BLITECAST Forecaster	4	0.8 ab	1.3 a	17.5 b	11.8 b	18.0 bc	3.0 a	8.3 a	28.8 a	346.1 ab
7- to 10-day intervals	6	1.0 ab	1.5 a	18.8 b	12.3 b	16.3 bc	10.3 ab	25.0 a-c	43.8 ab	504.0 bc
TOMCAST Forecaster	3	1.3 b	2.5 a	20.0 b	22.5 c	25.0 c	17.5 b	30.0 bc	47.5 ab	669.4 c

[x]Column means with a letter in common are not significantly different (LSD t-Test; *P*=0.05).
[y]Based on a visual estimation of the percentage of foliage infected.
[z]BWS = Bravo WeatherStik SC.

Table 2.2. Forecasting ratings for foliar infection symptoms on Vlaspik cucumbers from 0-100% on a grower cooperator commercial field in 2020

		2020						
		Foliar infection (%)[y]						AUDPC
Treatment[z]	Application No.	12-Aug	17-Aug	23-Aug	30-Aug	7-Sep	15-Sep	
Untreated Control	--	7.0 b	20.0 c	50.0 c	48.8 b	78.8 d	97.3 c	1837.1 d
Ranman SC 2.75 fl oz + BWS 2 pt *alternated with* Orondis Opti SC 2.5 pt *alternated with* Zampro SC 14 fl oz + BWS 2 pt								
7 Day Interval	6	2.3 a	0.8 a	10.5 ab	15.0 a	26.8 a	31.3 a	523.9 a
5 Day Interval	9	2.0 a	10.3 b	17.5 b	15.0 a	32.5 ab	35.5 a	689.6 b
TOMCAST Forecaster	3	1.5 a	8.5 b	17.3 b	18.3 a	36.3 b	48.8 a	788.3 b
BLITECAST Forecaster	3	0.3 a	0.5 a	7.0 a	18.3 a	61.3 c	77.5 b	989.5 c

[x]Column means with a letter in common are not significantly different (LSD t-Test; *P*=0.05).
[z]BWS = Bravo WeatherStik SC.

Table 2.3. The average, minimum, maximum temperatures, total rainfall, and relative humidity over the course of the spray season in the two year study period.

Year, week no. (Julian Days)	Total Rainfall (mm)	Average Temperature (˚C)	Minimum Temperature (˚C)	Maximum Temperature (˚C)	Relative Humidity (%)
2019 (231-279)					
1 (231-236)	4.1	21.2	10.8	32.2	80.5
2 (237-243)	27.9	19.9	11.8	29.5	76.6
3 (244-250)	8.6	19.9	7.9	27.9	68.3
4 (251-257)	15.2	22.7	17.8	29.5	58.1
5 (258-264)	4.1	24.8	24.6	24.9	75.1
6 (265-271)	46.2	18.4	10.0	28.3	73.2
7 (272-279)	42.4	14.8	4.4	27.2	84.8
2020 (209-249)					
1 (209-214)	23.4	22.8	14.6	31.0	75.1
2 (215-221)	51.3	19.0	10.2	31.4	82.5
3 (222-228)	3.3	23.9	13.3	35.2	74.7
4 (229-235)	0.3	21.3	10.4	32.0	67.5
5 (236-242)	38.1	23.3	15.9	33.4	79.2
6 (243-249)	4.1	20.7	10.2	34.7	74.3

Table 2.4. Harvest totals for the forecasting trials in both 2019 and 2020

Treatment	**Number of Applications**		**Harvest totals**			
			Weight in kg[w]			
	2019	**2020**	**2019[y]**		**2020[z]**	
Untreated			3.2	b	8.8	b
5-day	8	9	7.6	a	14.1	a
7-day	6	6	8.7	a	13.2	ab
TOMCAST	3	3	7.9	a	15.0	a
BLITECAST	4	3	5.8	ab	12.5	ab

[w]Kilograms. Harvests were taken at sizes 3A-3Bs the average size for commerical producers

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

[y]Four harvests were taken from the middle 10ft of each treatment

[z]Two harvests were taken from the middle 10 ft of each treatment

FUTURE DIRECTION

I believe that the evaluation of single fungicide products is beneficial to the growers for recommendations. This trial should continue as long as downy mildew is a problem cucurbit growers face. Fungicide products that are labeled for control of *P. cubensis* should be added and removed as necessary to keep the plot both manageable and effective.

The evaluation of forecasting models versus calendar-based spray applications should be modified. The first modification to occur should be the harvest. I believe that the most effective way to show better differences in the yield, would be to harvest the "crooks and nubs" or the misshaped fruit. The fruit become misshaped under lack of water, and *P. cubensis* reduces the amount of water the plant can take up causing more misshaped fruit. The next modification I would make would be the addition of more forecasting models. This would allow for a more opportunities to find a forecasting model that fits *P. cubensis.* I would also use multiple DSVs for each forecasting model to see if a reduction in the DSVs would help contribute to the management of disease.

APPENDIX

The appendix contains supplementary graphs and figures for chapters one and two

Figure 1. The foliar infection percentage of the single fungicide trial in 2019 on the last rating date (9/27/2019)

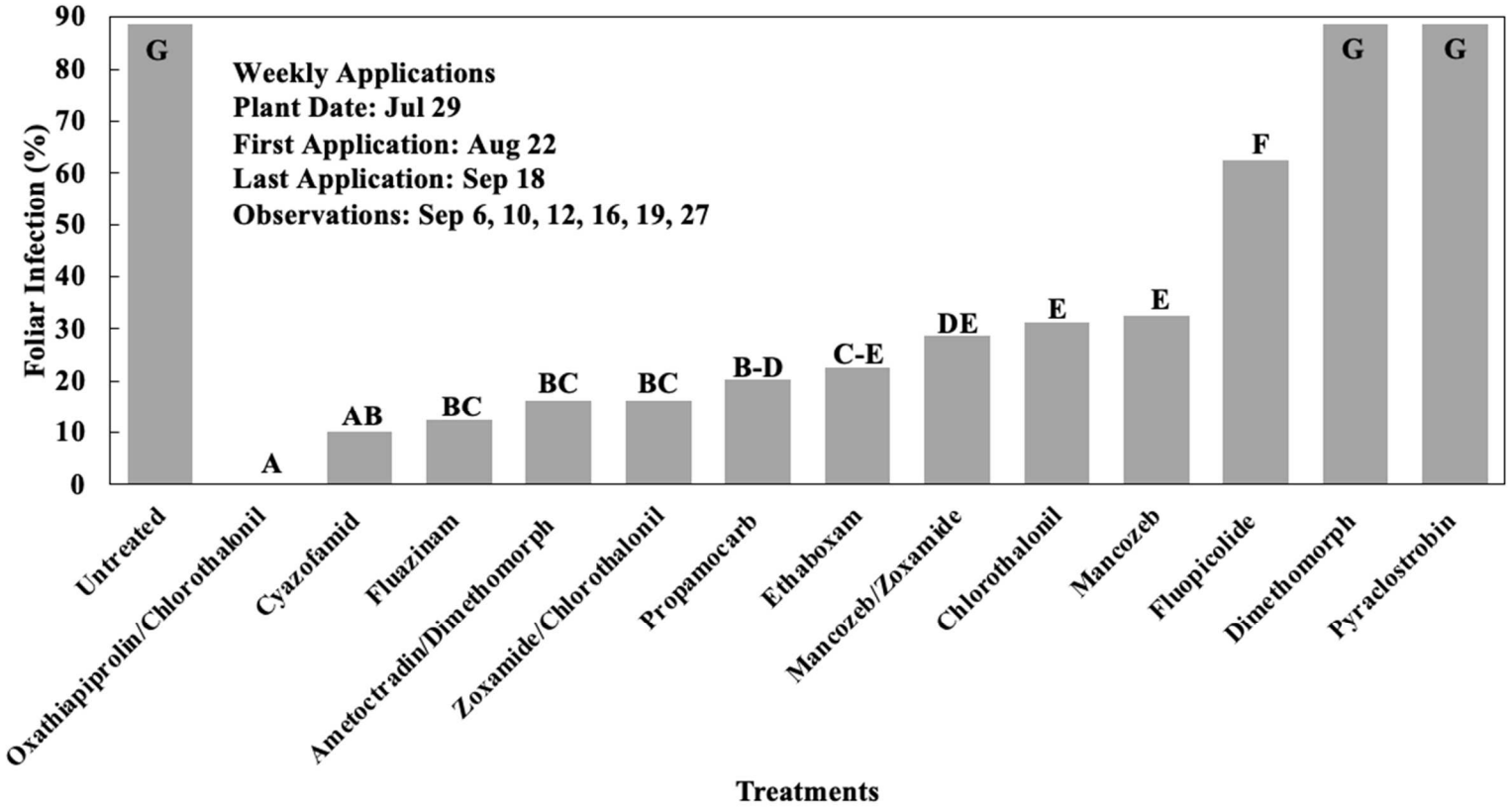


Figure 2. The AUDPC of the single fungicide trial in 2019

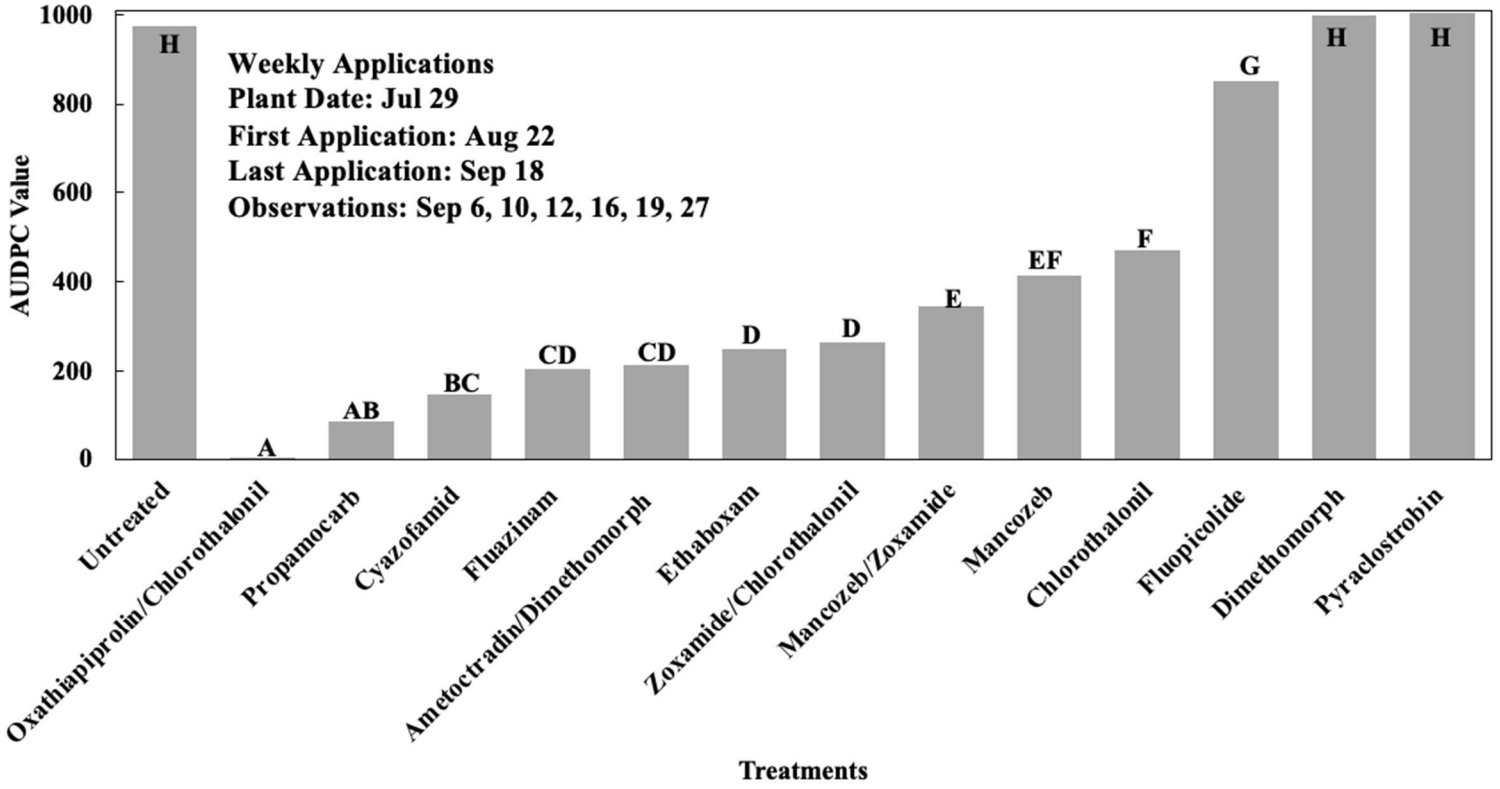
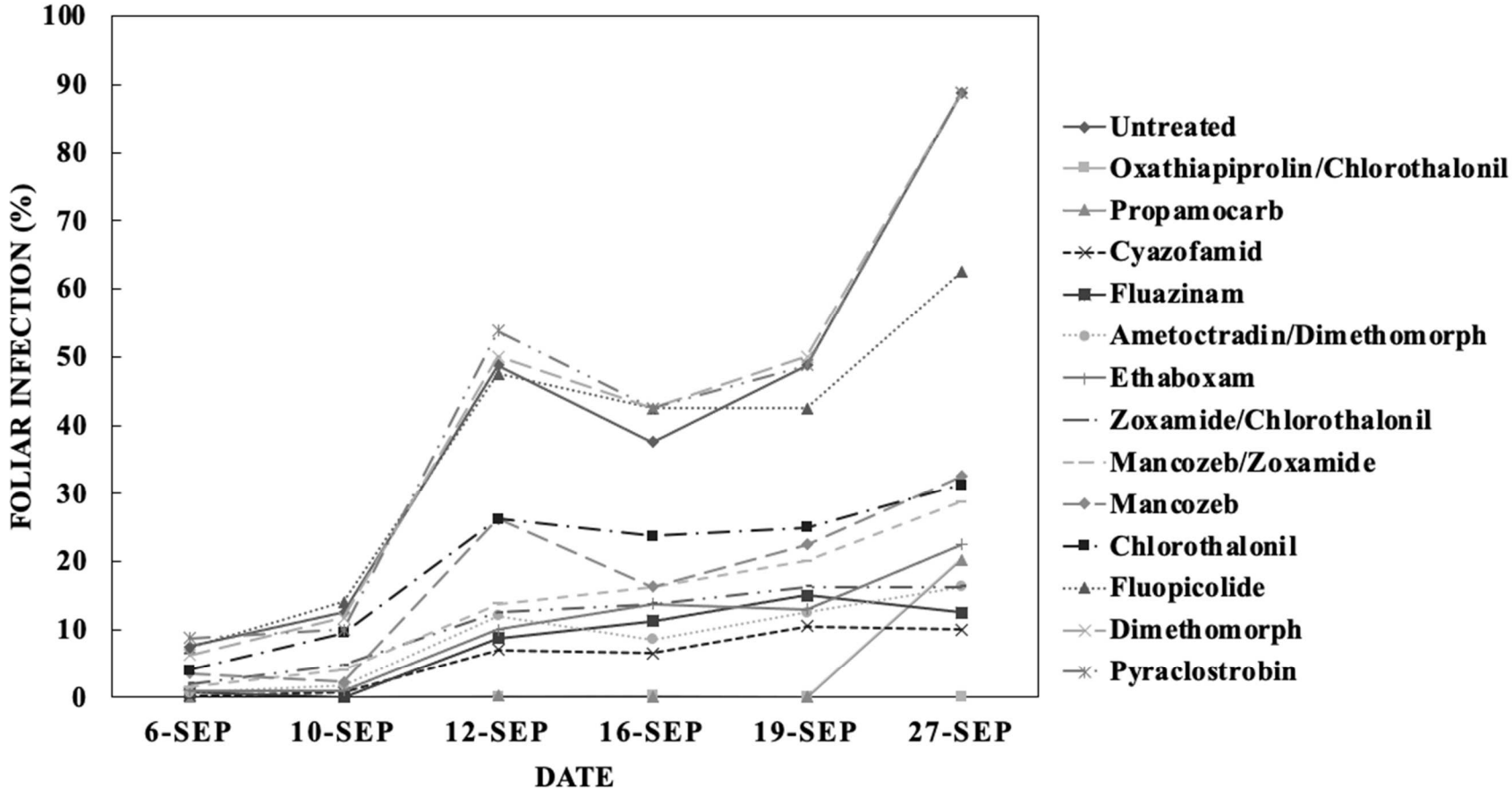


Figure 3. The foliar infection of the single fungicide trial in 2019 on the MSU Plant Pathology Farm



The percentage of foliar infection was rated visually.

Figure 4. The foliar infection percentage of the program trial in 2019 on the last rating date (9/19/2019)

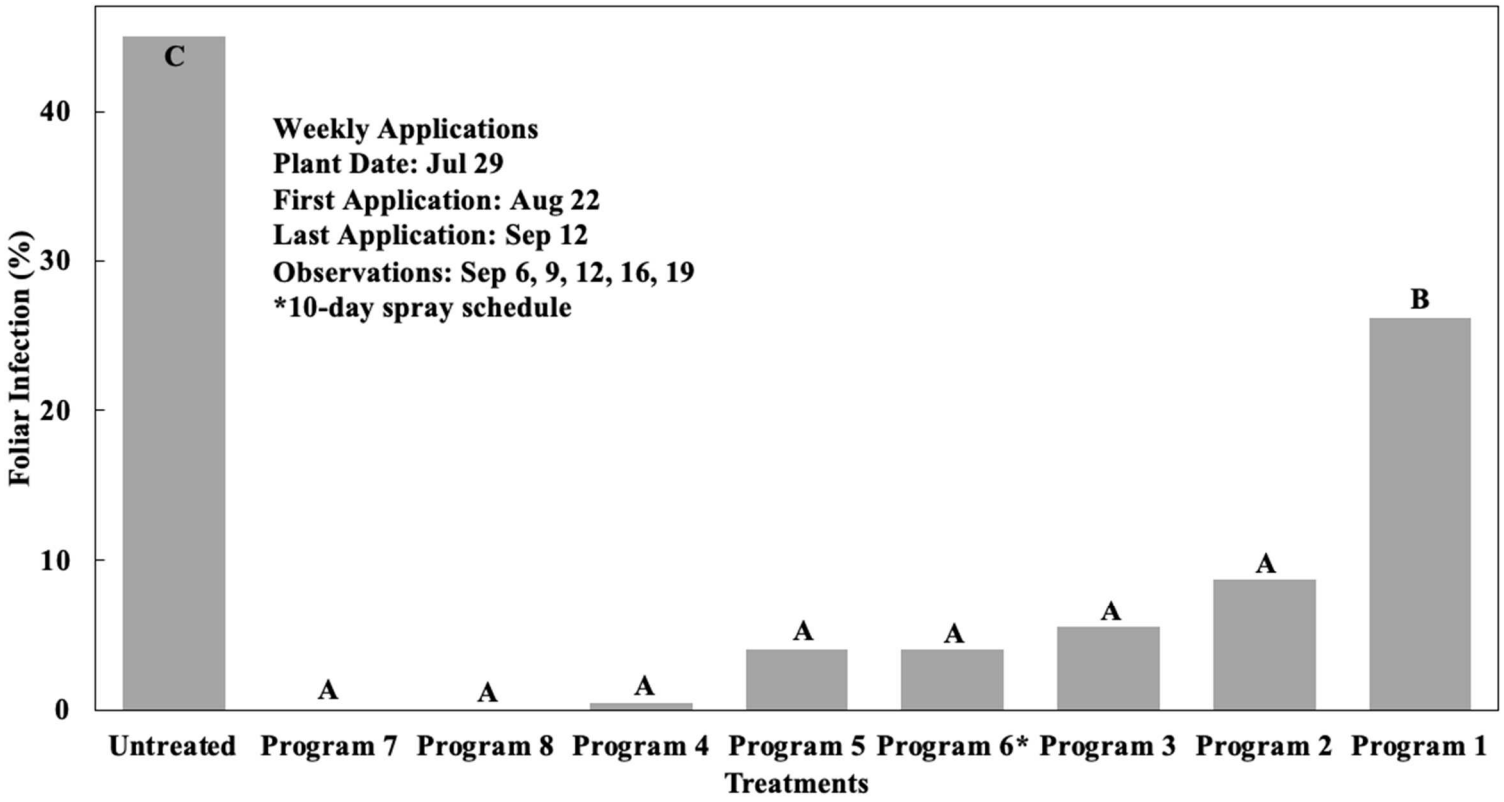
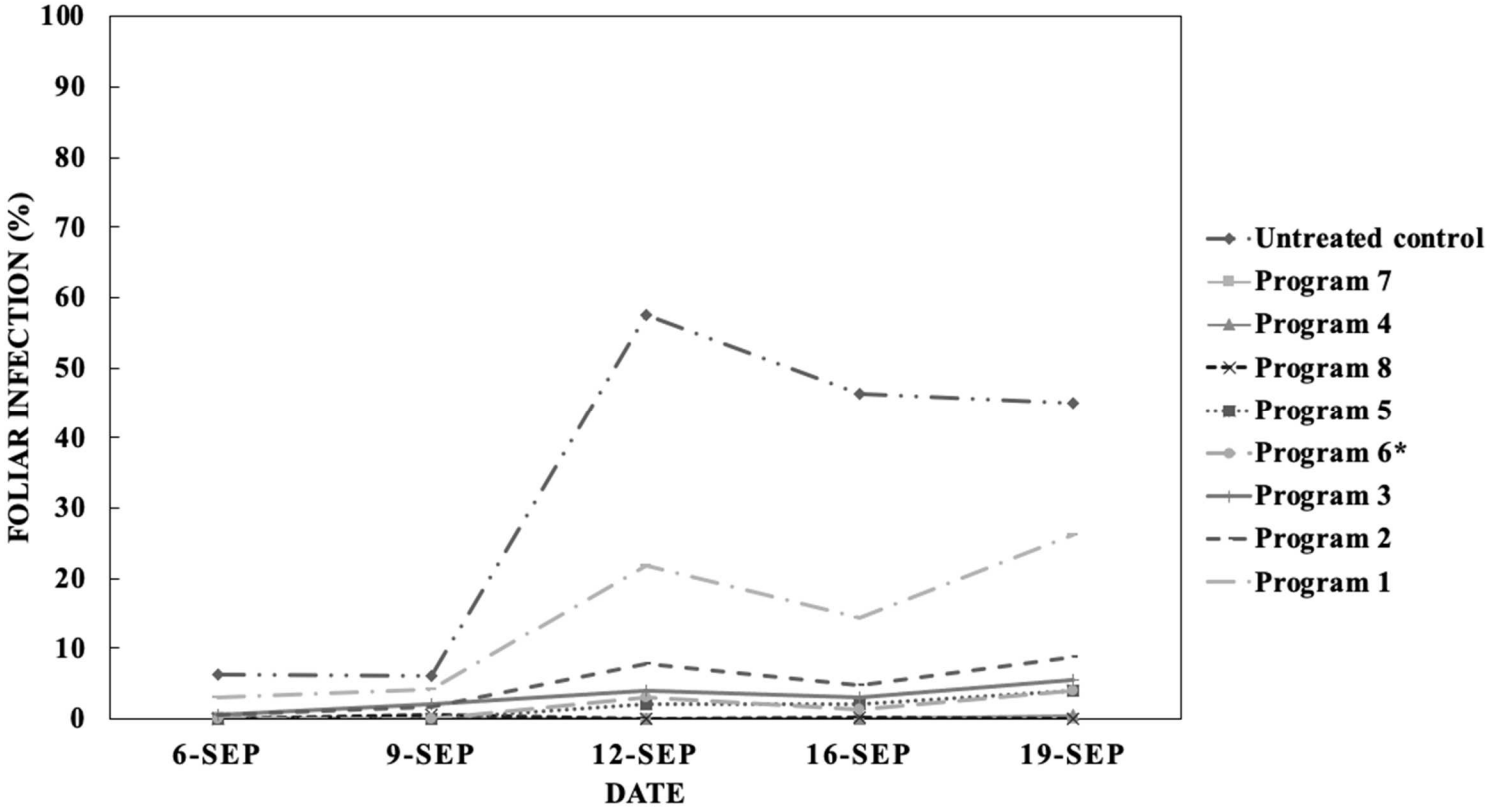


Figure 5. The foliar infection of the 2019 programs trial on the MSU Plant Pathology Farm.



The percentage of foliar infection was rated visually.

Figure 6. The AUDPC of the program trial in 2019

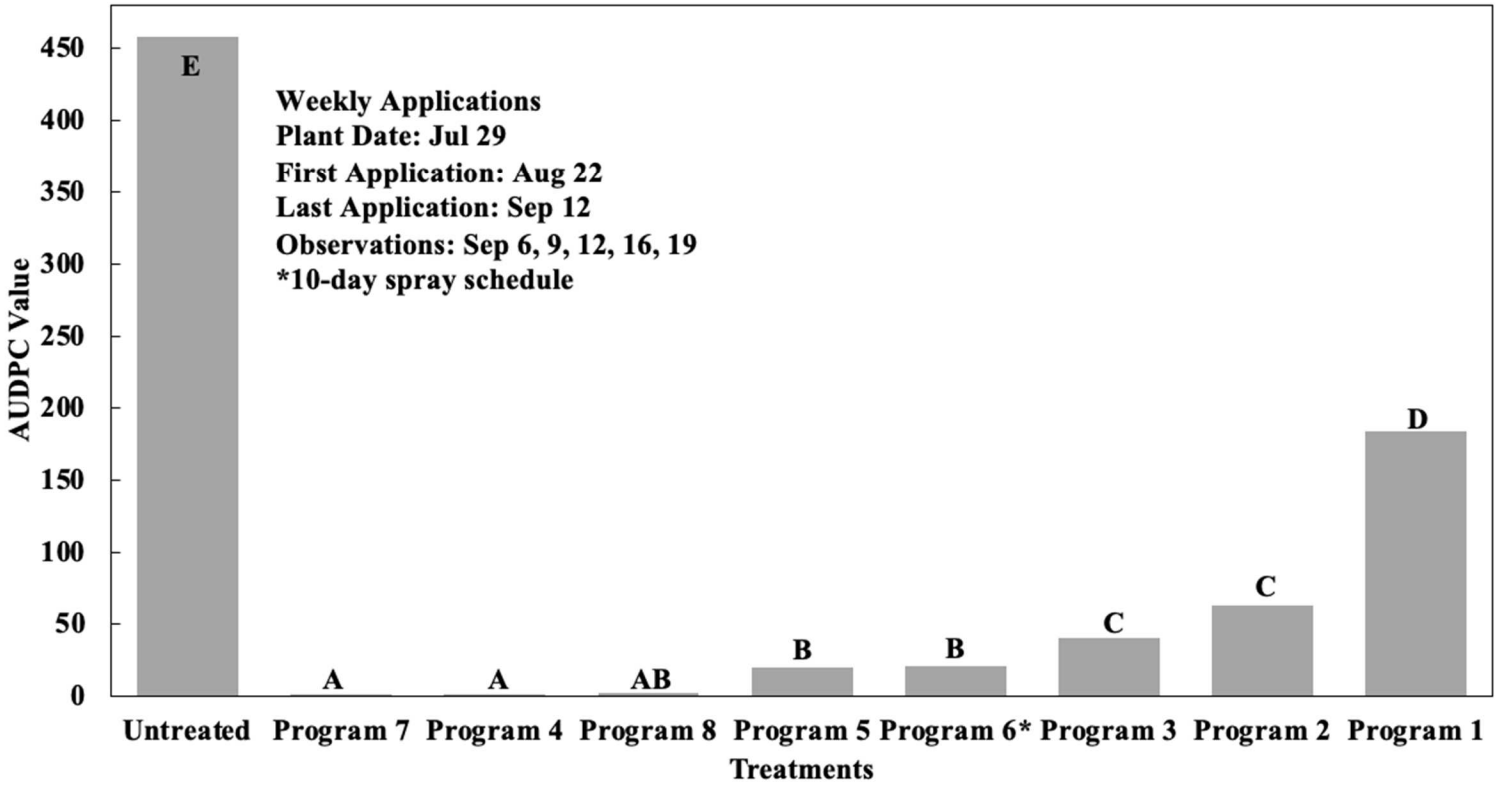


Figure 7. The foliar infection percentage of the single fungicide trial in 2020 on the last rating date (8/30/2020)

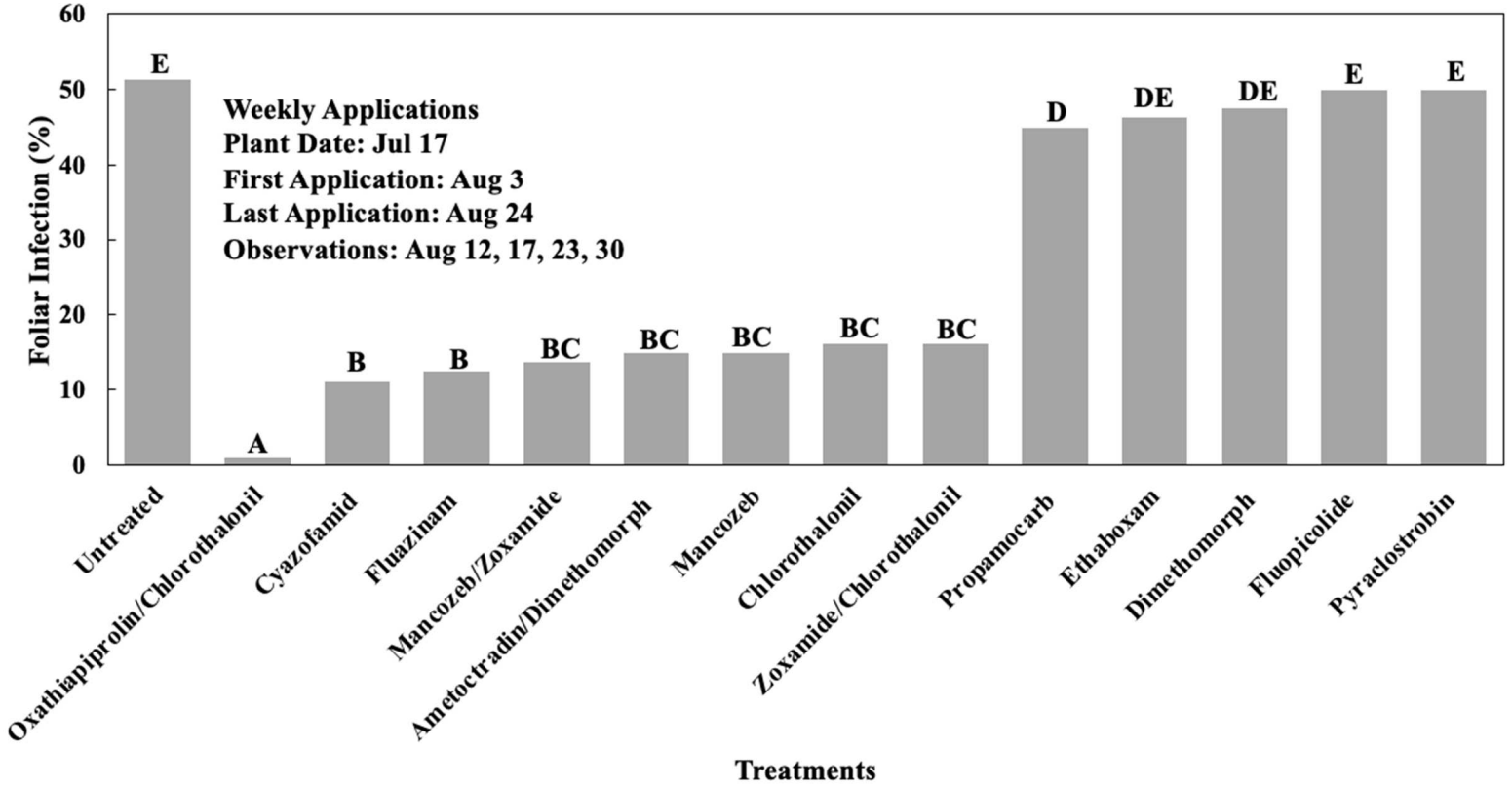


Figure 8. The AUDPC of the single fungicide trial in 2020

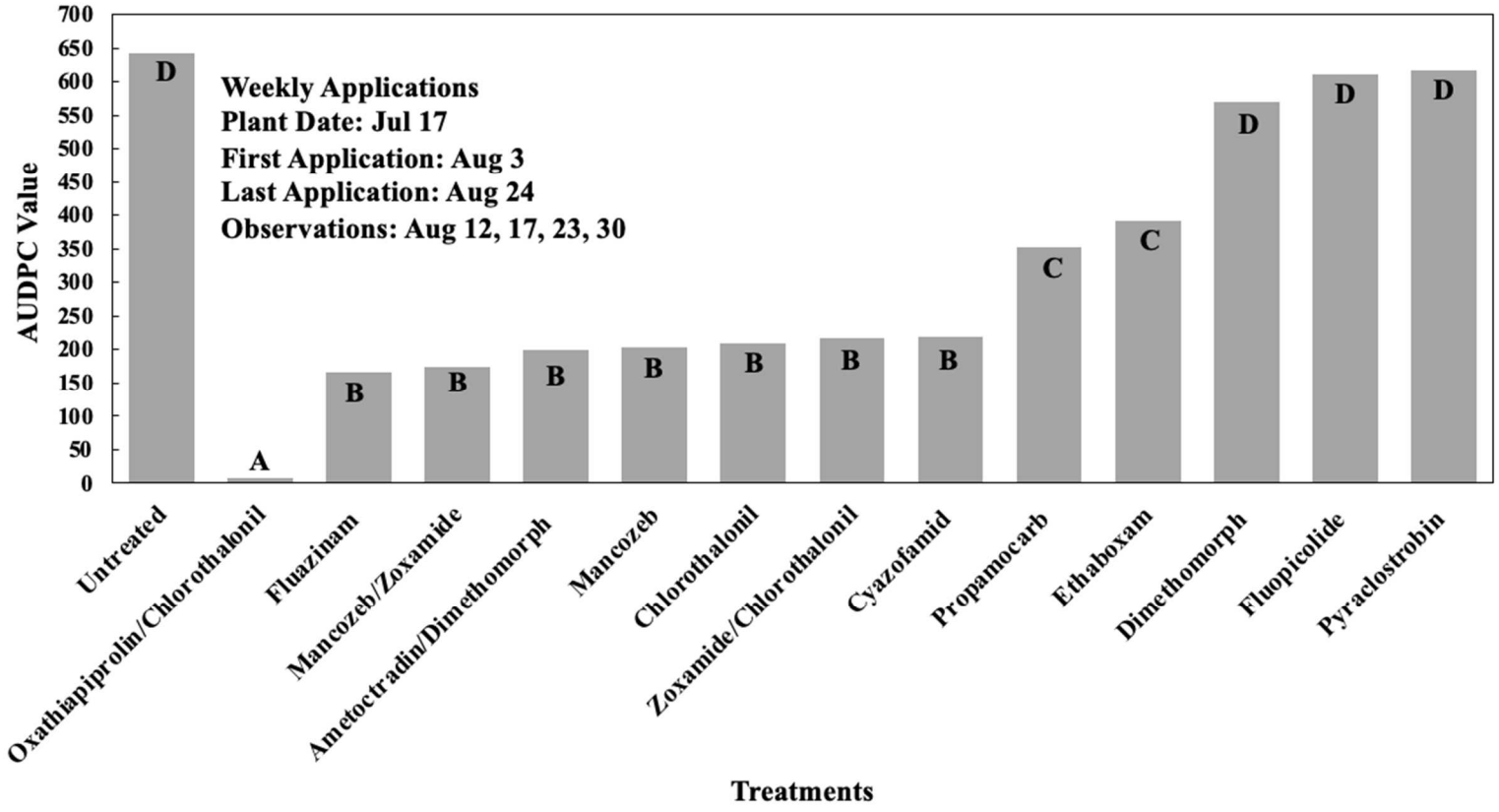
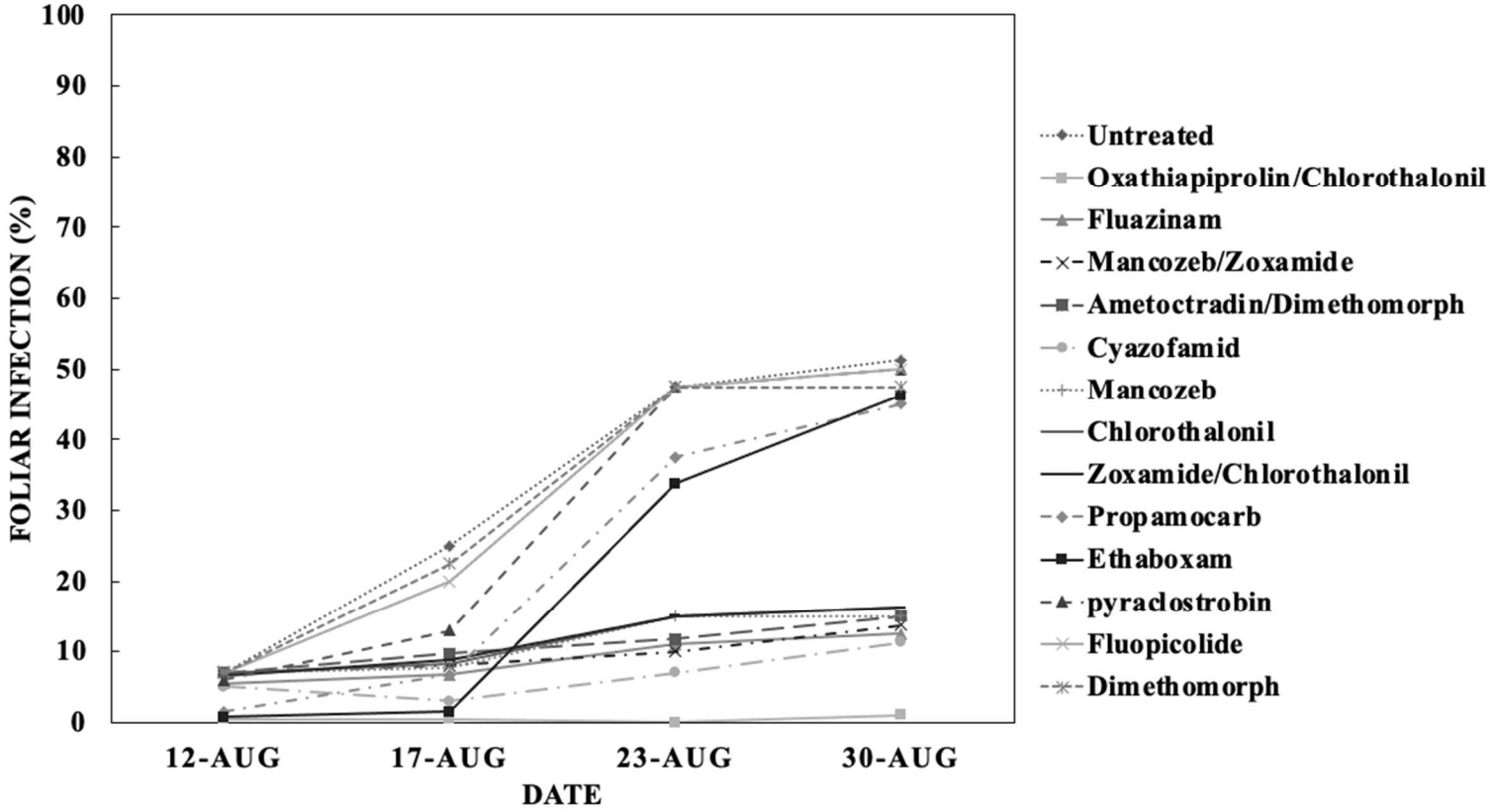


Figure 9. The foliar infection evaluation of the 2020 single fungicide trials on a grower cooperator farm in Merrill, MI



The percentage of foliar infection was rated visually.

Figure 10. The foliar infection percentage of the program trial in 2020 on the last rating date (8/30/2020)

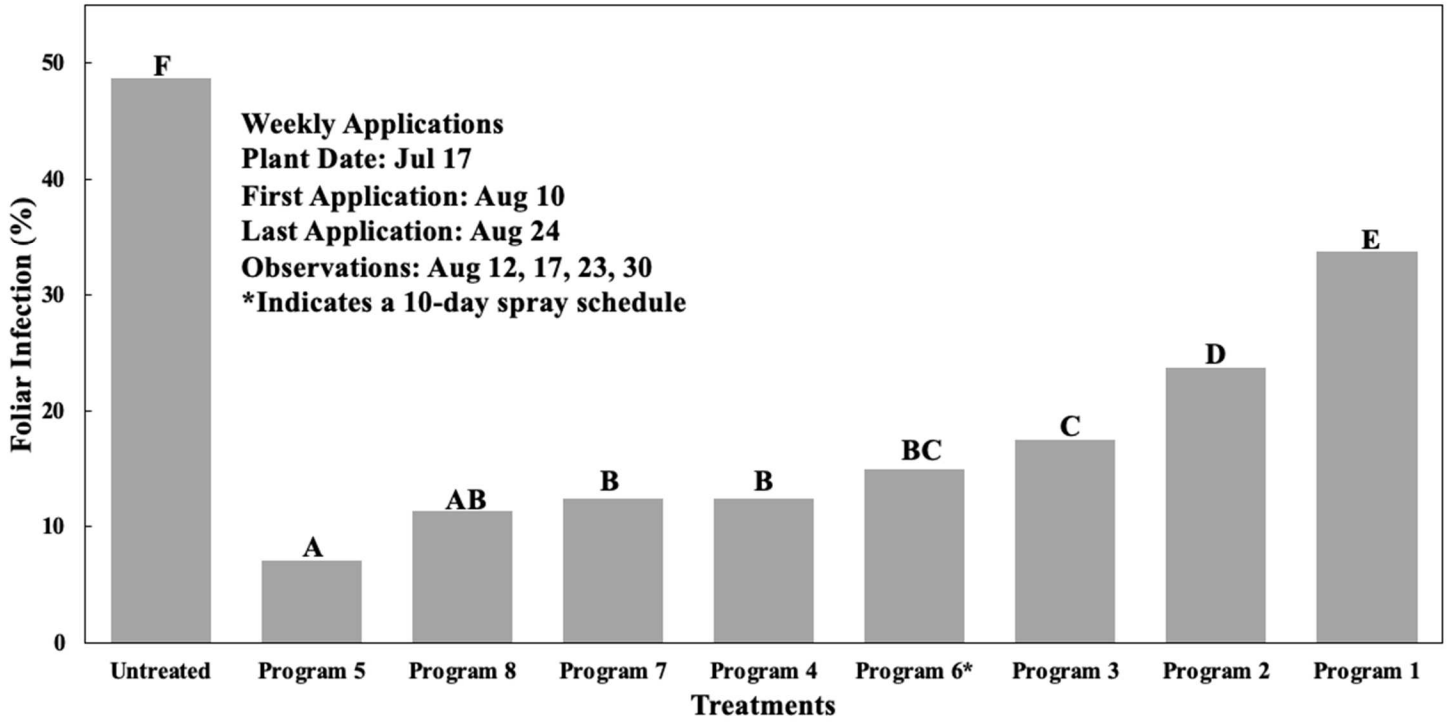


Figure 11. The AUDPC of the program trial in 2020

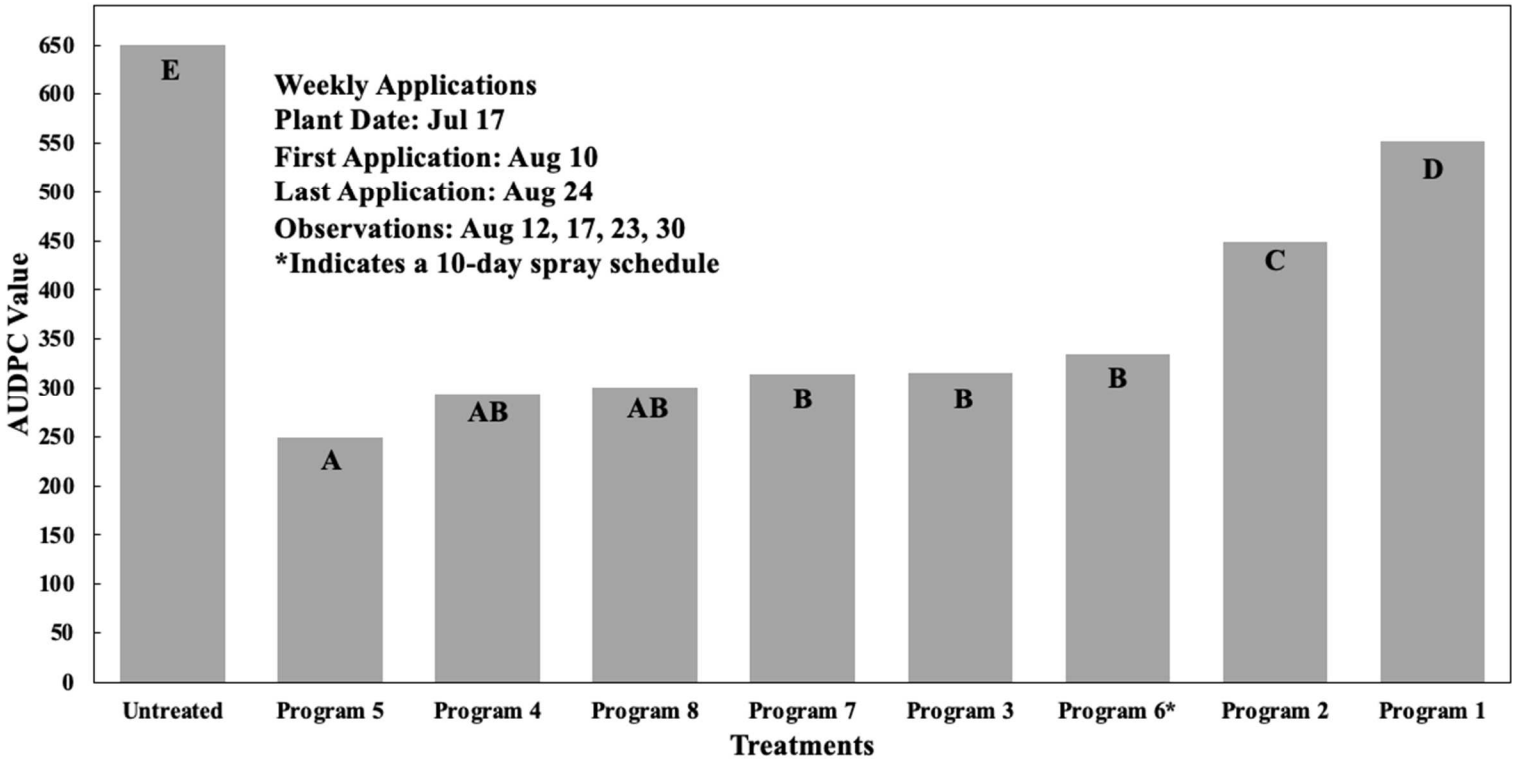
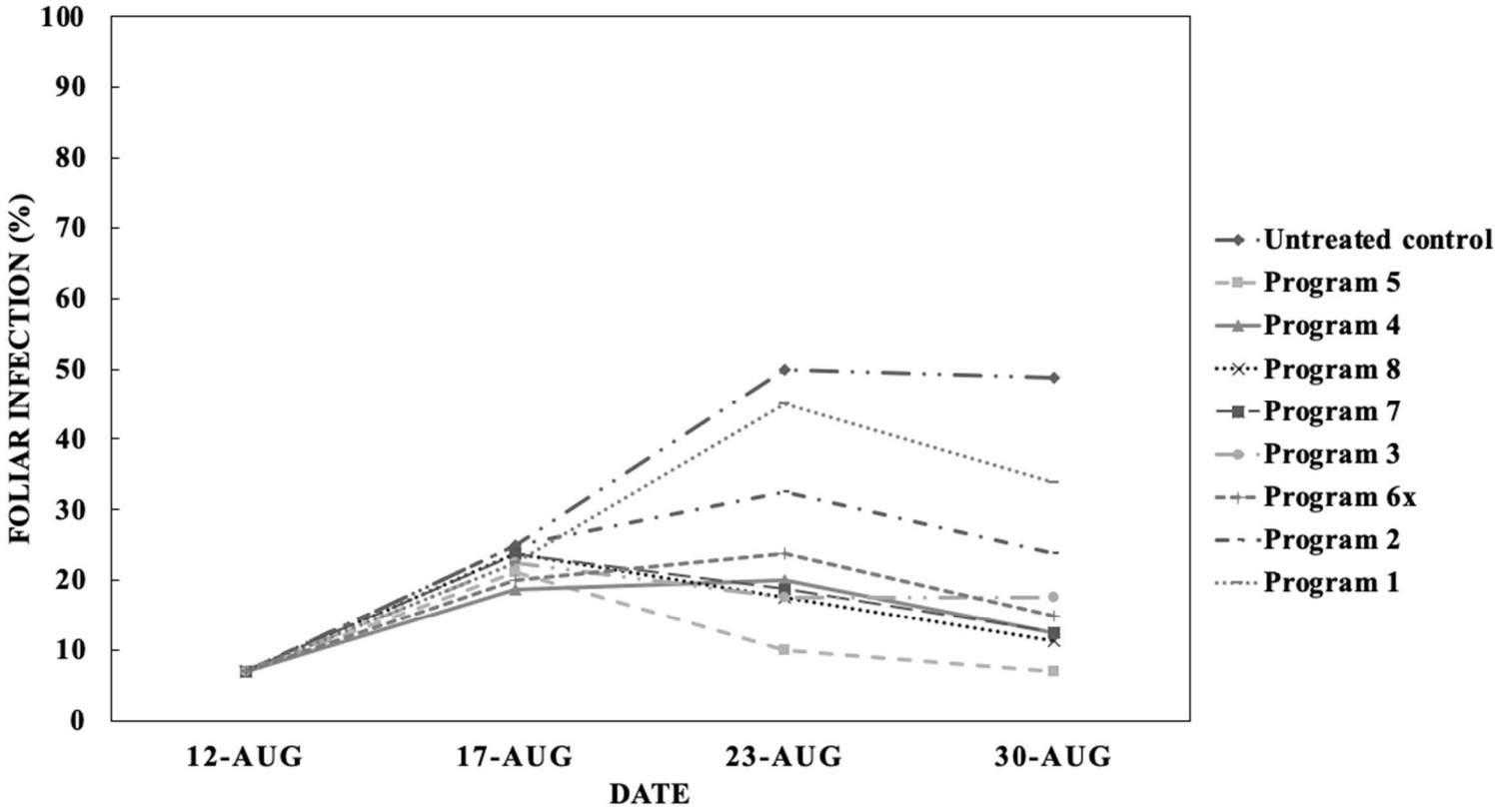


Figure 12. The foliar infection of the 2020 programs trial on a grower cooperator in Merrill, MI



The percentage of foliar infection was rated visually.

Figure 13. This shows the number of applications made according to each of the treatments in 2019.

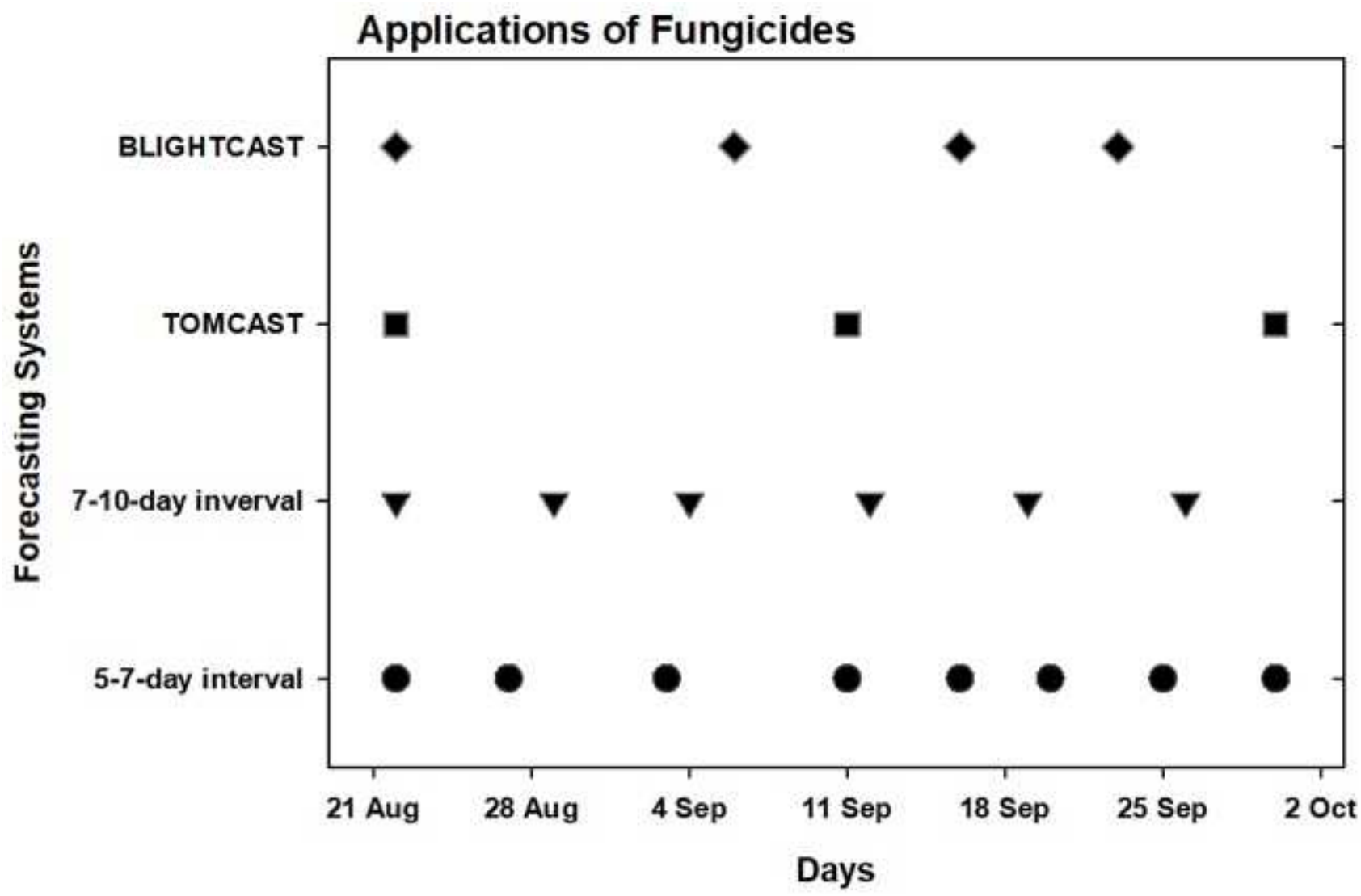


Figure 14. The foliar necrosis of the untreated control, 5-7 day, and TOMCAST over the course of the 2019 season. This graph indicates the differences between the, the 5-7 day interval, and the TOMCAST forecasting model applications shown by the triangles.

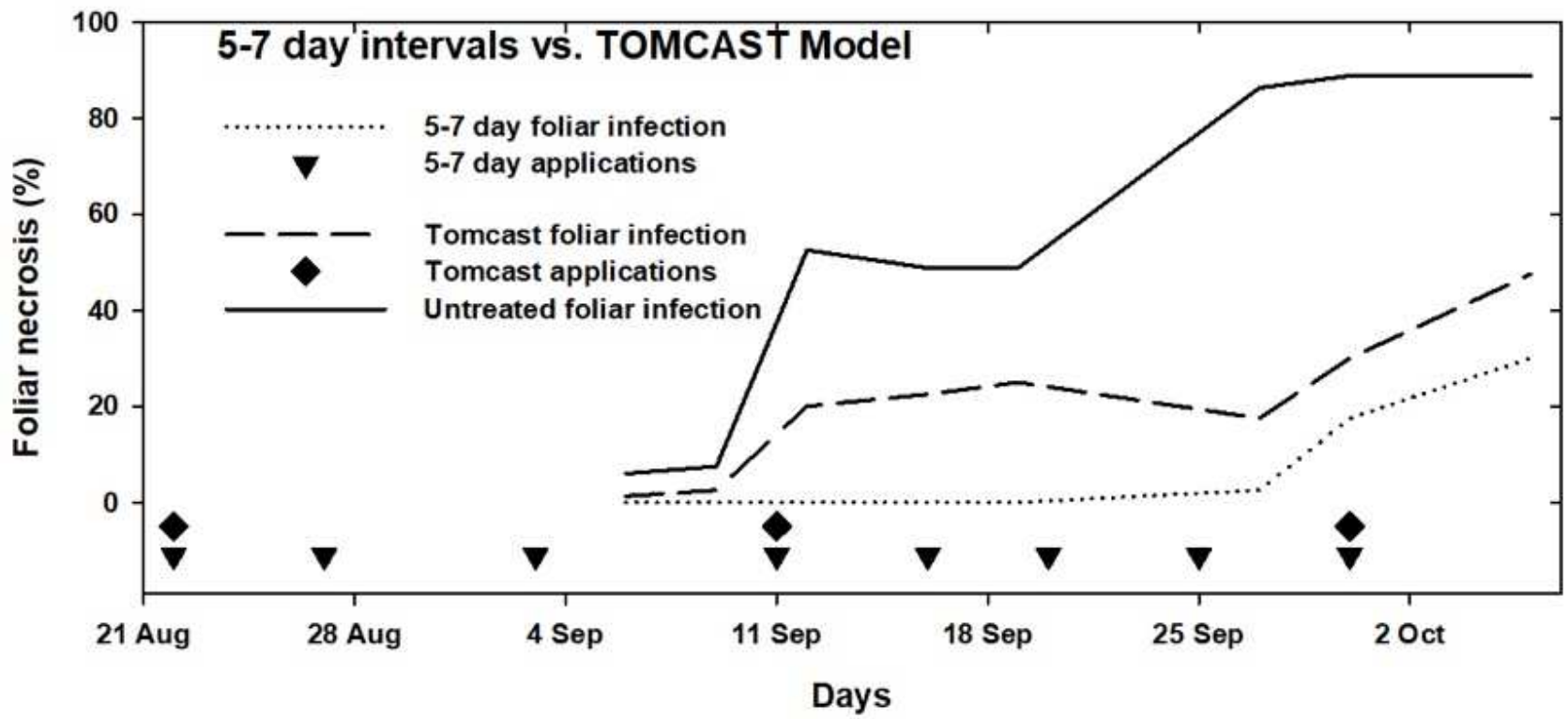


Figure 15. This graph compares the foliar necrosis of the untreated, 5-7 day interval, and BLITECAST in 2019. It also shows the applications of the 5-7 day interval with the downward triangles. The BLITECAST model has its applications indicated by the diamonds

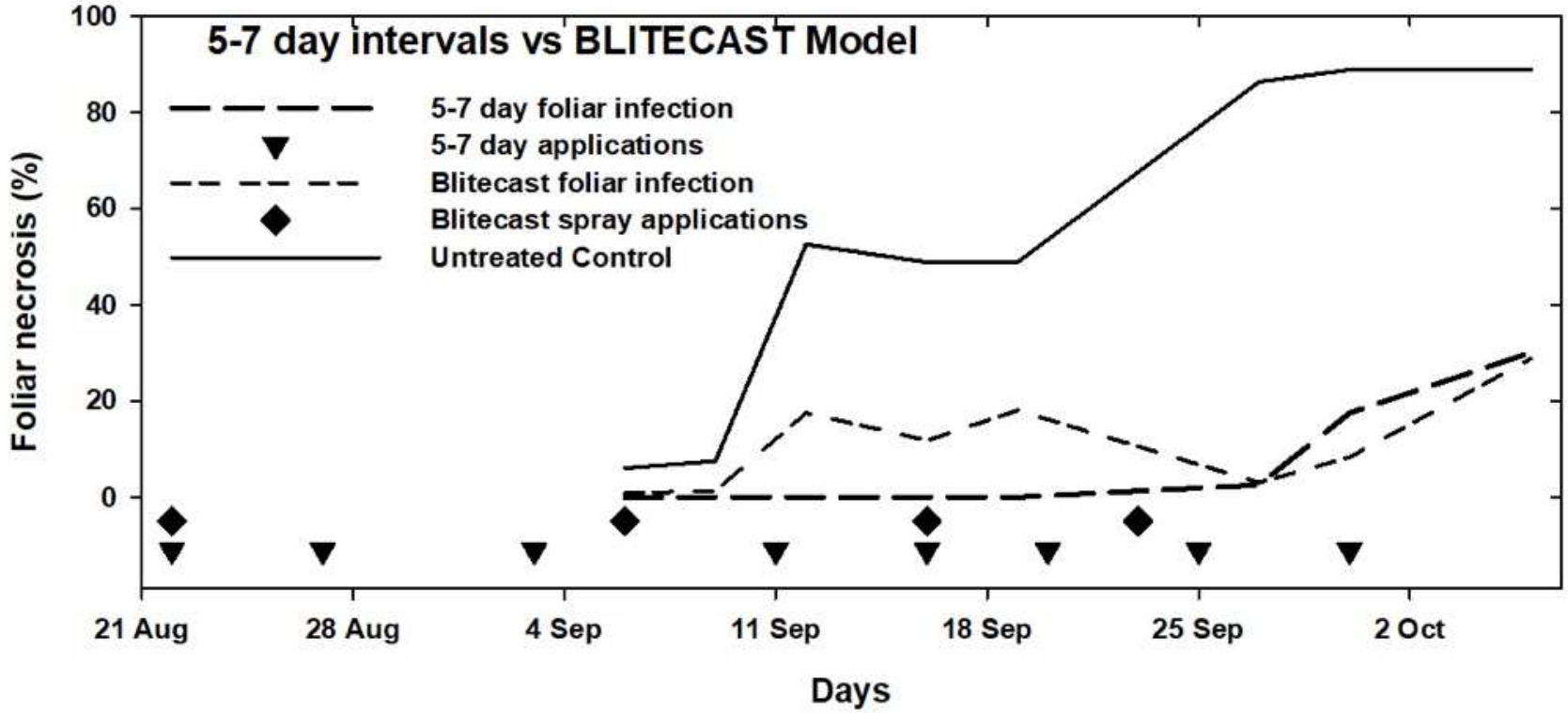


Figure 16. The graph shows the comparison of the untreated control and the 5-7 day spray interval in 2019. The spray date applications are indicated by the triangles at the bottom of the graph.

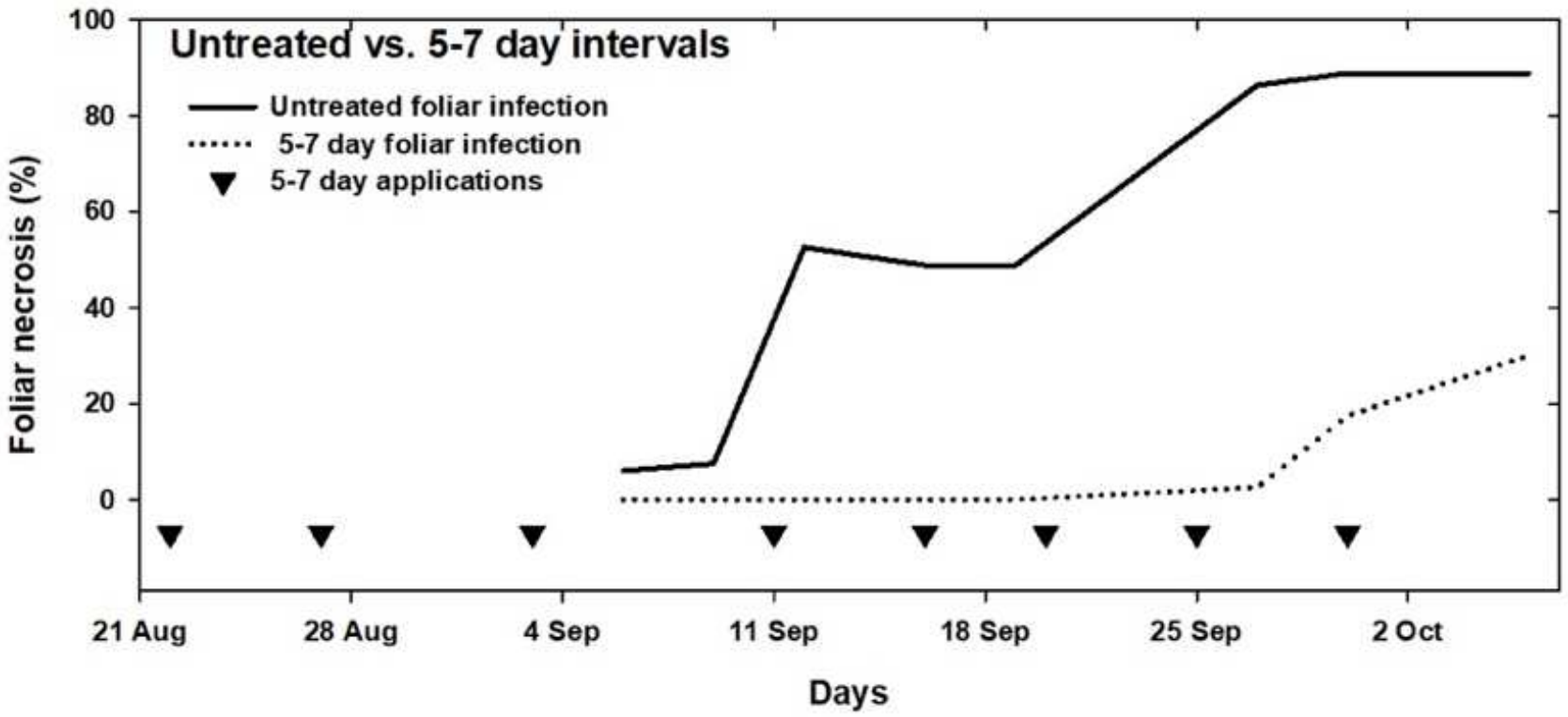


Figure 17. The foliar necrosis of the untreated, 5-7 day interval, and the 7-10 day interval in 2019. The application of fungicides are indicated by the triangles (5-7 day) and the diamonds (7-10 day).

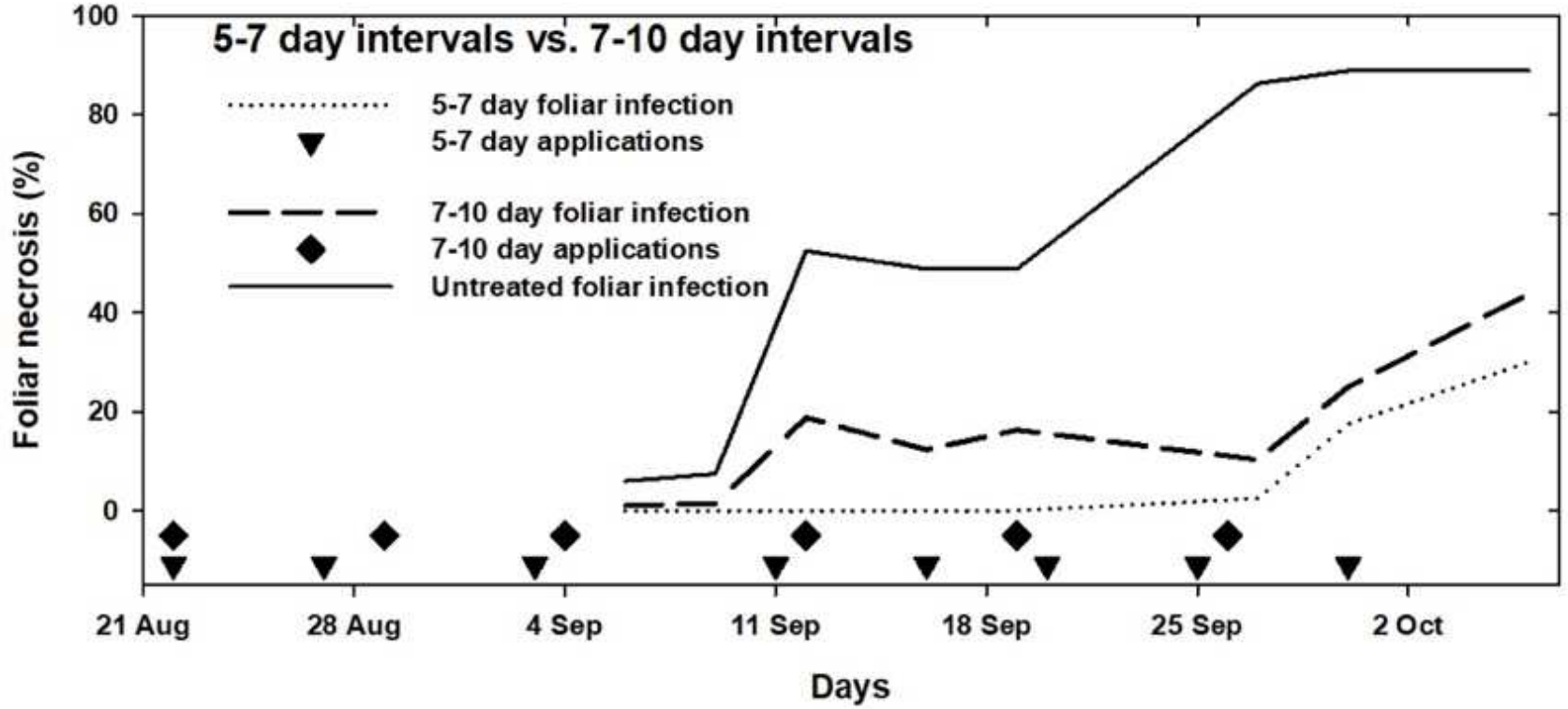


Figure 18. The comparison between the applications of each of the treatments over the course of the 2020 field season

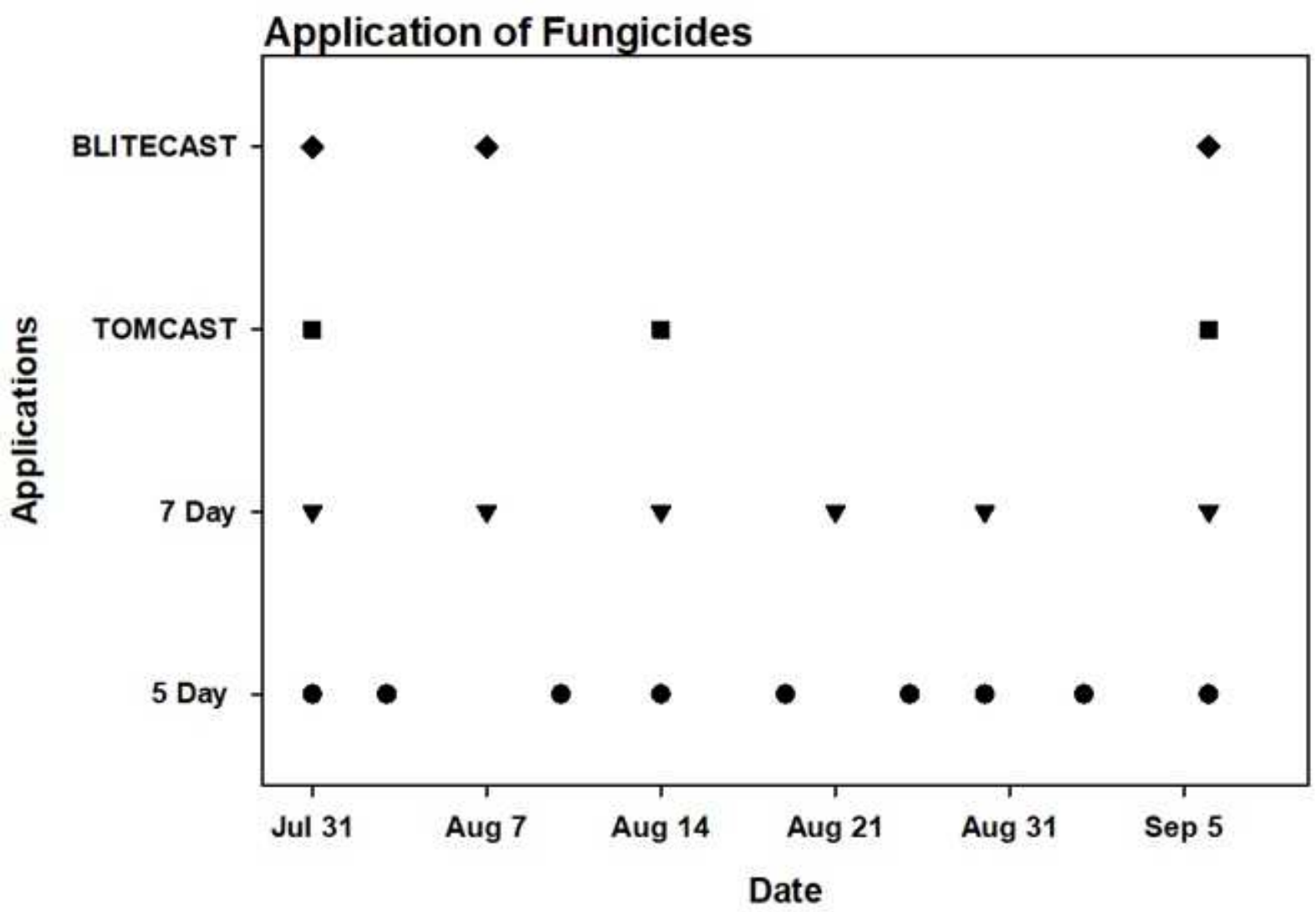


Figure 19. This shows the progression of foliar necrosis over the course of the 2020 field season for the untreated, 5-day interval, and BLITECAST. The application dates are shown as circles for the 5 day, and triangles for the BLITECAST model.

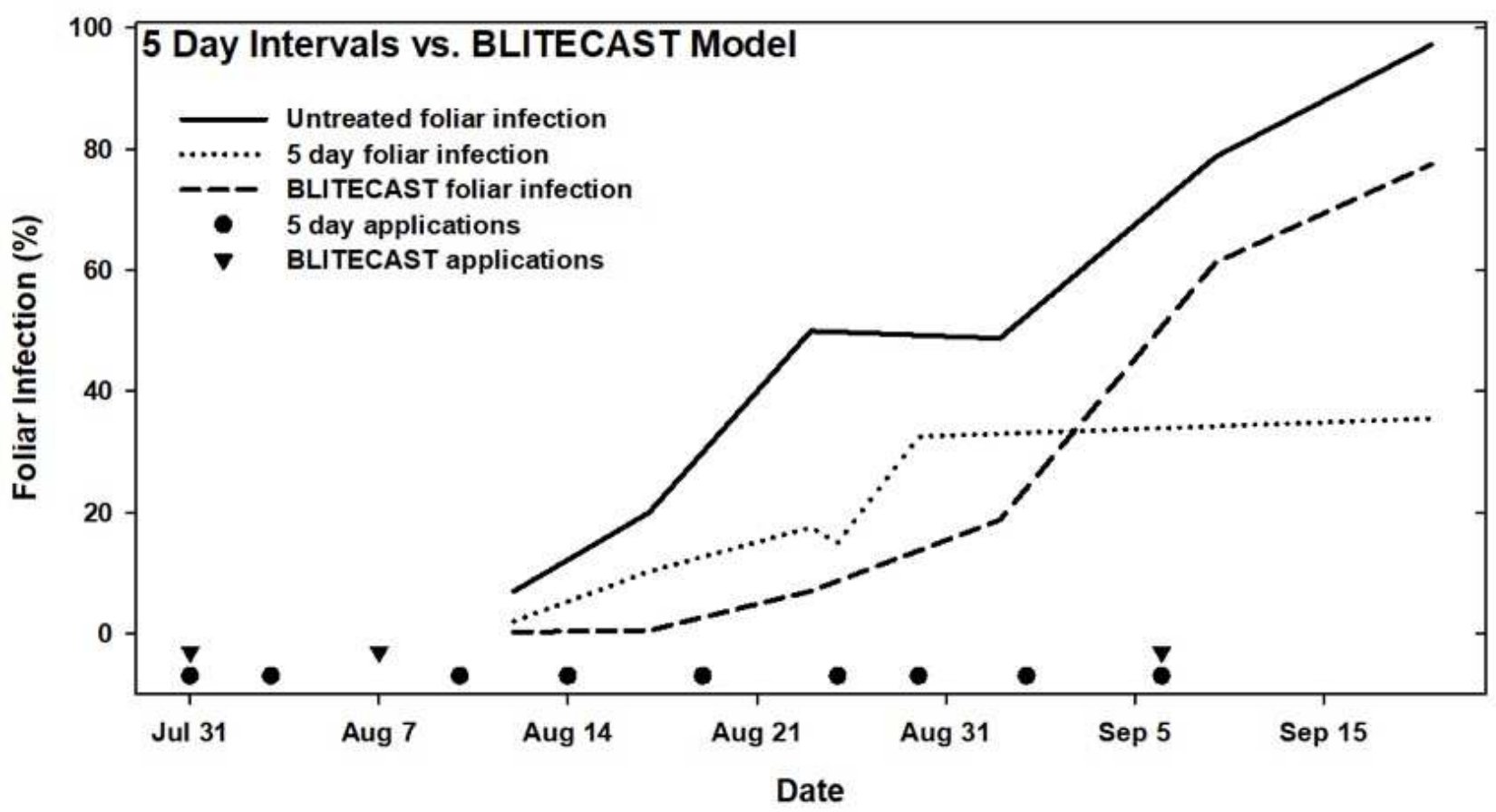


Figure 20. The foliar necrosis progression over the 2020 field season of the untreated control and the 5 day treatment. The triangles indicating when the fungicides applications occurred.

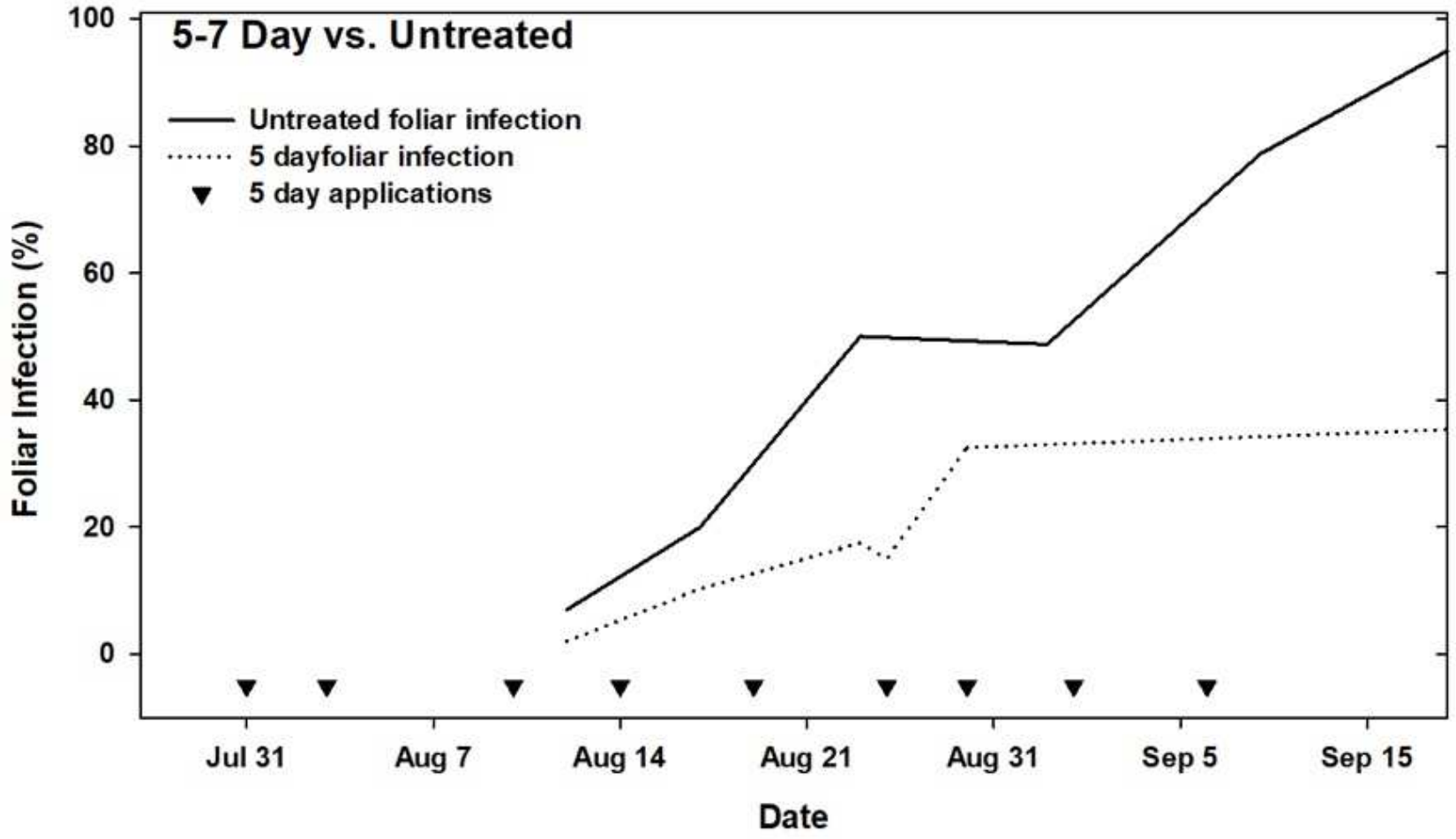


Figure 21. This shows the foliar necrosis of the untreated control, the 5-day, and TOMCAST in 2020. The application dates are indicated with circles at the bottom of the graph, and the triangles indicate the TOMCAST fungicide applications.

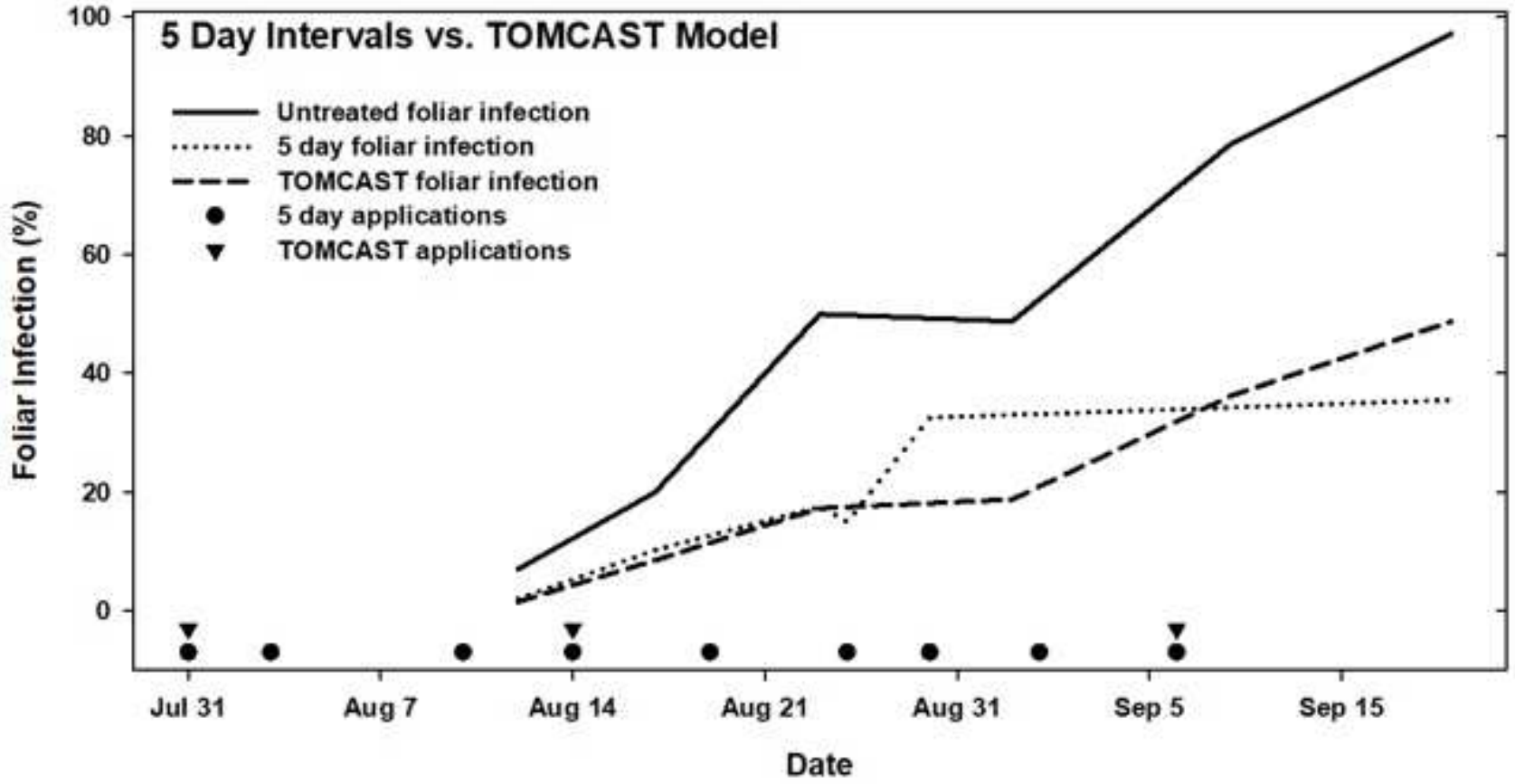
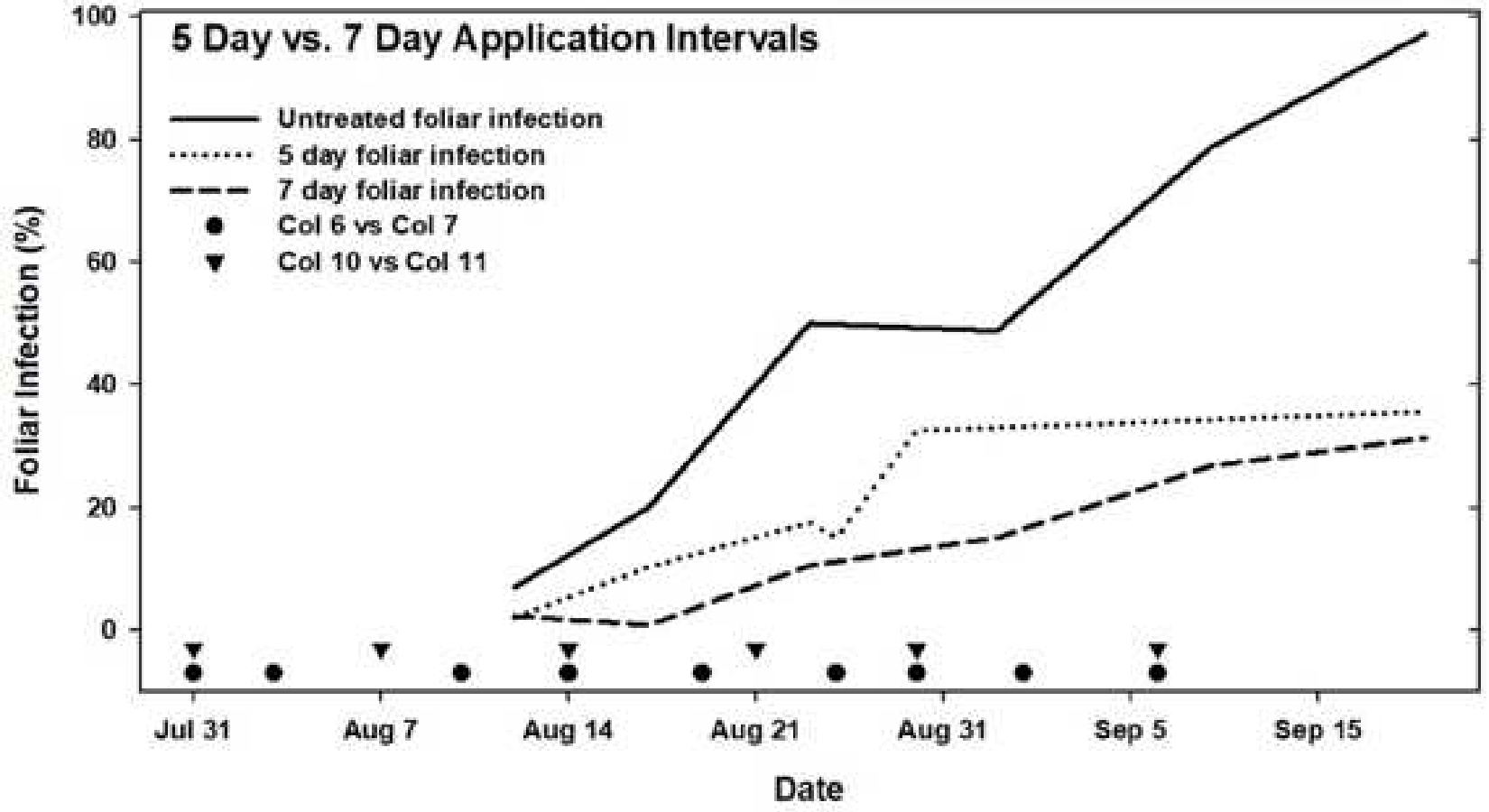


Figure 22. The graph shows the foliar necrosis over the course of the 2020 season based on the untreated control, 5-day, and 7-day application treatments. Fungicide applications are indicated for the 5 day treatment by circles, and 7 day with triangles.



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