SENSITIVITY OF *ALTERNARIA* SPECIES TO TEN SINGLE-SITE MODE OF ACTION FUNGICIDES

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Early blight caused *by Alternaria solani* and brown spot caused by the small-spored *Alternaria* spp., *Alternaria alternata, Alternaria arborescens,* and *Alternaria tenuissima* are observed annually in midwestern potato production areas. The use of foliar fungicides remains a primary management strategy. However, *Alternaria* spp. have developed reduced-sensitivity and/or resistance to many single-site fungicides such as quinone outside inhibitor (QoI), succinate dehydrogenase inhibitor (SDHI), and anilinopyrimidines (AP) fungicides in recent years.

Boscalid, fluopyram, solatenol, and adepidyn are EPA-registered SDHI fungicides applied commercially to a variety of crops including potato. High intrinsic activity was observed in fluopyram, solatenol and adepidyn to *A. solani* isolates. Adepidyn and solatenol reduced disease severity caused by *A. solani* in field evaluations. Molecular characterization of 2018 *A. solani* field isolates determined that the frequency of the D123E and H134R SDH-mutations increased. In contrast, the H278R/Y and H133R SDH mutations were found at low frequency.

Adepidyn demonstrated the highest intrinsic activity against the small-spored *Alternaria* spp. but high intrinsic activity was also observed with boscalid, fluopyram, and solatenol. In vivo experiments demonstrated that adepidyn, solatenol, and fluopyram were more effective at managing *A. arborescens* and *A. tenuissima* than boscalid. Under greenhouse conditions, adepidyn and solatenol reduced brown spot severity caused by *A. alternata* to a greater extent than did fluopyram and boscalid. Results of these studies determined that accurate pathogen identification of small-spored *Alternaria* spp. may be important for brown spot management.

Fludioxonil and cyprodinil exhibited a higher efficacy against of *A. solani* isolates when compared to pyrimethanil in greenhouse assays. Fludioxonil and cyprodinil were also highly

efficacious against the *Alternaria* spp. evaluated and appear to be a good addition into fungicide rotation programs for early blight and brown spot management. Anilinopyrimidine (AP) (pyrimethanil and cyprodinil) and phenylpyrrole (PP) (fludioxonil) fungicides have demonstrated high intrinsic activity against other pathogens. Determining the efficacy of these fungicides on *Alternaria* spp. is important to the potato industry.

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"If we knew what we were doing, it wouldn't be called research"—A. Einstein

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Pathogen Introduction

Alternaria spp. are ubiquitous and can be destructive plant pathogens. *Alternaria solani* Sorauer and *Alternaria alternata* (Fr.) Keissler are among the few economically important species of *Alternaria*. *Alternaria solani*, *Alternaria alternata*, *Alternaria arborescens* E.G. Simmons and *Alternaria tenuissima* (Kunze) Wiltshire are all ascomycete fungal pathogens. No teleomorphs have been found in any of these *Alternaria* spp. (van der Waals et al., 2004), however, high levels of genetic diversity have been suggested to have occurred through parasexual activity (van der Waals et al., 2004). Mutations also may be contributing to the high level of genetic diversity, as *A. solani* and the small-spored *Alternaria* spp. can produce a high number of spores in a short time period (Petrunak and Christ, 1992).

Alternaria solani was first described in 1882 and was originally named *Macrosporium solani*. In 1897 the name was changed to *A. solani*. *Alternaria solani* affects plants in the Solanaceous family such as pepper, eggplant, and nightshade but are found primarily and are economically important in potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) and can be further classified as either genotype I or genotype II (Ellis, 1971; Leiminger et al., 2013; van der Waals, 2001). *Alternaria solani* can be found in most potato production fields throughout the United States (Wharton et al., 2011). The primary determinant of susceptibility in Solanaceous crops is the age of the plant or how quickly the plant will mature (Rotem, 1994). *Alternaria solani* is more severe near the end of the growing season due to the pathogen preference of infecting senescing tissue on the lower canopy (Ferrin, 2015; van der Waals, 2001).

Alternaria alternata has been identified on over 380 hosts (Farr and Rossman, 2016). Diseases caused by *Alternaria alternata* are known by different names based on the host plant affected. *Alternaria alternata* infection on potato, tomato, and citrus is known as brown spot, while infection on pomegranate is known as black spot. Infection on banana, sorghum, and walnut by *A. alternata* is known as leaf spot or leaf blight. In tomato, *A. alternata* can cause up to 20% yield loss; however, there have been reports of 70-80% yield loss in potato fields (Miles and Fairchild, 2013; Soleimani and Kirk, 2012; van der Waals, 2001). Disease severity caused by *A. alternata* on potato tubers, known as black pit, has been reported in storage at around 10% (Boyd, 1972).

Alternaria arborescens was first described in 1975 and was originally classified as *Alternaria alternata* (Fr.) Keissler f. sp. *lycopersici*, but in 1999 the name was changed to *A. arborescens* (Simmons, 2000; Simmons, 2007). *Alternaria arborescens* causes infection on tomato known as stem canker disease, which causes dark-brown to black colored lesions on the lower stem (Gilchrist and Grogan, 1975). *Alternaria arborescens* was first described on potato in 2015 (Tymon et al., 2016).

Alternaria tenuissima was first described in 1818 as *Helminthosporium tenuissimum*, it was changed to *Macrosporium tenuissimum* in 1832, and was officially classified as *Alternaria tenuissima* (Nees & T. Nees: Fr.) Wiltshire in 1933 (Simmons, 2007). *Alternaria tenuissima* causes leaf spotting disease on blueberry and Alternaria blight or brown spot on potato (Milholland, 1973; Zheng and Wu, 2013).

All three of these small-spored *Alternaria* spp. (*A. alternata, A. arborescens,* and *A. tenuissima*) make up the *Alternaria* complex that causes *Alternaria* late blight on pistachio and have been identified as casual agents of brown spot on potato in the Pacific Northwest (Avenot

and Michailides, 2015; Tymon et al., 2016). They are considered opportunistic pathogens (Karaoglanidis et al., 2011). All four *Alternaria* spp. (*A. solani*, *A. alternata*, *A. arborescens*, and *A. tenuissima*) prefer warm and wet environments, resulting in high disease pressure in many potato and tomato growing regions (Ferrin, 2015; Soleimani and Kirk, 2012). In the Midwest, brown spot caused by the small-spored *Alternaria* spp., *Alternaria alternata* has been reported with increasing frequency in commercial potato fields (Fairchild et al., 2013; Ding et al., 2018). This increase is most likely due to the Midwest's optimum weather including the warm summer days and cool nights with dew formation and the increased potato production.

Taxonomy and Etiology

Alternaria is considered one of the most diverse genera of plant pathogens (Weir et al., 1998). *Alternaria* spp. are distributed worldwide. The genus *Alternaria* has been reclassified numerous times. The *Alternaria* genus was originally established in 1817 with *A. tenuis* (Rotem, 1994). This classification was later disputed and described as the genus *Macrosporium* and was differentiated from *Cladosporium*, *Helminthosporium*, and *Sporodesmium* (Rotem, 1994). After many years of debate, the genus *Alternaria* was differentiated from *Macrosporium* morphologically (Rotem, 1994). No member of the genus *Alternaria* has a known teliomorphic stage. Early *Alternaria* literature assumed that *Pleospora herbarum* (Pers.) Rabenh. was the ascogenous stage, but when the *P. herbarum* was grown from pure cultures no *Alternaria* conidia were produced (Rotem, 1994). Members of the genus *Alternaria* can be identified by their ovoid shaped conidia and further segregated into different groups based on the morphological differentiation of conidia. The formation of conidial chains, the absence or presence of beaks, as well as the length of the beaks and conidia bodies, also can be useful tools for morphological distinction.

Conidial chains of *A. alternata* are classified as Longicatenate, where long chains of up to 10 or more conidia are formed with short or no beaks (Rotem, 1994). Conidia of *A. alternata* possess extremely short or no beaks and have body lengths ranging from 25-53 µm. Conidia of *A. arborescens* form in long chains of up to 10 or more, also Longicatenate, with short or no beaks. Conidia of *A. arborescens* either can possess extremely small beaks or no beaks at all. Conidia of *A. tenuissima* form in short spore chains, referred to as Brevicatenatae, with beaks that range from short to long. Conidial body lengths of *A. tenuissima* range from 15-43 µm. Conidia of *A. solani* usually form individually, referred to as Noncatenatae, and have long beaks (Rotem, 1994). *Alternaria solani* conidial beak lengths range from 80-118 µm and have a conidial body length ranging from 83-114 µm.

Morphological classification is an unreliable method for species identification, and examples of misidentification have been particularly noted among the small spored *Alternaria* spp. (Roberts et al., 2000; Tymon et al., 2016). There are species of *Alternaria* that do not fit perfectly into one category, for example, *Alternaria brassicae* has been reported to form very short chains in culture, but not on host plants and has been placed in the Noncatenatae category. Overlap in morphological features can make it difficult to determine the identity of the *Alternaria* spp. solely through morphological methods. However, beak and conidial body lengths particularly *A. alternata*, are highly variable and do not always fall within the described ranges. This variability likely has been the culprit for many misidentifications, as is often the case with *A. tomatophila* misidentifications as *A. solani* (Simmons, 2000). Conidia from *A. tomatophila* and *A. solani* can be hard to differentiate because the beak lengths overlap. *Alternaria tomatophila* beak lengths can range from 99-217 µm, and *A. solani* range from 80-118 µm

(Gannibal et al., 2014). The conidia body length also has overlapping ranges for both *A. solani* and *A. tomatophila* at 109-115 μm and 83-114 μm, respectively.

Symptomatology

Alternaria solani, A. arborescens, A. tenuissima, and *A. alternata* are all polycyclic pathogens which produce small necrotic lesions on foliage and stems that can eventually coalesce. These pathogens can also infect tubers. Early blight of potato, caused by *A. solani,* is primarily a foliar disease, but can cause symptoms on tubers. Foliar symptoms appear as dark brown, circular lesions resembling a target board (Franc and Christ, 2001; van der Waals, 2001). Early blight lesions are initially restricted by the leaf veins, but over time may coalesce. Toxin production may result in chlorotic halos around lesions (van der Waals, 2001). Foliar early blight symptoms on potatoes begin to intensify at the flowering stage (van der Waals, 2001). Lesions are usually found on older leaves first, as the pathogen prefers senescing tissue. Therefore, earlier maturing plants, or those with nutrient or some other stress, tend to be more susceptible, which is where the disease name early blight originated (Thomas, 1948; van der Waals, 2001).

Tuber symptoms of early blight mainly appear in storage. The tubers display irregular grey, brown, purple, or black sunken lesions with elevated borders (Franc and Christ, 2001). Tuber infections generally occur when wounds inflicted during harvest come into contact with infected foliage (Rotem, 1994). Using suberization and wound periderm formation, tubers surface wounds can heal within two days when humidity is high and the temperature is above 15°C to prevent pathogen invasion (Nnodu et al., 1982; Rotem, 1994). However, while most commercial storage facilities keep a relatively high humidity, the temperatures are usually a bit lower than that needed for rapid tuber wound healing.

Alternaria alternata causes brown leaf spot of potato and black pit on tubers (Pearson and Hall, 1975). *Alternaria alternata* is an opportunistic pathogen that is commonly found on wounded or stressed host plants. Foliar symptoms can be mistaken for those of *A. solani*. The pathogen causes small brown-black lesions about 2 to 3 millimeters in diameter on both the foliage and tuber (Pearson and Hall, 1975). These small lesions can coalesce making them appear similar to early blight but lack the chlorotic halo commonly associated with *A. solani* infection. Brown leaf spot symptoms are observed earlier in the season than early blight and are more predominant in the upper canopy, while early blight is more frequent in the lower canopy (Ding et al., 2018).

Alternata arborescens causes stem canker disease on tomato and can also cause infection on the leaves and fruits of susceptible tomato cultivars (Gilchrist and Grogan, 1975). *Alternaria arborescens* was isolated from symptomatic tissue associated with other tomato diseases such as black mold of tomato, which causes sunken black lesions on the fruit (Morris et al., 2000). *Alternaria arborescens* produces an AAL toxin (originally, *Alternaria alternata* f. sp. *lycopersicum*), that causes many of the symptoms in susceptible tomato cultivars such as the foliar symptoms, fruit lesions, and stem cankers (Tymon et al., 2016). *Alternaria arborescens* causes brown spot on potato and is often misidentified as *A. alternata* (Tymon et al., 2016).

Alternaria tenuissima causes disease on several plant spp., such as tomato leaf spot, and foliar diseases in China on pepper, blueberry, potato, and wheat (Zheng et al., 2015). *Alternaria tenuissima* is an opportunistic pathogen that causes small brown-black lesions on the foliage and stems (Agamy et al., 2013). *Alternaria tenuissima* is commonly found with other *Alternaria* pathogens such as *A. alternata* or *A. arborescens* on pistachio and potato (Ma et al., 2003; Tymon et al., 2016).

Alternaria species have been reported in every region around the globe (Rotem, 1994). Conidia of *A. solani*, *A. alternata*, *A. tenuissima* and *A. arborescens* produced from early blight or brown spot lesions are spread to new plants via wind, rain or irrigation, humans, animals and machinery, among other things (Chaerani and Voorrips, 2006). The larger conidial bodies and longer beaks of *A. solani* allow them to float in the air and travel further compared to the small-spored *Alternaria* spp. (Gregory, 1973). Dispersal of conidia of all these *Alternaria* spp. is high during drier, warmer, and windier conditions. The conidia attach to leaf tissue, germinate and germ tubes penetrate the epidermis directly or through the stomata and other natural openings or wounds (Rotem, 1994). Conidia are produced on conidiophores when environmental conditions are favorable. These pathogens overwinter in the plant debris from the previous season, as well as on seeds (van der Waals, 2001). When early blight and brown spot lesions expand on plant foliar tissue, photosynthesis is inhibited as the leaves become chlorotic and eventually necrotic (Rotem, 1994).

Biology of *Alternaria*

The most *Alternaria* species are considered to be diurnal sporulators, which describes a pathogens ability to adapt to fluctuations of light and temperature (Rotem, 1994). *Alternaria solani* is a diurnal sporulating fungus where sporulation is influenced by two phases. The first is known as the inductive phase when conidiophores are formed. The second terminal phase leads to the formation of conidia (Rotem, 1994). The optimum sporulation temperature for *A. solani* is 20°C with 16h of light plus eight hours of darkness (Douglas, 1972). For *A. solani* in the field, sporulation is heaviest when rain or heavy dew occurs (Rotem, 1994). *Alternaria solani* germination has been shown at 2°C if the duration of the wet period is long enough (Rotem,

1994). *A. alternata, A. arborescens,* and *A. tenuissima* do not require these two phases for sporulation (Rotem, 1994).

Conidia from all known *Alternaria* spp. can begin to form when conidiophores are stressed (Rotem, 1994). Stress occurs when dryness is induced, temperature is reduced, or exposure of the conidiophores to brief near ultraviolet light (NUV). However, stressing conidiophores may not be enough to induce sporulation. *Alternaria solani* can produce conidia in the tens of thousands, and *A. alternata, A. arborescens,* and *A. tenuissima* in the hundreds of thousands over the same of growing time (Rotem, 1994). The optimum temperature for *A. alternata* sporulation is 27°C and it does not require any free moisture or alternating periods of light and dark (Pearson and Hall, 1975). *Alternaria alternata* has a very similar life cycle to *A. solani*. The conidia are dispersed by wind, water, and other forms of movement (Chaerani and Voorrips, 2006). However, due to the lack of beaks on the *A. alternata* conidia, the pathogen does not travel as far in the wind and has been shown to cause heavy infection in a single location. Like *A. solani, A. alternata* can also overwinter in infected tubers, crop debris, and alternative hosts (Rotem, 1994).

A wide variety of toxins are produced by select *Alternaria* spp., for instance Alternaric acid, a non-host specific toxin, has been shown to be produced by some isolates of *A. solani* (van der Waals, 2001). Solanapyrone A, B, and C were also identified as toxins produced by *A. solani* that, along with alternaric acid, can induce necrotic symptoms similar to early blight (Chaerani and Voorrips, 2006). There is no apparent correlation between the strain virulence and toxin production (van der Waals, 2001). Toxin production also has been observed in the small spored *Alternaria* spp. and has been used to differentiate species (Andersen et al., 2002). *Alternaria arborescens* is known to produce two AAL toxins, as well as alternariol, alternariol

monomethylether, and tenuazonic acid. Alternariol (AOH), alternariol monomethylether (AME), and tenuazonic acid are mycotoxins that have carcinogenic properties in vitro and can cause low acute toxicity in animals (Andersen et al., 2002; Paterson and Lima, 2015). *Alternaria tenuissima* produces AOH, AME, tenuazonic acid, and tentoxin. Tentoxin can cause chlorosis in germinating seedlings and can inhibit ATP synthesis and hydrolysis (Paterson and Lima, 2015).

Epidemiology

Many solanaceous plants such as hairy nightshade (*Solanum nigrum* L.), horse nettle, (*Solanum carolinensis* L.), and chili (*Capsicum frutescens* L.) can be hosts for *A. solani*, but the most economically important hosts are potato, tomato, and eggplant (van der Waals, 2001). There also have been records of non-solanaceous hosts such as cucumber (*Cucumis sativus* L.), zinnia (*Zinnia elegans Jacq*.) and wild cabbage (*Brassica oleracea* L.) (van der Waals, 2001). *Alternaria solani* has been reported globally in all potato growing regions from Iceland, to Argentina, to Africa, but is more prevalent in the temperate and tropical zones (Ellis, 1971; Tsedaley, 2014). *Alternaria solani* is widely distributed throughout the United States, but is not commonly seen in dryer southern and far western states (van der Waals, 2001). This is because Midwestern areas have frequent dew formation at night, allowing the wet-dry periods to occur more often (Pasche et al., 2004). *Alternaria solani* infection is more frequent in warmer environments (Rotem, 1994).

Alternaria alternata is a necrotroph with endophytic tendencies, making it a common secondary colonizer found on damaged plants. It is globally distributed, with a very wide host range. It has been found on citrus tree leaves in Peru (Marín et al., 2006) and China (Wang et al., 2010), and causing Alternaria late blight of pistachio in Australia (Ash and Lanoiselet, 2001) and California (Avenot and Michailides, 2007), and Alternaria leaf blight on *Gloriosa superba* in

India (Maiti et al., 2007), Alternaria black spot of pomegranate in Israel (Ezra et al., 2009), and Alternaria leaf spot on English walnut (Belisario et al., 1999).

Alternaria arborescens is a necrotroph that is a common secondary colonizer found in conjunction with foliar pathogens. This pathogen is most commonly associated with stem canker of tomato but it has also been isolated from pistachio (*Pistacia vera* L.), apple (*Malus domestica* Borkh), English walnut (*Juglans regia* L.), common hazel (*Corylus avellane* L.), cherry (*Prunus avium* L. (L.)), barley (*Hordeum vulgare* L.), pear (*Malinae pyrus* L.), and rice (*Oryza sativa* L.) (Andersen et al., 2002; Andrew et al., 2009; Akhtar et al., 2014; Ma et al., 2003).

Alternaria tenuissima is a necrotroph that is also a common secondary pathogen found with other foliar pathogens and has been isolated from hosts such as pistachio (*Pistacia vera* L.), apple (*Malus domestica* L.), English walnut (*Juglans regia* L.), common hazel (*Corylus avellane* L.), cherry (*Prunus avium* L. (L.)*)*, barley (*Hordeum vulgare* L.), pear (*Malinae pyrus* L.) grapefruit (*Citrus paradise* L.), and tobacco (*Nicotianeae nicotiana L.*) (Andersen et al., 2002; Andrew et al., 2009; Ma et al., 2003).

Disease Management

Cultural management

Both cultural and chemical management strategies can be implemented to protect crops from infection by *Alternaria* spp. Crop rotation and proper plant nutrition are examples of the cultural protection practices (van der Waals, 2001). Well-drained fields and nutrient rich soil can also reduce the susceptibility to diseases caused by *Alternaria* spp.. High doses of nitrogen, phosphorus, and potassium added in the soil proved to reduce early blight in tomatoes and potatoes in greenhouse conditions (Rotem, 1994). However, the addition of these nutrients into the field had little to no effect. The use of overhead irrigation systems can increase the

development of early blight by extending the leaf wetness period required for conidial dispersion, germination, and sporulation (van der Waals et al., 2004). Therefore, to reduce early blight infection, irrigation should be applied as to not extend the wet period. Forage crops and grains are the best crops to use in a three- to five-year crop rotation (Chaerani and Voorrips, 2006; van der Waals, 2001). However, crop rotation is relatively useless for *A. alternata* because of its wide host range. *A. solani, A. alternata, A. arborescens,* and *A. tenuissima* can survive in the previous season's debris; therefore, the removal or burial of infected plant debris can be effective to reduce disease severity. The removal of weed hosts also can reduce inoculum. Pathogen infection on tubers mainly occur after harvest and placed into storage where the flesh wounds are vulnerable. Therefore, minimizing injury to tubers and fruits during harvest is the best way to prevent storage rot.

While there are many cultural practices that can aid in the management of early blight and brown spot, foliar fungicide applications have had the most success in overall control of *A. solani* (Pasche and Gudmestad, 2008). However, due to the expense of fungicide applications, the effect on the environment and the development of fungicide resistance, it is recommended that fungicides only be applied using an integrated pest management strategy (Bradley and Pedersen, 2011). With the use of fungicides, cultivar resistance is also important to consider, although, cultivar resistance to early blight has not been well characterized and cultivar resistance to brown spot is currently unknown (Pelletier and Fry, 1990). Temporary resistance can be observed by using late-maturing potato varieties (Rotem, 1994). Plants producing abundant foliage and a lower yield have been shown to be more resistant to the pathogen, but this is not a viable management practice (Rotem, 1994).

Fungicides

The most common and effective early blight and brown spot management practice is the application of foliar fungicides starting six to seven weeks after planting (Christ and Maczuga, 1989). However, fungicide resistance research has been single-site mode of action chemistries are highly effective in managing *Alternaria* spp. when compared to multi-site modes of action; however, it is important to monitor the pathogen for insensitivity or resistance (Pasche and Gudmestad, 2008). Mode of action refers to the specific cellular process inhibited by a particular fungicide. Fungicides with similar modes of action are grouped by the Fungicide Resistance Action Committee (FRAC) to aid in prolonging fungicide efficacy (FRAC, 2019). To reduce the development of fungicide resistance, the use of tank mixtures and alternations of multi-site modes of action paired with single-site fungicides have been recommended (Horsfield et al., 2010). The use of single-site fungicides throughout a growing season is effective at reducing disease severity, but it is costly and resistance can develop quickly (Pasche et al., 2004; Shahbazi et al., 2010). Therefore, using a combination of protectant fungicides (multi-site mode of action fungicides) as well as specialty fungicides (single-site mode of action fungicides) has been shown to be more effective at providing early blight disease management (Yellareddygari et al., 2016). Specialty fungicides, such as quinone-outside inhibiting (QoI) and succinate dehydrogenase inhibiting (SDHI), utilized in rotation with standard protectant fungicides such as mancozeb or chlorothalonil, can increase early blight disease control and potato yield (Yellareddygari et al., 2019). Protectant fungicides were found to be less effective during the latter part of the growing season for early blight management, whereas specialty fungicides are effective for the entire season (Yellareddygari et al., 2018). The utilization of both protectant and single-site fungicides for early blight management is important, but the fungicide application

timing can also influence the effect on yield. During the early bulking stages of potato growth (stages 3-4) each percentage increase in early blight disease severity results in greater yield loss, when compared to the same increase in disease severity at the late bulking stages (stages 4-5). Therefore, the application of specialty fungicides are more important during early bulking.

Protectant fungicides

Protectant fungicides have a multi-site mode of action and are generally less expensive than the single-site specialty fungicides (Yellareddygari et al., 2016). Multi-site fungicides also have a lower risk of developing resistance but are less effective when utilized under high disease pressure (Gudmestad et al., 2013; Pasche and Gudmestad, 2008). Chlorothalonil is a multi-site dithiocarbamate fungicide that inhibits the formation of the sulfur-containing enzymes (Sujkowski, 1995). Mancozeb is a common ethylenebisdithiocarbamates (EBDC) fungicide that works by breaking down to cyanide, in-turn interfering with the sulfhydryl groups in the pathogen (Georgopolus, 1977). Most foliar application programs utilize both multi-site and specialty fungicides to manage any potential foliar pathogen threats.

Specialty fungicides

Specialty fungicides Fungicide resistance in plant pathogenic fungi has become a major threat in crop production since the introduction of the single-site fungicides

Quinone outside Inhibitors (QoI)

Quinone outside Inhibiting fungicides (QoI) have a single-site mode of action that causes an interruption of the electron transport system of the cytochrome bc1 complex (cytb gene) (Avenot and Michailides, 2010). QoIs were derived from a natural fungicidal chemical, β-methoxyacrylic acid, produced by a range of Basidiomycete wood-rotting fungi (Bartlett et al., 2002). QoI fungicides belong to FRAC group 11 and are commonly used for broad-spectrum

protection against a variety of plant pathogens belonging to the Ascomycota, Basidiomycota, and Oomycota (Bartlett et al., 2002; Fairchild et al, 2013).

In 1998, an emergency exemption was granted by the Environmental Protection Agency (EPA) under section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act. This exemption was for the use of the QoI fungicide azoxystrobin for the control early blight of potato in Minnesota, North Dakota, Nebraska, and Wisconsin (Federal Register, 1998; Pasche et al., 2005). Azoxystrobin received full registration in the United States for potato in 1999 (Pasche et al., 2005). That year, azoxystrobin was the world's most widely sold fungicide, grossing approximately $415 million (Bartlett et al., 2002).

Other QoI chemistries registered on solanaceous crops include famoxadone, fenamidone, trifloxystrobin, and pyraclostrobin, registered in 2003, 2004, 2001, and 2002, respectively (FRAC, 2019). Famoxadone and fenamidone, unlike azoxystrobin, trifloxystrobin, and pyraclostrobin, are non-strobilurin QoI fungicides belonging to the subclass oxazolidinedione (FRAC, 2019). Famoxadone and fenamindone have the same mode of action as the strobilurin QoI fungicides, but are structurally different.

All QoI fungicides are classified as high-risk for resistance development due to the single-site mode of action (FRAC, 2019). *Alternaria solani* and *A. alternata* are classified as medium- and high-risk pathogens, respectively, due to the single-site point mutation that can occur (FRAC, 2019). In 2000, reduced sensitivity of azoxystrobin was observed in *A. solani* isolates collected from potato fields in Nebraska. A reduction in early blight disease control by azoxystrobin was detected in North Dakota and Minnesota in 2001 and reduced sensitivity was confirmed in the pathogen (Pasche et al., 2005). By 2006 it was prevalent in all United States potato production areas (Pasche et al., 2004; Pasche et al., 2005; Pasche and Gudmestad, 2008).

Reduced-sensitivity/resistance to QoI fungicides has been detected among a variety of pathogens in addition to *A. solani* (Pasche et al., 2004) including *Venturia inaequalis* (Lesniak et al., 2011), *Botrytis cinerea* (Ma and Michailides, 2005), and *Ascochyta rabiei* (Wise et al., 2008). The reduction in fungicide sensitivity in *A. solani* is due to a single-point mutation resulting in an amino acid shift from phenylalanine with leucine at position 129 (F129L) in the cytb gene in the mitochondria. There are three codon combinations that can result in the F129L mutation. TTA and CTC are the two most common codon combinations, and TTG is the least common (Rosenzweig et al., 2008). *Alternaria solani* isolates possessing the F129L mutation display reduced-sensitivity to the fungicide, resulting in a 12- to 15- fold reduction in sensitivity to azoxystrobin and pyraclostrobin, respectively (Pasche et al., 2005; Pasche and Gudmestad, 2008).

Resistance to QoI fungicides in *Alternaria alternata, A. arborescens*, and *A. tenuissima* is the result of the G143A mutation (Ma et al., 2003). The G143A mutation (substitution of glycine with arginine at position 143 in the cytb gene) cause complete resistance. The G143A mutation conveyed a 1143- fold reduction in sensitivity to azoxystrobin in *A. alternata* isolates (Karaoglanidis et al., 2011). Cross resistance with other QoI fungicides was confirmed in *P. grisea* isolates that contained the G143A mutation but not isolates with the F129L mutation (Kim et al., 2003).

In European isolates of *A. solani*, there are two different genotypes within the cytb gene structure, genotype I and genotype II. The F129L mutation is not restricted to either genotype (Leiminger et al., 2013). Isolates evaluated from Europe were found to be genotype I and did not possess the F129L mutation and are the most prevalent genotype (Leiminger et al, 2013). Genotype II isolates, which are the more prevalent in the United States possessed the F129L

mutation (Bauske et al., 2018). The F129L mutation is not restricted to a certain genotype but in genotype II isolates an intron between codon 131 and 132 was found, which the genotype I isolates lack (Leiminger et al., 2013).

Succinate Dehydrogenase Inhibitors (SDHI)

Succinate dehydrogenase inhibiting fungicides (SDHI) inhibit the succinate dehydrogenase (Sdh) at complex II in the mitochondrial respiratory chain (Avenot and Michailides, 2010). Due to this site of action there is no cross-resistance between other chemical classes and the SDHIs (Avenot et al., 2008). SDHIs were first registered in the United States in 2003 and belong to FRAC group 7 (Avenot et al., 2014; Miles et al., 2014). Due to the single-site mode of action of the SDHI fungicides, they are at medium- to high-risk for resistance development (De Miccolis Angelini et al., 2014). The use of SDHIs have grown in popularity since the development of resistance to QoIs (Miles et al., 2014).

Currently, there are 19 SDHI active ingredients that have been used on a variety of crops to manage various pathogens (Stevenson et al., 2019). Boscalid was registered on potatoes to manage early blight in 2005. Resistance was reported in *A. solani* isolates recovered from Idaho in 2009 (Wharton et al., 2012) and was prevalent throughout most of the United States by 2012 (Gudmestad et al., 2013). Fluopyram was registered in 2012 to manage early blight on potato. Reduced sensitivity has developed to SDHI fungicides boscalid and fluopyram in a variety of pathogens such as *A. alternata* (Avenot et al., 2014), *A. solani* (Pasche et al., 2005, Bauske et al., 2018), *B. cinerea* (Fernández-Ortuño et al., 2013), *Blumeriella jaapii* (Outwater et al., 2019), and *Didymella bryoniae* (Thomas et al., 2012). Currently, reduced sensitivity to boscalid and fluopyram has been reported in isolates of *A. alternata, A. solani, B. cinerea,* and *P. xanthii* (Avenot et al., 2008; Gudmestad et al., 2013; Miazzi and McGrath, 2008).

Two SDHI fungicides, solatenol (benzovindiflupyr) and adepidyn (pydiflumetofen), were registered for use on potato in 2015 and 2018, respectively. Resistance/reduced-sensitivity have not been reported to these fungicides.

The Sdh complex is made up of four subunits: a flavoprotein (SdhA), an iron sulfur protein made up of three iron-sulfur clusters (SdhB), and two membrane anchored proteins (SdhC and SdhD) (Avenot and Michailides, 2010). There are five single point mutations known to occur in the three *AsSdh* genes (*A. solani* succinate dehydrogenase) that cause SDHI resistance in *A. solani* (Mallik et al., 2014). Two mutations are known on the *AsSdhB* gene. The first is an exchange of a histidine (H) for a tyrosine (Y) at position 278, known as H278Y, and the second is a histidine (H) exchanged for an arginine (R) at position 287, known as H287R. The H287Y mutation was found in isolates that displayed a moderate, high, or very high resistance to boscalid and penthiopyrad (Mallik et al., 2014). The H278R mutation was found in isolates that displayed a moderate resistance to boscalid and penthiopyrad (Mallik et al., 2014). The mutations on the *AsSdhB* gene are slightly different in *A. solani* (H278R and H278Y) compared to the corresponding *AaSdhB* gene in *A. alternata* (H277R and H277Y). A single mutation is known in the *AsSdhC* gene, the exchange of histidine (H) to arginine (R) at position 134 (H134R) and the same mutation on the *AsSdhC* gene (H134R) can be found in the corresponding *AaSdhC* gene (Avenot et al., 2009). *Alternaria solani* isolates carrying this mutation are moderately to very highly resistant to boscalid and highly to very highly resistant to penthiopyrad (Mallik et al., 2014). The final two mutations are located on the *AsSdhD* gene. The first is an exchange of a histidine (H) to an arginine (R) at position 133 (H133R). Isolates containing this mutation display moderate to very high resistance to boscalid and high to very high resistance to penthiopyrad (Mallik et al., 2014). The final mutation on the AsSdhD gene is

the exchange of an aspartate (D) to a glutamic acid (E) at position 123 (D123E). These isolates fall into the very high resistance category for boscalid and penthiopyrad (Mallik et al., 2014). The same two mutations on the *AsSdhD* gene (D123E and H133R) can be found in the corresponding *AaSdhD* gene (*A. alternata* succinate dehydrogenase D gene) (Avenot et al., 2009).

Fluopyram, has been shown to encourage the development of the highly aggressive D123E SDH mutation (Bauske et al., 2018). It is believed that since fluopyram is a fluorinated SDHI, it binds differently in the mitochondrial complex (Gudmestad et al., 2013). The potential development of cross resistance within this class of fungicides is highly concerning in regards to the *Alternaria* spp..

Demethylation inhibitors (DMIs)

Demethylation inhibitors (DMIs) were first introduced in the 1970s and are classified as sterol biosynthesis inhibitors (FRAC Group 3) (Thomas et al., 2012). DMIs work by inhibiting the biosynthesis of ergosterol, a necessary element in the plasma membrane of certain fungi (Kunz et al., 1997). Difenoconazole and metconazole are single-site fungicides first registered on potato in 2011 and have been effective at managing *A. alternata* and *A. solani* (Fonseka and Gudmestad, 2016). Reduced-sensitivity in metconazole has been detected in *Colletotrichum truncatum*, while other species (*Colletotrichum fructicola, C. siamense, C. nymphaeae*, and *C. fioriniae*) remained sensitive (Chen et al., 2016). However, the previously listed *Colletotrichum* spp. were sensitive to difenoconazole (Chen et al., 2016). DMI resistance has been reported in other fungi, in the Ascomycota, including *V. inaequalis*, *Mycosphaerella graminicola*, and several powdery mildew pathogens (Thomas et al., 2012). Currently, there is no known resistance or reduced sensitivity of the DMI chemical class to the *Alternaria* spp..

AnilinoPyrimidines (AP)

Anilinopyrimidines (AP) fungicides have a single site mode of action and belong to FRAC group 9 (Latorre et al., 2002; Miles et al., 2014). Anilinopyrimidine fungicides inhibit the methionine biosynthesis and other amino acids in pathogens and were found to inhibit the secretion of hydrolytic enzymes in *B. cinerea*, though the primary mode of action has not yet been resolved (Cabras et al., 1997; Fernández-Ortuño et al., 2013; Petsikos-Panayotarou et al., 2003).

Pyrimethanil was originally registered in the United States in 2004 as a postharvest fungicide for pome fruits and was registered for early blight disease control for potato in 2005 (Li and Xiao, 2008). Pyrimethanil is considered a medium-risk resistance fungicide (Li and Xiao, 2008). When used in rotation with other chemistries, AP fungicides including pyrimethanil have been successful in preventing disease on pathogens such as *B. cinerea* and *V. inaequalis* (Köller et al., 2005; Zhao et al., 2010). Over time, resistance to AP fungicides has been detected in *B. cinerea* (Fernández-Ortuño et al., 2013), *Penicillium expansum* (Xiao et al., 2011), and *V. inaequalis* of apple (FRAC, 2019). *Alternaria solani* and *A. alternata*, isolates with reduced-sensitivity to pyrimethanil have been only found in recent years at a low frequency; therefore, pyrimethanil is still regarded as effective in the management of early blight and brown spot (Fairchild et al., 2013; Fonseka and Gudmestad, 2016).

The AP fungicide cyprodinil was introduced in 1995 and found to have both protective and curative action against certain pathogens (Knauf-Beiter et al., 1995). As with pyrimethanil, cyprodinil has been shown to be very successful at preventing disease caused by *B. cinerea* and *V. inaequalis* and is also considered to be a medium-risk resistance fungicide (Li and Xiao, 2008).

Phenylpyrroles (PP)

Phenylpyrrole (PP) fungicides inhibit conidial germination, mycelial growth, and are reported to induce germ tube distortions and cell bursting, though the primary mode of action has not been confirmed (Fernández-Ortuño et al., 2013; Kanetis et al., 2007; Li and Xiao, 2008). It has been demonstrated fludioxonil has induced cell death by improperly activating the Hog1-type mitogen-activated protein kinase (MAPK) (Li et al., 2014). This indicates that the primary mode of action is most likely the disruption in the MAPK pathway, which disrupts mycelial growth and conidial germination (Kuang et al., 2011; Li et al., 2014).

PPs are obtained from the antibiotic pyrrolnitrin, which is produced by many *Pseudomonas* spp. (Errampalli, 2004). Fludioxonil is a protective, broad spectrum fungicide effective against fungi in the Ascomycota and Basidiomycota and was first registered in 2002 in the United States. It has a low to medium resistance risk (Li et al., 2014). Fludioxonil was first registered on potato as a seed treatment in 2002 and was then registered as a potato post-harvest fungicide in 2012 (EPA.gov). Fludioxonil has proven to be effective against the *Alternaria* spp. brassicae, brassicicola, and japonica (Iacomi-Vasilescu et al., 2004). However, fludioxonil resistance was detected in 2008 on potato in the dry rot fungus *Fusarium sambucinum* (Ganchango et al., 2011).

Summary

Alternaria spp. are a common plant and human pathogen. *Alternaria solani* causes the disease early blight on potatoes and resistance has been reported on QoI and SDHI fungicides. The small-spored *Alternaria* spp. cause the disease brown spot on potatoes and resistance has been reported on QoI and SDHI fungicides. These fungicide classes are commonly paired with multisite fungicides in early blight foliar management programs and it is important to monitor

the effectiveness of these single-site fungicides to safeguard the chemistry. Identifying additional chemicals that contain different modes of action from currently registered fungicides for the management of early blight will further aid in safeguarding the fungicides and increase the arsenal of early blight management options.

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CHAPTER ONE: IMPACT OF SDHI FUNGICIDES ON THE FREQUENCY OF SDH MUTATIONS

Abstract

Early blight caused by *Alternaria solan*i is observed annually in all midwestern potato production areas. The use of foliar fungicides remains a primary management strategy. However, *A. solani* has developed reduced-sensitivity or resistance to many single-site fungicides such as quinone outside inhibitor (QoI, FRAC group 11), succinate dehydrogenase inhibitor (SDHI, FRAC group 7), and anilinopyrimidine (AP, FRAC group 9) fungicides in recent years. Boscalid, fluopyram, solatenol, and adepidyn are EPA-registered SDHI fungicides, used commercially on a variety of crops, including potato. Conidial germination assays were used to determine if a shift in sensitivity has occurred in the SDHI fungicides. *Alternaria solani* isolates collected prior to the commercial application of SDHI fungicides (baseline) and were compared to recently collected isolates (non-baseline). Greenhouse evaluations were conducted to evaluate the efficacy of the boscalid, fluopyram, solatenol, and adepidyn. Field trials were conducted to determine the effects application of these SDHI fungicides would have on the frequency of SDH mutations. Fluopyram, solatenol and adepidyn had high intrinsic activity against *A. solani* when compared to boscalid, based on in vitro assays. The application of adepidyn and solatenol resulted in greater early blight control than the application of boscalid and fluopyram in greenhouse experiments. In field experiments, disease severity was significantly lower with all fungicide treatments at both locations compared to the non-treated control. Molecular characterization of *A. solani* isolates collected from the field trials determined that the frequency of the D123E- and H134R-mutations are increasing in response to more recently developed

SDHI fungicides. In contrast, the H278R/Y- and H133R-mutations decreased to the point of being nearly absent in these field experiments.

Introduction

Early blight caused by, *Alternaria solani*, causes economic losses in potatoes across all United States growing regions annually. Potato yield losses from 20 to 30% have been recorded in the United States during severe early blight epidemics (Christ and Maczuga, 1989; Shtienberg et al., 1990), with the greatest yield losses occurring during early bulking (growth stages III to IV, weeks 7 to 9) (Yellareddygari et al., 2018). The primary method of early blight management is through the application of foliar fungicides. Single-site mode of action fungicides such as the succinate dehydrogenase inhibitors (SDHI, FRAC group 7) are highly efficacious for managing early blight (Pasche and Gudmestad, 2008). Therefore, the application of single-site mode of action fungicides during early tuber bulking is important to limit yield losses from early blight (Yellareddygari et al., 2016; Yellareddygari et al., 2018).

SDHI fungicides were first introduced in the late 1960s and the usage in foliar application programs has been increasing. EPA-registered SDHI fungicides boscalid, fluopyram, solatenol, and adepidyn have been used commercially on a variety of crops including potato. Boscalid was registered for early blight disease control on potato in 2005 and resistance was detected in *A. solani* in 2009 and 2010 (Fairchild et al., 2013; Gudmestad et al., 2013). Fluopyram was registered on potato for early blight disease control in 2012 and reduced-sensitivity was reported in 2014, with evidence of reduced-sensitivity in greenhouse and field settings determined in 2015 (Bauske et al., 2018a; Mallik et al., 2014). Reduced-sensitivity and/or resistance to boscalid and fluopyram also has been identified in a variety of pathogens including *Alternaria alternata* (Avenot et al., 2014), *Botrytis cinerea* (Fernández-Ortuño et al., 2013), *Blumeriella jaapii*

(Outwater et al., 2019), various *Colletotrichum* spp. (Ishii et al. 2016), and *Didymella bryoniae* (Thomas et al., 2012). Solatenol and adepidyn were labeled for use on potato in 2015 and 2018, respectively. Baseline studies have been established for solatenol in *Bipolaris maydis* (Hou et al., 2018), *Colletotrichum* spp. (Ishii et al., 2016), and *Venturia inaequalis* (Villani et al., 2016). Adepidyn baseline studies have been established for *Cercospora zeae-maydis* (Neves and Bradley, 2019), *Fusarium asiaticum* (Hou et al., 2017), and *Sclerotinia sclerotiorum* (Duan et al., 2019). Currently, there has been no reports of reduced-sensitivity or resistance to either solatenol or adepidyn. Sensitivities to these fungicides have not been determined for baseline *A. solani* isolates. Without known baseline sensitivities there is no way to determine if reduced-sensitivity or resistance has developed in a fungal population.

The five single point mutations conveying reduced-sensitivity to boscalid in *A. solani* have been found on three *Sdh* genes (Mallik et al., 2014). Two mutations have been identified on the *AsSdhD* gene (D123E and H133R), one on the *AsSdhC* gene (H134R) and two on the *AsSdhB* gene (H278R and H278Y) (Mallik et al., 2014). In a 2011-2012 *A. solani* survey, *AsSdhB* H278Y- and H278R-mutants were recovered at the highest frequencies across all sampled United States potato producing regions (Mallik et al., 2014). In contrast, *AsSdhC* and *AsSdhD* H133R-, H134R-, and D123E-mutants were recovered at lower frequencies and were more regionally specific (Mallik et al., 2014). Results from a 2013-2015 survey indicated that the H134R-mutant was predominate (50, 36, 27%, respectively), while the presence of the H278Y-mutant increased over these three years (18, 38, and 40%, respectively) (Bauske et al., 2018b). The H278R-mutant decreased over the three-year period, where in 2015 only 1% of the 562 collected isolates possessed the mutation (Bauske et al., 2018b). Over the three-year period, the H133R-mutant increased slightly from 14 to 16% and the D123E-mutant increased from 4 to 12% (Bauske et al.,

2018b). No predicted or realized fitness penalties were detected among *A. solani* isolates carrying these mutations (Bauske et al., 2018c).

The objectives of this study were to determine (i) the sensitivity of an *A. solani* baseline population and non-baseline isolates collected from 2010 to 2015 to SDHI fungicides, (ii) the impact of the SDH mutations have on the efficacy of SDHI fungicides boscalid, fluopyram, solatenol, and adepidyn, and (iii) the impact of newly registered SDHI fungicides on the frequency of the SDH mutations in *A. solani* isolates under field conditions.

Materials and Methods

Alternaria solani isolates collection and maintenance

Alternaria solani isolates were recovered from foliage submitted to the Gudmestad Laboratory from potato growing regions across the United States. Fifty-seven *A. solani* isolates with no exposure to SDHI fungicides (baseline) collected from 1998 to 2002 were obtained from long-term cryogenic storage (Appendix A). One hundred and twelve *A. solani* isolates with exposure to SDHI fungicides (non-baseline) were isolated from early blight infected potato foliage submitted to the Gudmestad laboratory in 2010, 2013, and 2015. These 112 non-baseline isolates were composed of 21 to 22 *A. solani* isolates possessing each of the five known SDH mutations (H133R, H134R, H278R, H278Y, and D123E). Four isolates with no SDH mutations (SDHI-sensitive/wildtype) were also included in this non-baseline population.

Foliar sections with lesions characteristic of early blight were surface sterilized in a 10% bleach solution for one min. and rinsed in sterile, distilled water. Tissue sections were aseptically excised from the edge of the foliar lesion using a sterile scalpel blade and transferred to a 1.5% unamended agar media (water agar) and incubated at room temperature (22 ± 2°C) for three to four days until conidia were produced. Purification of the isolates was performed by transferring

a single conidium from the plate using a sterile glass needle to solid clarified V8 (CV-8) medium (Campbell's V8 juice, 100 ml; $CaCO_3$, 1.5g; agar, 15g; and distilled water 900 ml) amended with 50 mg/ml of ampicillin and incubating under 24h fluorescent light at room temperature (22 ± 2°C) for seven days (Pasche et al., 2004). To preserve isolates in long-term cryogenic storage, a 4-mm diameter sterilized cork borer was used to remove circular sections of media with *A. solani* conidia and mycelia and placed into screw-top centrifuge tubes. The caps were loosely screwed on to the tubes, tubes were labeled, and placed in a closed container with silica gel for two to three days to remove excess moisture. After drying, the tubes were capped tightly, sealed with Parafilm, and stored in a -80°C ultra-freezer. Herbarium specimens were made for each tissue sample from which *A. solani* isolates were obtained.

Characterization of SDH mutations

To identify mutations present in *A. solani* isolates, DNA was extracted using the Omega Mag-Bind® Plant DNA Plus Kit (Omega Bio-tek Inc., Norcross, GA) with the KingFisher™ Flex Purification system (Thermo Fisher Scientific Inc., Waltham, MA). Using a sterile toothpick, spores were scraped from the seven-day-old pure *A. solani* cultures into a 2 ml screw-top tube with 1 ceramic bead and 500 ml of CSPL Buffer. The tube was placed in the FastPrep instrument (MP Biomedicals) and agitated at a speed of 6.00 m/s for 40-sec to homogenize the spores and buffer. The tubes were incubated at 56°C for 30-min. while the additional buffer plates were prepared.

A total of six plates were used for the KingFisher system. The first of the five buffer plates (plate 2) contained 500 µl of CSPW1, the second buffer plate (plate 3) contained 500 µl of CSPW2 buffer. Plates 4 and 5 each contained 500 µl of SPM wash buffer and the final buffer plate (plate 6) contained 200 µl of elution buffer. After the 30-min incubation, the tubes were

centrifuged at 14,000 g for 10 min, 400 µl of supernatant was transferred to a clean 96-well plate (plate 1) and 5 µl of RNaseA was added to each well. After incubation at room temperature for 10 min, 400 µl of isopropanol and 15 µl of Mag-Bind particles were added to each well of plate 1. The plates were placed in the KingFisher and the DNA extraction program Omega Plus 1 was activated. Samples were incubated at room temperature for 5 min., vortexed for 90 sec, and rested for 90 sec. Next, the Mag-Bind® particles were collected in 5 rounds of 5 sec intervals and moved into plate 2. The beads were released into the buffer and samples were vortexed for 60 sec, rested for 30 sec, and vortexed again for 30 sec before beads were collected as previously described (5 rounds of 5 sec) and moved to plate 3. The samples and beads go through two separate rounds of SPM wash buffer (plates 4 and 5), collected, and set at room temperature for 10 min. to dry. The dry beads were released into plate 6 at 65°C and gently vortexed for 30 sec and allowed to rest for 5 min. The samples were gently vortexed for 30 sec and the beads collected from the plate and discarded. The DNA was transferred to 0.5 ml labeled snap-cap tubes and stored in the -20°C freezer.

The H134R and H133R mutations in the *AsSdhC* and *AsSdhD* genes, respectively, were detected using previously described PCR methods (Mallik et al., 2014). Multiplex PCR assays were performed using 25 µl volume consisting of 20 ng of DNA, 2 mM $MgCl_2$, 0.2 mM dNTP, 5 µM SdhBSen-F, 5 µM SdhBSen-R, 3 µM SdhC-F, 3 µM SdhC-R1, 5 µM SdhD-F, and 5 µM SdhD-R1 primers and 1 U of Go Taq polymerase (Promega Corp., Madison, WI). The multiplex was performed in a T100 Thermal cycler (Bio-Rad, Hercules, CA) with an initial preheat of 95°C for 2 min. followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 1 min. A final extension at 72°C for 7 min. was executed at the end of the program. A 475-bp product or a 72-bp product amplified when a mutation existed in

AsSdhC or *AsSdhD* genes, respectively. Amplification of a single 235-bp product amplified alone, indicated no mutation in the *AsSdhB* gene.

If no product was amplified in the multiplex PCR, further evaluation was conducted with a MAMA-PCR to determine if a mutation was present in the *AsSdhB* gene (H278R or H278Y) (Mallik et al., 2014). The H278R mutation was detected with MAMAB1-F and MAMABM-R primers and the H278Y mutation was detected with MAMAB1-F and MAMABR-R primers. MAMA-PCR assays were performed using 25 µl volume consisting of 20 ng of DNA, 1.5 mM MgCl2, 0.2 mM of each primer, and 1 U of Go Taq polymerase. The MAMA-PCR program for amplifying *AsSdhB* mutations was the same as the multiplex program, as previously described, except an annealing temperature of 60°C was used. An additional PCR assay was performed if no amplifications were expressed in the multiplex or MAMA-PCR assays to detect the presence of the D123E mutation. This additional assay was completed using a 23 µl volume consisting of 20 ng of DNA, 1.5 mM $MgCl_2$, 0.5 mM dNTP, 5 µM of each primer, and 1 U of Go Taq polymerase. The thermal cycler PCR program was as described for the MAMA-PCR program. A 127-bp product amplified in the presence of the D123E mutation. All amplified products were separated by gel electrophoresis in a 1.2% agarose gel.

In vitro sensitivity of *Alternaria solani* isolates to SDHI fungicides

A study was performed to determine the in vitro sensitivity of *A. solani* baseline and non-baseline isolates using a conidial germination inhibition assay. The baseline and non-baseline *A. solani* isolates were assayed in 18 trials, with eight to ten isolates included in each trial. Internal control isolates (13-1, an *A. solani* wild-type isolate, and 526-3, an *A. solani* QoI reduced-sensitive isolate containing the F129L mutation) were used in each trial to determine assay reproducibility (Wong and Wilcox, 2002).

All isolates were grown on CV-8 agar for seven to nine days at 22 ± 2°C under 24h fluorescent light (Pasche et al., 2004). A sterile glass rod was used to scrape conidia from the agar surface using distilled H_2O. The conidia concentration was adjusted to a 2 x 10^5 conidia/ml using a hemocytometer. One-hundred-fifty microliters was added to the surface of each fungicide amended media plate and spread using a sterile glass rod. Media containing 2% laboratory-grade agar (A360-500 Fischer Scientific, Pittsburgh, PA) was amended with technical formulation of either boscalid (99% active ingredient; BASF Corporation, Research Triangle Park, NC), fluopyram (97.78% active ingredient; Bayer CropScience, Raleigh, NC), solatenol (97% active ingredient; Syngenta Crop Protection, Greensboro, NC), or adepidyn (98.3% active ingredient; Syngenta Crop Protection, Greensboro, NC) was dissolved in acetone, to reach final concentrations of 0.01, 0.1, 1, 10, 100 µg/ml. A no-fungicide control was included and all acetone concentrations in the fungicide amended-media were 0.1% by volume. Salicylhydroxamic acid (SHAM) was added at 100 µg/ml to the media to prevent *A. solani* from overcoming the activity of the SDHI fungicides through an alternative oxidative pathway. Media was incubated at 21 ± 2°C in the light for 4h. Percentage spore germination (fifty conidia for each treatment) was estimated using a compound microscope at 100 x magnification. A conidium was classified as germinated if one germ tube was at least the length of the conidium, or if multiple germ tubes developed from a single conidium. This study was performed twice with two replicates per trial.

In vivo efficacy of boscalid, fluopyram, solatenol, and adepidyn

Alternaria solani isolates were selected for in vivo sensitivity assays based on the low and high EC_{50} values produced by the solatenol and adepidyn in vitro sensitivity results (Table 1.1). Efficacy of SDHI fungicides boscalid, fluopyram, solatenol and adepidyn against early

blight was assayed under greenhouse conditions using a twenty-four-hour preventative test (Pasche et al., 2004). The Orange Pixie tomato cultivar (Tomato Growers Supply Company, Fort Myers, FL) was chosen because of its susceptibility to early blight, its compact size compared to potato plants, and the resistance of leaves to dehisce when severely infected.

Three tomato seeds were sown in a single 10 cm^3 plastic pot containing Sunshine Mix LC1 (Sun Gro Horticulture Inc., Bellevue, WA). After emergence, plants were thinned to acquire two uniformly sized plants per pot. When the plants reach a height of 15 to 20 cm and the first three leaves were fully expanded they were treated with commercial formulations of boscalid (Endura ®, BASF Corporation, Research Triangle Park, NC), fluopyram (Luna ® Privilege, Bayer CropScience, Raleigh, NC), solatenol (Aprovia ®, Syngenta Crop Protection, Greensboro, NC) or adepidyn (Miravis ™, Syngenta Crop Protection, Greensboro, NC). Fungicide concentrations of 0, 0.1, 1, 10, and 100 µg/ml of the active ingredient were applied to the plants to obtain a dose-response curve. Fungicides were applied using a Generation II Research Sprayer (Devries Manufacturing, Hollandale, MN) at approximately 400 kPa.

A 50 ml suspension of 2.0 x 10^5 conidia/ml was prepared from 10 to 12-day old cultures of *A. solani* grown under 24h fluorescent light at 22 ± 2°C on CV-8 medium and applied to plants using a Preval paint-spray gun (Preval Sprayer Division, Prevision Valve Corporation, Yonkers, NY). Inoculated plants were placed in humidity chambers (Phytotronic Inc.; 1626D) at >95% RH at 22 ± 2 °C for 24 hours. The plants were transferred to confinement chambers (plastic cages with an open ceiling) on the greenhouse benches to avoid cross-contamination from other isolates.

Table 1.1. *Alternaria solani* isolates assayed for in vivo sensitivity to boscalid, fluopyram, solatenol, and adepidyn

Isolate	State of origin	SDH mutation	Collection Year
1545-1	Nebraska	D123E	2015
1393-18	North Dakota	D123E	2013
1580-5	Minnesota	D123E	2015
1545-5	Nebraska	H278Y	2015
1339-5	North Dakota	H278Y	2013
1341-3	Nebraska	H278Y	2013
1583-9	Minnesota	H133R	2015
1367-12	Michigan	H133R	2013
1537-8	Minnesota	H133R	2015
1346-2	North Dakota	H278R	2013
1327-1	Michigan	H278R	2013
1322-7	Wisconsin	H278R	2013
1528-21	Nebraska	H134R	2015
1567-4	Michigan	H134R	2015
1580-8	Minnesota	H134R	2015
1577-3	Minnesota	SDHI Sensitive	2015
1553-3	Texas	SDHI Sensitive	2015
13-1	Nebraska	SDHI Sensitive	1998

The greenhouse temperature was maintained at 25 ± 2 °C and plants were watered daily. Early blight severity was visually rated at 6-, 9- and 12-days post-inoculation by estimating percentage infected leaf area on the first three true leaves and recorded as diseased tissue percentage. This in vivo experiment was performed twice with two samples (two plants per pot) and three replicates (three pots) and per isolate at each fungicide concentration.

Effective SDHI fungicides on early blight severity and frequency of SDH mutations

Two field trials were conducted in 2018 to determine the impact of solatenol applied in-furrow at planting, and adepidyn as a foliar application on the development of SDH mutations.

The trials were performed under irrigated conditions in Inkster and Larimore, ND, using the early blight and brown spot susceptible cv. Ranger Russet.

The experiment consisted of 10 treatments with four replicates in a randomized complete block design. Plants were grown in four row plots, approximately 3.6 m wide and 9 m long (Appendix B). Two treatments consisted of the standard protectants chlorothalonil and mancozeb applied at 7-day intervals throughout the growing season. Two treatments developed to be similar to foliar fungicide programs followed by commercial grower in North Dakota. These consisted of tank mixes of multiple applications of single-site foliar fungicides with standard protectant fungicides (Pasche and Gudmestad, 2008). The single-site foliar fungicide in one treatment was fluopyram/pyrimethanil, and the other was adepidyn/fludioxonil. Five treatments with in-furrow applications were solatenol alone, solatenol mixed with two rates of the biological control *Bacillus subtilis* (Serenade Soil, Bayer CropScience), fluopyram alone, and fluopyram mixed with the higher rate of *Bacillus subtilis*. In-furrow applications were directed at the seed-piece using a planter-mounted CO_2 sprayer with a single nozzle with a 6501 tip. Foliar fungicides were applied with a water volume of 560 liters/ha and a pressure of 375 kPa. Foliar disease percentage was recorded in the center two rows at approximately 7-day intervals, beginning approximately 60-70 days after planting. These disease severity evaluations were recorded on a 0-100% diseased leaf tissue scale for 11 weeks, terminating one week following the final foliar application (Pasche and Gudmestad, 2008).

All treatments were inoculated using four *A. solani* isolates, two isolates containing the H278Y mutation, and two isolates classified as wild-type (no F129L mutation/ no SDH mutations). Isolates were grown in CV8 media under constant fluorescent light for two weeks at room temperature (22 ± 2°C). Distilled water was added to the cultures, and conidia were

dislodged with a glass rod and diluted into a 0.25% gelatin solution to a concentration of 6.7 x 10^3 conidia/ml. This suspension was applied twice using customized ATV application equipment to the outside two rows of each four-row treatment at a rate of 104 mL/row in mid-July and early-August (approximately 2 weeks apart). Immediately following the final foliar disease severity rating, approximately 10 infected leaves were sampled arbitrarily from all four replicates of each treatment, including the non-treated control. The leaves were placed in unsealed plastic bags inside a cooler and transported back to the Gudmestad Laboratory. The infected leaf tissue was transferred to 1.5% unamended agar media and isolations were made as described above (Holm et al., 2003). Thirty-five to 41 *A. solani* isolates from each treatment were collected, DNA was extracted, and examined using PCR, as previously described.

Statistical analysis

The effective concentration where fungal germination is inhibited by 50% (EC_{50} value) was calculated using the percentage reduction in germination relative to the non-fungicide-amended controls and regressed against the $\log_{10}$ fungicide concentration. Using the Statistical Analysis System (SAS version 9.4, SAS Institute Inc., Cary, NC), the concentration determined to reduce germination by half compared to the 0 µg/ml concentration was deduced from the 50% intercept (EC_{50} value) (Pasche et al., 2004). Isolates with EC_{50} values of <0.01 and >100 were analyzed as 0.01 and 100 µg/ml, respectively. The experiments were analyzed with an F-test to determine homogeneity of variance among experiments. Assay reproducibility was determined using the approximate limits for a 95% confidence interval for two internal controls included in every trial (Wong and Wilcox, 2002). Trials were included in the final analysis if the internal control EC_{50} values were within the 95% confidence interval. Mean separation was determined using Fisher's protected least significant difference (LSD) test (P = 0.05). Pearson correlation

coefficients were calculated to compare the in vitro fungicide EC_{50} values for baseline and non-baseline *A. solani* isolates. A resistance factor (Rf) was calculated for each fungicide by dividing the mean EC_{50} value of the non-baseline isolates by the mean EC_{50} value of the baseline isolates.

Greenhouse in vivo experiments were arranged as split-plot randomized complete block designs with *A. solani* isolates as the main plot and fungicide concentrations as split-plots. For every isolate at all fungicide concentrations (0, 0.1, 1, 10, and 100 µg/ml), disease severity data was transformed to percentage disease control using the formula: [1 – (% diseased tissue / % diseased tissue in non-treated plants) x 100] (Gudmestad et al., 2013; Pasche et al., 2004). Disease control data was utilized for further statistical analyses and the Levene's test was used to determine homogeneity of variance between two independent experiments (Milliken and Johnson, 1992). Analysis of variance (ANOVA) was conducted for isolate x fungicide at each fungicide concentration using SAS. F-tests were used on the combined data to detect differences at each fungicide concentration. Area under the dose-response curve (AUDRC) (similar to the area under the disease progress curve across all doses of fungicide) was calculated to determine if there were significant differences in early blight control provided by boscalid, fluopyram, solatenol, and adepidyn.

For field trials, the area under the disease progress curve (AUDPC) was calculated using the early blight severity percentage, recorded as previously described above (Shaner and Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [[(W_{i+1} + W_i)/2][t_{i+1} - t_i]]$$

W_i is the percentage foliar disease severity at the i^{th} observation, t_i the time in days at the i^{th} observation and n the total number of observations. The relative area under the disease progress curve (RAUDPC) was calculated for each treatment of the replicated trials from each

year by dividing AUDPC values by the total area of the graph and analyze using ANOVA (Proc GLM SAS version 9.4, Cary, NC). Fisher's protected LSD test (P = 0.05) was used to differentiate mean RAUDPC values. SDHI-mutant frequency field data was unbalanced and therefore required transformation before analysis. Back-transformed data are presented here.

Results

In vitro sensitivity of *Alternaria solani* to SDHI fungicides

Independent analysis of variance of in vitro fungicide sensitivity experiments for boscalid, fluopyram, solatenol, and adepidyn determined that variances were homogenous (P = 0.7506) and the experiments were combined for further analysis (Appendix C). EC_{50} values of *A. solani* baseline isolates for boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 0.97, <0.01 to 1.31, <0.01 to 0.23, and <0.01 to 0.08 µg/ml, respectively. The mean fungicide sensitivities of baseline *A. solani* isolates to boscalid and fluopyram (0.20 and 0.14 µg/ml, respectively) were significantly higher than for solatenol and adepidyn (0.03 and 0.01 µg/ml, respectively) (Table 1.2).

EC_{50} values of *A. solani* non-baseline isolates for boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 65.03, <0.01 to 8.64, <0.01 to 3.43, and <0.01 to 0.69 µg/ml, respectively. The mean fungicide sensitivities of the 112 non-baseline *Alternaria solani* isolates evaluated here were more sensitive to solatenol (0.17 µg/ml) and adepidyn (0.05 µg/ml) than to fluopyram (0.65 µg/ml) and boscalid (3.99 µg/ml), but *A. solani* isolates were more sensitive to fluopyram than boscalid (Table 1.2). Furthermore, all mean fungicide sensitivities for the baseline were significantly lower than the non-baseline for each respective fungicide (Table 1.2).

Table 1.2. In vitro fungicide sensitivity (mean EC_{50} values) baseline and non-baseline *Alternaria solani* isolates to boscalid, fluopyram, solatenol, and adepidyn.

Alternaria solani population	Mean fungicide EC_{50} values (µg/ml)				LSD $_{P=0.05}$ [y]
	Boscalid	Fluopyram	Solatenol	Adepidyn	
Baseline (n = 57)	0.20 b A	0.14 b B	0.03 b C	0.01 b C	0.030
Non-baseline (n = 112)	3.99 a A	0.65 a B	0.17 a B	0.05 a B	0.630
LSD $_{P=0.05}$ [y]	0.80	0.08	0.02	0.005	

[Y] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letters represent no significant difference between the SDHI fungicides within the baseline/non-baseline. Columns containing the same lowercase letters represent no significant difference between the baseline and non-baseline means within the SDHI fungicide.

Fifty-five of 112 *A. solani* non-baseline isolates fell outside of the *A. solani* baseline range for boscalid (Figure 1.1). Twenty of 112 *A. solani* non-baseline isolates fell outside of the *A. solani* baseline range for fluopyram. Twelve of 112 *A. solani* non-baseline isolates fell outside of the *A. solani* baseline range for solatenol. Twenty-two of 112 *A. solani* non-baseline isolates fell outside of the *A. solani* baseline range for adepidyn.

Across the five SDH mutations evaluated, boscalid had the lowest intrinsic activity (Table 1.3). Across all SDH mutations, adepidyn had the highest intrinsic activity except for the H278R. Similarly, fluopyram had the lowest intrinsic activity against *A. solani* isolates with the D123E, H133R, and H278Y mutations, compared to isolates with the H278R and H134R mutations (Table 1.3). Results of the in vitro fungicide sensitivity assays demonstrate a substantial shift in sensitivity between the mean EC_{50} values of the boscalid baseline and non-baseline isolates (Rf 23.3). The Rf for *A. solani* non-baseline isolates compared to the baseline isolates in response to fluopyram, solatenol, and adepidyn were 5.6-, 5.8-, and 5.9-fold, respectively.

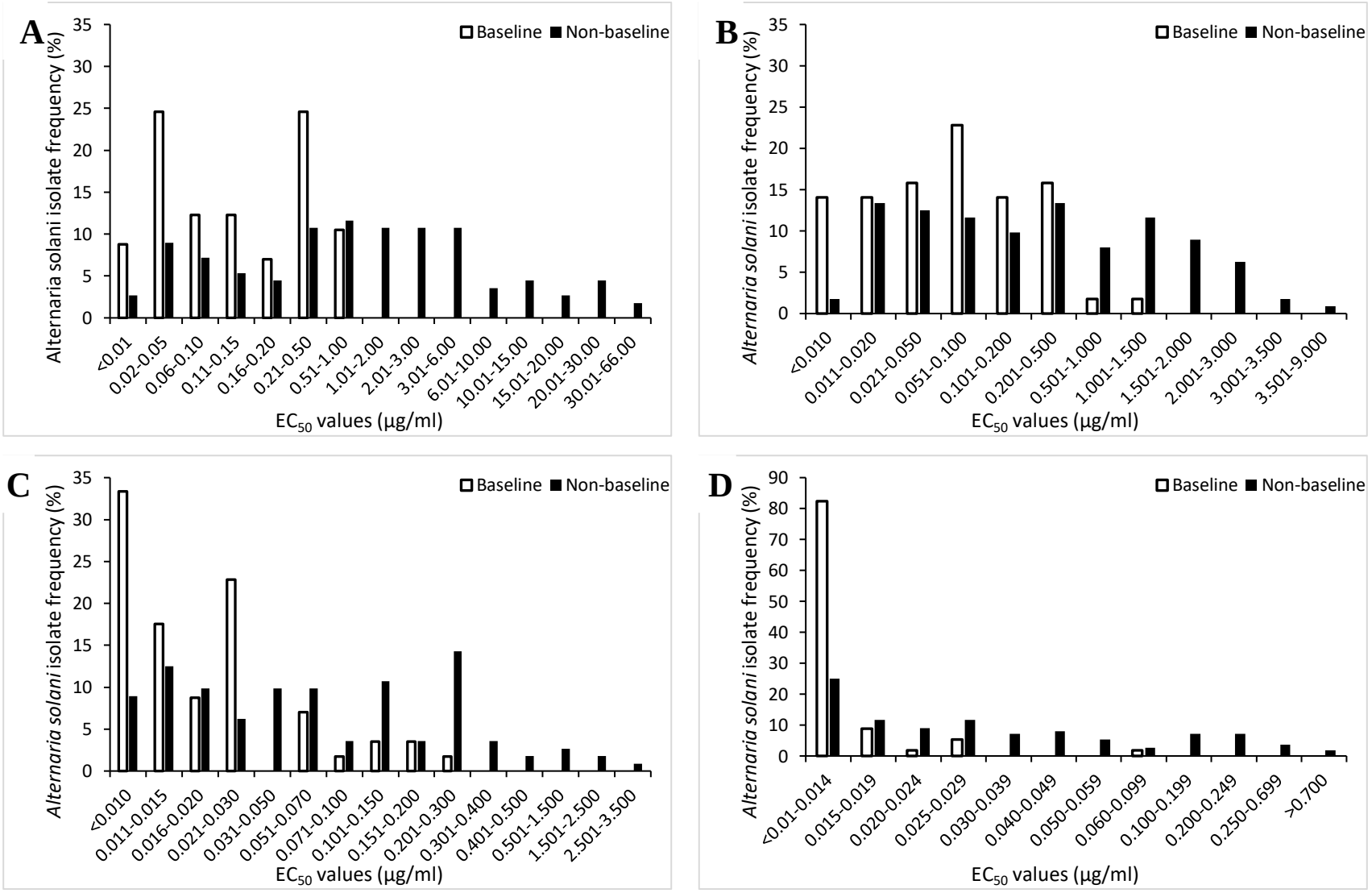

Figure 1.1. Frequency distribution of sensitivity of 57 baseline and 112 non-baseline *Alternaria solani* isolates to the SDHI fungicides (A) boscalid, (B) fluopyram, (C) solatenol, and (D) adepidyn. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

Table 1.3. In vitro fungicide sensitivity (mean EC_{50} values) of the succinate dehydrogenase (SDH) mutations within non-baseline *Alternaria solani* isolates to boscalid, fluopyram, solatenol, and adepidyn.

SDH mutations (number of isolates)	Mean fungicide EC_{50} values (µg/ml)				LSD $_{P=0.05}$
	Boscalid	Fluopyram	Solatenol	Adepidyn	
H278R (n = 22)	4.28 b A	0.27 c B	0.02 c B	0.03 d B	2.244
H278Y (n = 21)	3.29 b A	0.90 a B	0.36 a B	0.07 a B	0.951
H134R (n = 22)	2.71 b A	0.67 b B	0.16 b B	0.05 bc B	0.732
H133R (n = 21)	6.54 a A	1.00 a B	0.14 b B	0.06 b B	1.304
D123E (n = 22)	6.31 a A	0.89 a B	0.31 a B	0.04 cd B	2.245
SDHI-Sensitive (n = 4)	0.13 c B	0.09 c B	0.03 c A	0.01 e A	0.046
LSD $_{P=0.05}$ [z]	0.012	1.947	0.986	0.060	

[z] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letters represent no significant difference between the fungicides within the SDHI-mutation and columns containing the same lowercase letters represent no significant difference detected between SDH mutations within the SDHI fungicide.

Among *A. solani* baseline isolates, there was no significant correlation between the EC_{50} values for adepidyn and boscalid (r = 0.1350, P = 0.3170), boscalid and fluopyram (r = 0.2552, P = 0.0554), or adepidyn and fluopyram (r = 0.1040, P = 0.4410) (Table 1.4). There was a strong and significant correlation in fungicide sensitivity among *A. solani* baseline isolates to adepidyn and solatenol (r = 0.6041, P = <0.00001). A weak but significant correlation was observed in the fungicide sensitivity of baseline *A. solani* isolates to boscalid and solatenol, (r = 0.3535, P = 0.0070), and fluopyram and solatenol (r = 0.2610, P = 0.0499) (Table 1.4). A significant but weak correlation between adepidyn and fluopyram was observed in the sensitivity of non-baseline *A. solani* isolates (r = 0.2009, P = 0.0337), and between adepidyn and solatenol (r = 0.3097, P = 0.0009) (Table 1.2). There was no significant correlation observed between the other

SDHI fungicides (Table 1.4). These cross-sensitivity assessments indicate that adepidyn sensitive *A. solani* isolates will also be sensitive to solatenol and fluopyram.

Table 1.4. Relationship between the EC_{50} values of *Alternaria solani* baseline and non-baseline isolates between SDHI fungicides

	Alternaria solani	
	Baseline	Non-baseline
SDHI fungicides[y]	r[z]	r
Adep vs Bos	0.1350	-0.0151
Adep vs Flu	0.1040	0.2009*
Adep vs Sol	0.6041***	0.3097**
Bos vs Flu	0.2552	-0.0406
Bos vs Sol	0.3535**	0.0553
Flu vs sol	0.2610*	0.0829

[y] Adep=Adepidyn; Bos=Boscalid; Flu=Fluopyram; Sol=Solatenol.
[z] One asterisk (*) indicates P value < 0.05, two (**) P value < 0.01, three (***) P value < 0.0001

Impact of SDH mutations in *Alternaria solani* isolates provided by SDHI fungicides in the greenhouse

Independent analysis of greenhouse disease control experiments for boscalid, fluopyram, solatenol, and adepidyn determined that variances were homogenous (P = 0.8158), and experiments were combined for further analysis (Appendix D). A significant interaction was observed between the main plot (isolate) and subplot (fungicide concentration) for percentage disease control by all fungicides (P < 0.0001). Disease severity, as represented by AUDRC, was significantly higher when boscalid was applied to manage *A. solani* isolates possessing all SDH mutations (Table 1.5). The application of fluopyram resulted in significantly lower AUDRC values with isolates possessing the H134R, and H278R mutations when compared to the SDHI-sensitive (wildtype) *A. solani* isolates. Solatenol provided significantly lower levels of control of *A. solani* isolates possessing the H278R, H133R, H134R, and D123E mutations. Significantly lower AUDRC and levels of disease control with adepidyn were observed in *A. solani* isolates

possessing all the SDH mutations except H278Y. Based on AUDRC across SDH mutations, boscalid provided significantly less disease control than fluopyram, solatenol, and adepidyn, but solatenol and adepidyn provided higher disease control than fluopyram. Furthermore, across all mutations, *A. solani* isolates were significantly more sensitive to adepidyn than to solatenol and adepidyn provided the highest amount of disease control of isolates with all SDH mutations when compared to the other SDHI fungicides (Table 1.5).

Table 1.5. Mean area under the dose response curve (AUDRC) for *Alternaria solani* isolates possessing an SDH mutation (H278R, H278Y, H134R, H133R, D123E, or SDHI-sensitive) among the SDHI fungicides boscalid, fluopyram, solatenol, and adepidyn

SDH mutations	Mean AUDRC				
	Boscalid	Fluopyram	Solatenol	Adepidyn	LSD $_{P=0.05}$[x]
H278R (n = 3)	6825.8 c	7178.1 b	8655.7 d	9587.9 b	863.5
	C	C	B	A	
H278Y (n = 3)	8222.6 ab	8402.4 a	9729.7 ab	9949.5 a	639.0
	C	C	B	A	
H134R (n = 3)	6589.6 c	6077.9 c	9550.7 b	9496.4 b	983.7
	B	B	A	A	
H133R (n = 3)	7052.7 c	8791.6 a	9205.5 c	9587.9 b	439.6
	C	B	AB	A	
D123E (n = 3)	7353.0 bc	8627.6 a	9235.8 c	9670.7 b	721.2
	C	B	AB	A	
SDH-sensitive (n = 3)	8855.2 a	9217.5 a	9820.7 a	9938.4 a	528.4
	C	B	A	A	
LSD $_{P=0.05}$	1147.9	834.2	263.6	186.6	
Combined *A. solani* isolates (n = 18)	7483.2	8049.2	9366.4	9700.8	313.6
	D	C	B	A	

[x] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed between the fungicides. Columns containing the same lowercase letter indicate no significant differences exist between the SDH mutations within the fungicide.

Early blight severity and SDH mutation frequency under field conditions

Results from field trials conducted at Larimore and Inkster, North Dakota in 2018 demonstrated there were significant differences among fungicide treatments in the control of early blight (Appendix E). At both locations, all fungicide treatments provided significantly better control of early blight compared to the non-treated control plots (Non-trt) but, the chlorothalonil, mancozeb, grower's standard with fluopyram and pyrimethanil, and grower's standard with adepidyn and fludioxonil treatments provided the best control of early blight (Figure 1.2). At the Larimore location, the grower standard with adepidyn and fludioxonil was the only treatment which resulted in significantly higher yield than the non-treated (Figure 1.3A). At the Inkster location, the application of mancozeb full-season was the only treatment resulting in significantly higher yield than the non-treated (Figure 1.3B). However, the yield resulting from the mancozeb treatment was not significantly higher than the grower standard with adepidyn and fludioxonil, solatenol in furrow with 4.7 l/ha, fluopyram in-furrow with 9.4 l/ha of serenade soil, and separate applications of QoI, SDHI-fluopyram mixed with and anilinopyrimidine-pyrimethanil, each mixed with standard protectants.

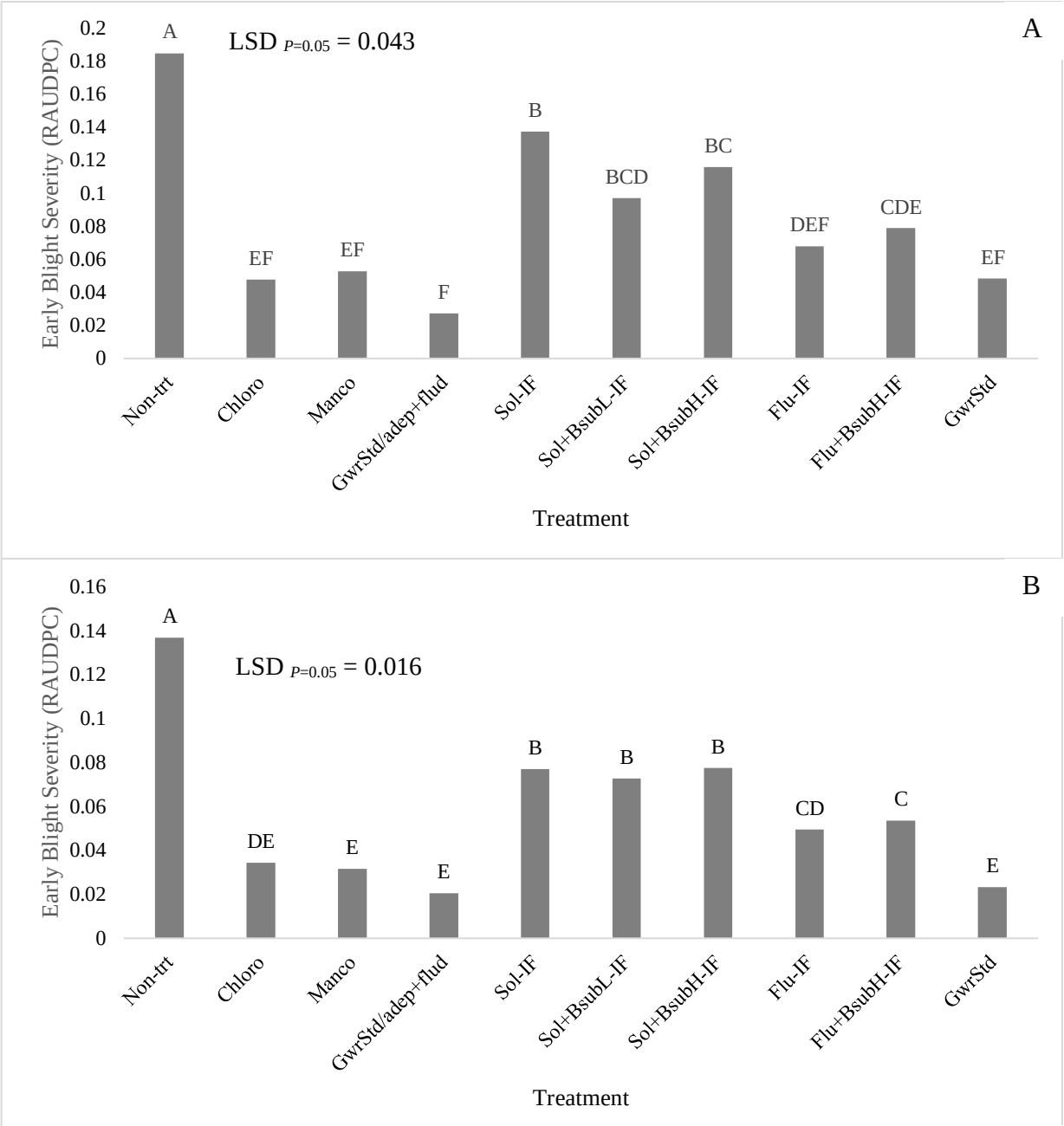

Figure 1.2. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from 2018 field trials conducted in (A) Larimore and (B) Inkster, ND. Treatments included a non-treated control (non-trt); Chlorothalonil (Chloro); Mancozeb (Manco); Separate applications of QoI, a new foliar SDHI and PP, and DMI fungicide each mixed with standard protectants (GwrStd/adep+flud); solatenol in-furrow (Sol-IF); solatenol in furrow with 4.7 l/ha and 9.4 l/ha of serenade soil (Sol+BsubL-IF and Sol+BsubH-IF, respectively); fluopyram in-furrow (Flu-IF); fluopyram in-furrow with 9.4 l/ha of serenade soil (Flu+BsubH-IF); and separate applications of QoI, SDHI-fluopyram mixed with and Anilinopyrimidine-pyrimethanil, each mixed with standard protectants (GwrStd). Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

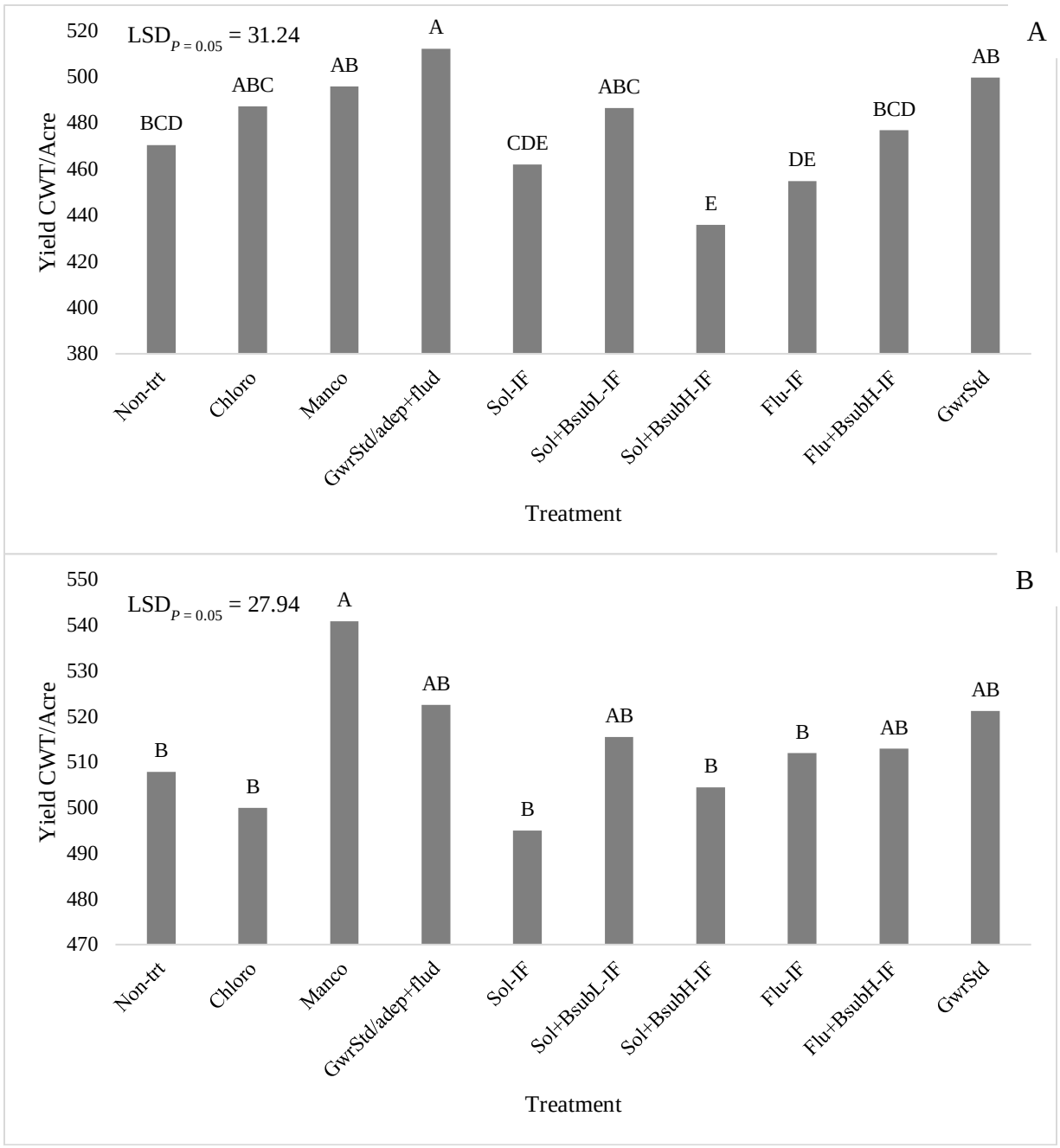

Figure 1.3. Yield data expressed in hundredweight (CWT) per acre from 2018 field trials conducted in (A) Larimore and (B) Inkster, ND. Treatments included a non-treated control (non-trt); Chlorothalonil (Chloro); Mancozeb (Manco); Separate applications of QoI, a new foliar SDHI and PP, and DMI fungicide each mixed with standard protectants (GwrStd/adep+flud); solatenol in-furrow (Sol-IF); solatenol in furrow with 4.7 l/ha and 9.4 l/ha of serenade soil (Sol+BsubL-IF and Sol+BsubH-IF, respectively); fluopyram in-furrow (Flu-IF); fluopyram in-furrow with 9.4 l/ha of serenade soil (Flu+BsubH-IF); and separate applications of QoI, SDHI-fluopyram mixed with and Anilinopyrimidine-pyrimethanil, each mixed with standard protectants (GwrStd). Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

All known *A. solani* SDH mutations were detected in isolates from these trials. *Alternaria solani* isolates possessing the H134R mutation were detected in all treatments at both locations. A single isolate possessing the H133R mutation was detected in the serenade soil treatment/solatenol in-furrow treatment at the Inkster, ND location (Table 1.6). At the Inkster location, *A. solani* isolates possessing the D123E mutation were observed in all treatments except the two which included applications of fluopyram (Flu-IF and Flu + BsubH-IF) (Table 1.6). The highest frequency of D123E-mutants was obtained from the mancozeb, chlorothalonil, and grower's standard with fluopyram and pyrimethanil treatments. However, at the Larimore location, the highest frequency of D123E-mutants was obtained from the solatenol in-furrow, and grower's standard with fluopyram and pyrimethanil treatments compared to the non-treated control plots. At both field locations, despite inoculating the plots with two SDHI-sensitive and two H278Y-mutant isolates, the prevalence of collected H278Y- and H278R-mutants were minimal. In both locations, the H278R-mutants were low among all treatments. At the Inkster location, *A. solani* isolates possessing the H278Y mutation were observed in the solatenol in-furrow and the grower's standard with adepidyn and fludioxonil treatments. However, at the Larimore location, the frequency of the H278Y-mutants was low among all treatments. The frequency of *A. solani* isolates with unknown SDH mutations was high among all treatments with the solatenol in-furrow with 4.7 l/ha treatment (Sol + BsubL-IF) being the highest at both locations.

Table 1.6. Frequency of *Alternaria solani* SDH mutations recovered from each treatment in 2018 from two locations[y]

Location, Treatments[w]	SDH mutations						
	D123E[x]	H133R	H134R	H278R	H278Y	No mutations	Unknown[y]
Larimore, ND							
Non-treated	5.8 c	0.0 a	46.9 bc	5.8 a	5.3 a	8.6 ab	27.7 bcd
Chlorothalonil	5.2 c	0.0 a	85.2 a	0.0 a	0.0 a	1.9 b	7.7 d
Mancozeb	17.6 abc	0.0 a	6.3 e	0.0 a	0.0 a	9.6 ab	63.5 abc
GrwStd/Adep+flud	20.2 abc	0.0 a	5.8 e	1.7 a	3.8 a	0.0 b	68.5 ab
Sol-IF	34.7 a	0.0 a	33.7 bcd	2.1 a	1.9 a	2.1 b	27.8 bcd
Sol + BsubL-IF	7.5 c	0.0 a	4.2 e	1.7 a	2.1 a	2.1 b	82.5 a
Sol + BsubH-IF	12.3 c	0.0 a	19.6 de	3.6 a	4.5 a	7.1 ab	57.4 abc
Flu-IF	6.9 c	0.0 a	35.2 bcd	0.0 a	1.8 a	0.0 b	56.1 abc
Flu + BsubH-IF	12.4 bc	0.0 a	53.3 b	0.0 a	0.0 a	21.2 a	13.1 d
GrwStd	33.6 ab	0.0 a	24.2 cde	6.0 a	0.0 a	13.9 ab	22.4 cd
$LSD_{P=0.05}$	21.3	0.0	24.4	6.6	7.2	14.5	41.3
Inkster, ND							
Non-treated	15.7 bc	0.0 a	27.7 a	1.8 a	9.8 ab	3.6 a	41.4 ab
Chlorothalonil	49.2 ab	0.0 a	8.7 a	1.6 a	1.6 ab	21.4 a	17.5 b
Mancozeb	56.3 a	0.0 a	10.4 a	0.0 a	0.0 a	16.7 a	16.7 b
GrwStd/Adep+flud	8.3 c	0.0 a	38.3 a	0.0 a	11.1 ab	6.1 a	36.1 ab
Sol-IF	10.4 bc	0.0 a	44.6 a	4.2 a	13.2 a	15.8 a	18.1 b
Sol + BsubL-IF	7.8 c	2.3 a	18.5 a	0.0 a	0.0 a	1.9 a	69.5 a
Sol + BsubH-IF	12.5 bc	0.0 a	21.8 a	0.0 a	5.0 ab	20.0 a	40.7 ab
Flu-IF	0.0 c	0.0 a	24.2 a	0.0 a	0.0 a	14.3 a	61.5 ab
Flu + BsubH-IF	0.0 c	0.0 a	36.7 a	0.0 a	0.0 a	8.7 a	54.6 ab
GrwStd	36.5 abc	0.0 a	30.8 a	0.0 a	0.0 a	0.0 a	32.7 ab
$LSD_{P=0.05}$	38.9	2.3	38.8	4.9	13.1	28.6	48.6

[w] Treatments included a non-treated control; Chlorothalonil; Mancozeb; Separate applications of QoI, a new foliar SDHI and PP mix, and DMI fungicide each mixed with standard protectants (GrwStd/adep+flud); solatenol in-furrow (Sol-IF); solatenol in furrow with 4.7 l/ha and 9.4 l/ha of serenade soil (Sol+BsubL-IF and Sol+BsubH-IF, respectively); fluopyram in-furrow (Flu-IF); fluopyram in-furrow with 9.4 l/ha of serenade soil (Flu+BsubH-IF); and separate applications of QoI, SDHI-fluopyram mixed with and Anilinopyrimidine-pyrimethanil, each mixed with standard protectants (GrwStd).

[x] Numbers followed by different lowercase letters within columns are significantly different according to Fisher's protected least significant difference test at the P = 0.05 level.

[y] Isolates could not be confidently characterized and are currently undergoing further examination.

Discussion

One of the goals of this study was to determine the sensitivity of the *A. solani* baseline and non-baseline isolates to four SDHI fungicides. These results indicate that there has been a shift in boscalid in the baseline *A. solani* isolates. Cross-sensitivity assays determined that isolates with reduced-sensitivity to solatenol also possess reduced-sensitivity to adepidyn. The EC_{50} values in the non-baseline *A. solani* isolates in response to solatenol were numerically higher among all SDH mutations except for the H278R mutation compared to the adepidyn EC_{50} values. However, the EC_{50} values for all five Sdh-mutants of fluopyram, solatenol, and adepidyn are significantly lower than the EC_{50} values of boscalid.

An additional goal of this study was to determine the impact of sensitivity shifts from the baseline to non-baseline isolates using greenhouse efficacy evaluations. Isolates were assessed in the greenhouse to determine if the new SDHI fungicides are more effective at controlling each of the five Sdh mutations than the older SDHI fungicides.

Results from greenhouse evaluations indicated that adepidyn provided the highest level of disease control among the SDHI fungicides for all the SDH mutants. Furthermore, solatenol provided significantly higher disease control in *AsSdhC* and *AsSdhB* mutants compared to fluopyram and boscalid. Previous studies suggest that solatenol may bind to the SdhD protein in the Sdh complex (Ishii et al., 2016). Significantly lower control of *A. solani* isolates possessing the H278R mutation (*AsSdhB* gene) was provided by solatenol when compared to the other SDH mutations and SDHI-sensitive isolates. Isolates possessing the D123E-mutation were controlled at lower levels compared to the SDHI-sensitive isolates in all fungicides except fluopyram. Previous aggressiveness studies determined that *A. solani* isolates possessing the D123E mutation are more aggressive in greenhouse conditions compared to the SDHI-sensitive isolates

(Bauske et al., 2018c). These differences could be explained by the differing number of isolates used in the greenhouse studies and the high levels of variability within *A. solani* (Bauske et al., 2018c; van der Waals et al., 2004; Woudenberg et al., 2015). In previous studies, a larger subset of *A. solani* isolates were evaluated in the greenhouse compared to the greenhouse isolates analyzed in this study (Bauske et al., 2016c). Utilizing a larger number of *A. solani* isolates provides a more comprehensive look at the aggressiveness, and response of the SDH mutants to the SDHI fungicides.

The final goal of this study was to determine the impact of newly registered SDHI fungicides on the frequency of the H278Y-mutant under field conditions. *Alternaria solani* isolates possessing the H278Y mutation were controlled as effectively as the SDHI-sensitive isolates across all SDHI fungicides, explaining why the H278Y mutation is not commonly seen in the field. *Alternaria solani* isolates possessing the H134R mutation were not controlled as effectively with boscalid and fluopyram compared to the SDHI-sensitive isolates. This is not surprising, a previous survey determined that the H134R-mutants are becoming predominate in the field after fluopyram was introduced into early blight foliar fungicide programs (Bauske et al., 2018b). Fluopyram has been shown to bind to a different region within the SDH complex compared to boscalid (Avenot et al., 2014). This suggests that the *A. solani* H134R-mutants may have a greater impact on the efficacy of fluopyram in the field.

The frequency of the D123E mutation has been reportedly increasing in potato field production areas in recent years (Bauske et al., 2018b). The D123E mutation was first identified in a Nebraska potato field in 2011 and was determined to possess very high resistance to boscalid and penthiopyrad, while remaining sensitive to fluopyram (Mallik et al., 2014). A recent study determined that isolates possessing a high level of resistance to boscalid also possessed a low

level of reduced-sensitivity to fluopyram (Bauske et al., 2018a). In that previous study, it was determined that in response to fluopyram, percentage disease control was significantly lower in D123E-mutants compared to other SDH mutations such as H278R, H133R, and H134R.

Fluopyram resistance was first reported in one out of 26 isolates collected in Idaho during the 2010 season; however, individual EC_{50} values were not reported in that study (Fairchild et al., 2013). In a 2011 survey, approximately 1.5% of collected isolates possessed the D123E mutation (Mallik et al., 2014). A recent survey of *A. solani* isolates collected across the United States potato production areas conducted in 2013, 2014, and 2015 determined the D123E-mutant frequency increased (Bauske et al., 2018b). In contrast, the *AsSdhB* mutations (H278R and H278Y) were the predominate SDH mutations in 2010-2011, but have been rapidly decreasing from the current *A. solani* population. However, in this study we have determined that the use of newer SDHI fungicides that do not bind to the SdhB protein may be the cause of the reduction of the *AsSdhB* mutants.

Fluopyram was an attractive replacement for boscalid in most fungicide application programs since it binds at a different sight in the Sdh complex. Therefore, in the presence of fluopyram, *AsSdhB*-mutants may have a fitness penalty that affects their ability to compete in the presence of the newly registered SDHI fungicides (solatenol and adepidyn) or fluopyram efficacy is not affected by the *AsSdhB*-mutants because of the different binding sites. Previous studies have determined that the *AsSdhB*-mutants affects the performance of boscalid while strengthening the binding of fluopyram (Avenot et al., 2014). Therefore, the lack of cross resistance among *A. solani* isolates between boscalid and fluopyram (Avenot et al., 2014; Gudmestad et al., 2013). This suggests that the use of boscalid will drive an increase in *AsSdhB*-mutants because of the lack of efficacy, while fluopyram will control *AsSdhB*-mutants. Recently,

it was demonstrated that the frequency of isolates with the D123E mutation increase when fluopyram is applied in furrow and the authors hypothesize this is due to the high selection pressure (Bauske et al., 2018a). This indicates that due to the increased use of fluopyram in early blight foliar fungicide programs that the presence of the *AsSdhB* (H278R and H278Y) mutations are decreasing while the *AsSdhC* (H134R) and *AsSdhD* (H133R and D123E) mutations are increasing (Bauske et al., 2018a).

In previous studies, *AsSdhB*-mutants were not significantly more aggressive than the SDHI-sensitive isolates (Bauske et al., 2018c). Due to the low frequencies of H278Y- and H278R-mutants collected in this study, these results support that of previous research that the *AsSdhB*-mutants do not possess a selective advantage in the presence of new SDHI fungicides.

The H134R- and D123E-mutants were detected in all treatments at both locations except in the two fluopyram in-furrow treatments at the Inkster location. The low frequency of the D123E-mutants at the Inkster location may be due to the lower disease pressure at this location compared to the Larimore location. The Inkster location has been used for fungicide assessment at early blight severity since 2009, while this is the first year the Larimore location was used. Interestingly, the D123E-mutants at the Inkster location in the grower's standard with the adepidyn/fludioxonil treatment were detected at a numerically lower frequency than at the Larimore location. However, the inverse was detected for the H134R-mutants, where the grower's standard with the adepidyn/fludioxonil treatment at Larimore detected numerically lower H134R-mutants than at Inkster. Overall, H134R-mutants were detected at a higher frequency than the other SDHI mutants at both locations among most of the treatments. Interestingly, the H134R-mutants were detected at a higher frequency in fluopyram in-furrow treatments while previous research determined that the application of in-furrow fluopyram

increases the frequency of *A. solani* isolates possessing the D123E mutation (Bauske et al., 2018a). However, in the current, study it was observed that fluopyram applied in-furrow had little or no impact on the frequency of *A. solani* D123E-mutants recovered from those treatments. In contrast, a numerically larger frequency of D123E-mutants were isolated from other treatments such as mancozeb, chlorothalonil, and the grower's standard (treatment of QoI, SDHI-fluopyram mixed with and anilinopyrimidine-pyrimethanil, each mixed with standard protectants). Based on results reported here, the D123E-mutation may not be driven by fluopyram in-furrow seed treatments, as previously reported (Bauske et al., 2018c).

With the registration of newer SDHI fungicides, adepidyn and solatenol, it is expected that the frequency of SDH mutations may shift. When boscalid was the primary SDHI used for early blight management, the prominent SDH mutations that appeared were the *AsSdhB* mutants (Mallik et al., 2014). Currently, boscalid is not used as frequently as other SDHI fungicides for early blight management. Therefore, it is possible that the SDH mutation frequency has shifted from the *AsSdhB* mutants being dominant to the *AsSdhC* (H134R) and *AsSdhD* (H133R and D123E) mutations. Based on the shits that have occurred to date, continued monitoring of *A. solani* populations is imperative to determine if current and new fungicide chemistries will be effective for early blight disease management (Mallik et al., 2014). Results from the studies reported here support previous studies that the lack of monitoring leaves the potato industry blind to the eventual impact of the very fluid *A. solani* population developing across the US.

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CHAPTER TWO: IN VITRO AND IN VIVO SENSITIVITY OF SMALL-SPORED *ALTERNARIA* SPP. TO SINGLE SITE FUNGICIDES

Abstract

Brown leaf spot caused by the small-spored *Alternaria* spp., *Alternaria alternata, Alternaria arborescens,* and *Alternaria tenuissima* have been reported with increasing frequency in commercial potato fields. Most potato cultivars have not been developed with resistance to small-spored *Alternaria* spp. Therefore, the use of foliar fungicides remains the primary management strategy. Conidial germination assays of small-spored *Alternaria* spp. were used to determine if a shift in sensitivity has occurred in the Quinone outside Inhibitor (QoI) or Succinate dehydrogenase inhibitor (SDHI) fungicides. Isolates of small-spored *Alternaria* spp. collected before QoIs and SDHIs were applied commercially (baseline) were compared to a population of recently collected small-spored *Alternaria* spp. (non-baseline). In vivo evaluation of the small-spored *Alternaria* spp. also was conducted to evaluate SDHI fungicide efficacy. Resistance factors (Rf) were calculated for each fungicide by dividing the mean EC_{50} value of the non-baseline isolates by the baseline isolates, for each *Alternaria* spp. *A. alternata* Rfs for QoI and SDHI fungicides ranged from 362- to 2368- and 6- to 7-fold, respectively. *Alternaria arborescens* Rfs for boscalid, fluopyram, solatenol, and adepidyn were 29-, 29-, 25-, and 2-fold, respectively. *Alternaria tenuissima* Rfs for boscalid and fluopyram were 9-, and 16-fold, respectively. For all three small-spored *Alternaria* spp., very low or no shift in sensitivity was detected for adepidyn and no shift in sensitivity to solatenol was observed in *A. tenuissima*. Greenhouse experiments demonstrated that adepidyn, solatenol, and fluopyram were more effective at managing non-baseline *A. arborescens* and *A. tenuissima* than boscalid. Under greenhouse conditions, adepidyn and solatenol provided better disease control for non-baseline

A. alternata isolates than fluopyram and boscalid. Results of these studies determined that accurate species identification in the small-spored *Alternaria* spp. can be important for disease management.

Introduction

Brown leaf spot caused by small-spored *Alternaria* spp. has been reported with increasing frequency in commercial potato fields (Ding et al., 2019; Fairchild et al., 2013). Brown leaf spot causes small lesions (pinpoint to 10 mm in diameter) on the foliage and can reduce tuber yield up to 18% if conditions are favorable (Droby et al., 1984) but has been considered a minor disease compared to early blight, caused by *Alternaria solani*. *Alternaria alternata* (Fr.) Keissler, *Alternaria arborescens* E.G. Simmons, and *Alternaria tenuissima* (Kunze) Wiltshire were isolated from potato foliage displaying leaf blight symptoms in the Pacific Northwest (Tymon et al., 2016a). These *Alternaria* spp. are morphologically identical but can be distinguished using molecular techniques.

In the current study, a preliminary survey was conducted with 241 small-spored *Alternaria* isolates collected between 1999-2017 from potato regions across the United States (CA, CO, ID, IL, IN, MI, ND, NE, NM, OH, TX, WA, and WI) using next-generation sequencing methods. Fifty-seven percent of these isolates were *A. alternata*, 17% were *A. tenuissima*, 9% were *A. arborescens*, and 17% of the isolates could not be conclusively identified (S.M. Budde-Rodriguez, *unpublished data*). It has been suggested that correct identification of plant pathogens is crucial in the development of accurate disease management strategies (Tymon et al., 2016a). For instance, some small-spored *Alternaria* spp., such as *A. arborescens*, can produce toxins, making correct spp. identification very important (Tymon et al., 2016a).

Small-spored *Alternaria* spp. in potato fields is viewed frequently as a secondary infection with *A. solani* being the dominate pathogen (Tymon et al., 2016b). In potato, *A. solani* has been investigated more extensively in the realm of fungicide sensitivity monitoring. Currently, there are no commercial potato cultivars resistant to early blight or brown leaf spot, so the primary management approach is foliar fungicide applications. Single-site fungicides such as quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs) are used for early blight and brown spot management in commercial potato growing regions. Single-site fungicides are generally considered to be high-risk for resistance development as resistance is based on a single target-site mutation. Reduced-sensitivity and/or resistance to QoI and SDHI fungicides has been documented in *A. solani* and *A. alternata* among multiple crops across the United States (Avenot and Michailides, 2007; Fairchild et al., 2013; Gudmestad et al., 2013; FRAC, 2019; Mallik et al., 2014; Miles et al., 2014; Pasche et al., 2004; Pasche et al, 2005; Pasche and Gudmestad 2008). *Alternaria alternata* isolates causing leaf spot of pistachio in California have developed resistance to QoI fungicides due to the G143A mutation in the cytochrome b gene (*cytb)*, rendering this fungicide class ineffective (Avenot and Michailides, 2007). Five-point mutations in *A. alternata* resulting reduced sensitivity to boscalid have been characterized across three subunits in the *Sdh* gene (Avenot et al., 2008; Avenot et al., 2009). Two mutations, the replacement of a histidine with tyrosine (H277Y) or arginine (H277R), have been identified in the *AaSdhB* gene (Avenot et al., 2008). One mutation, the replacement of a histidine with arginine (H134R), has been identified in the *AaSdhC* gene. Two mutations, the replacement of a histidine with arginine (H133R) and aspartate by glutamic acid (D123E), have been identified in the *AaSdhD* gene. While *A. alternata* and *A. solani* have been classified as high-risk and medium-risk pathogens, respectively, *A. arborescens* and *A. tenuissima* have not

been classified (FRAC, 2019). Monitoring a fungal population is crucial if fungicide resistance management is to be successfully achieved.

The objectives of this study were to (i) identify if a shift in sensitivity has occurred between baseline populations of small-spored *Alternaria* spp. with no exposure to QoI fungicides and non-baseline isolates collected in 2014 and 2015, (ii) identify if a shift in sensitivity has occurred baseline populations of small-spored *Alternaria* spp. with no exposure to SDHI fungicides and non-baseline isolates collected from 2011 to 2017, and to (iii) determine efficacy of SDHI fungicides: boscalid, fluopyram, solatenol, and adepidyn against small-spored *Alternaria* spp. in greenhouse evaluations.

Materials and Methods

Collection and maintenance of isolates

Isolates of small-spored *Alternaria* spp. were recovered from potato growing regions across the United States. Sixteen *A. alternata,* seven *A. tenuissima,* and three *A. arborescens* isolates collected from 1999 to 2002 with no exposure to QoI fungicides (baseline) were obtained from long-term cryogenic storage (Appendix F). Twenty *A. alternata,* ten *A. tenuissima,* and seven *A. arborescens* isoaltes collected from 1999-2004 with no exposure to SDHI fungicides (baseline) were obtained from long-term cryogenic storage (Appendix G). Twenty-eight *A. alternata* isolates, one *A. tenuissima,* and one *A. arborescens* isolate collected in 2015 with exposure to QoI fungicides (non-baseline), and twenty-nine *A. alternata*, ten *A. arborescens*, and twenty-two *A. tenuissima* isolates collected from 2011 to 2017 with exposure to SDHI fungicides (non-baseline) were submitted as foliar samples to the Gudmestad Laboratory (Appendix F, G). Foliar sections with lesions characteristic of early blight were surface sterilized in a 10% bleach solution for one min. and rinsed in sterile, distilled water.

Lesions were aseptically excised from the foliar surface using a sterile scalpel blade and transferred to an unamended 1.5% agar media (water agar) and incubated at room temperature (22 ± 2°C) for three to four days until conidia were produced.

Purification of the isolates was performed by transferring a single conidium from the plate using a sterile glass needle to solid clarified V8 (CV-8) medium (Campbell's V8 juice, 100 ml; $CaCO_3$, 1.5g; agar, 15g; and distilled water 900 ml) amended with 50 mg/ml ampicillin. Cultures were incubated under 24h fluorescent light at ambient temperature (22 ± 2°C) for seven days and examined for the presence of fungal growth consistent with that of small-spored *Alternaria* spp. For long-term cryogenic storage, media with fungal conidia and mycelia was excised using a 4-mm diameter sterilized cork borer and placed into screw-top centrifuge tubes. The caps were loosely screwed on to the tubes, tubes were labeled and placed in a closed container with silica gel for two to three days to remove excess moisture. After drying, the tubes were capped tightly, sealed with Parafilm, and stored at -80°C. Herbarium specimens were also prepared for each tissue sample from which *Alternaria* spp. isolates were obtained. The leaf specimens were labeled and pressed between cardboard and placed into a leaf press to be stored at ambient room temperature.

DNA extraction and species identification of *Alternaria* spp.

DNA was extracted from all the isolates using a modified cetyltrimethylammonium bromide (CTAB) method (Mallik et al., 2014). First, spores and mycelium were scraped from a seven-day-old pure culture of small-spored *Alternaria* spp. into an autoclaved mortar. Spores and mycelium were ground into a fine powder using liquid nitrogen and approximately 100 mg of the powder was transferred into a 2.0 ml XXTuff microvial (Bio Spec Products Inc., OK) consisting of 750 µl of Carson lysis buffer (100 mM Tris HCl [pH 9.5], 2% CTAB, 1.4 M NaCl, 1% PEG

8000, and 20 mM EDTA) accompanied with 2% β-mercaptoethanol and 2 µl RNAase. The tube was placed in a FastPrep homogenizer (MP Biomedicals LLC, OH) at a speed of 6.00 m/s for 40 sec. After centrifugation, tubes were incubated at 75ºC for 30 min. with inversions at 10-min intervals. Following incubation, tubes were centrifuged at 14,000 x g for 10 min. (Mallik et al., 2014). The supernatant was removed and placed into a new tube. Nucleic acids were extracted by adding an equal volume of phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol) (Sigma-Aldrich, St. Louis, MO). Following extraction, DNA was precipitated with an equal volume of isopropanol and washed with 95% ethanol. Finally, the DNA was reconstituted in glass-distilled RNAase-DNAase-free water (Growcells.com, Irvine, CA) at a final concentration of 10 ng/µl, and 0.2 µg RNAase (Qiagen Inc., Valencia, CA) was added.

Two sets of primers, OPA-F4/R4 and OPA-F5/R5, were designed to sequence isolates of small-spored *Alternaria* spp.. Primer design was based on previously published OPA 1-3 anonymous locus genomic sequence as a template and Primer 3Plus software to create the genomic library (Tymon et al., 2016a). The primer set OPA-F4 (CGAGCCACATGCTCTGGTTA), and OPA-R4 (AAGTCTAGATCGCTTGCGGG) amplified a 236 bp amplicon. OPA-F5 (TTCCACTTTGTCCCCTGCAA) and OPA-R5 (CGTATCTTCTCACGTCGGGC) amplified a 234 bp amplicon targeting two separate regions in the OPA 1-3 anonymous locus genomic sequence. The library was sequenced using Ion-Torrent next-generation semiconductor sequencing technology with an Ion Personal Genome Machine System (ThermoFisher Scientific, Waltham, MA). The sequences from all isolates were analyzed and compared using the Multalin web aligning program (http://multalin.toulouse.inra.fr/multalin/multalin.html hosted by Plateforme Bioinformatique

Genotoul). The isolates were identified as *A. alternata*, *A. arborescens*, or *A. tenuissima* based on the single-nucleotide polymorphisms (SNPs) (Tymon et al., 2016a).

In vitro sensitivity of small-spored *Alternaria* spp. to QoI fungicides

A study was performed to determine the in vitro sensitivity of small-spored *Alternaria* spp. baseline and non-baseline isolates to QoI fungicides using a conidial germination inhibition assay (Pasche et al., 2004). The 54 isolates of small-spored *Alternaria* spp. were assayed in nine trials, with four to eight isolates included in each trial. Internal control isolates (125-1, an *A. alternata* QoI sensitive isolate, and 1702-5, an *A. tenuissima* QoI reduced-sensitive isolate) were used in each trial to determine assay reproducibility (Wong and Wilcox, 2002). This study was performed twice with two replicates per trial.

Isolates were grown on CV-8 medium for seven to nine days at 22 ± 2°C under 24h fluorescent light. Media contained 2% laboratory-grade agar (A360-500 Fischer Scientific, Pittsburgh, PA). This medium was amended with a technical formulation of azoxystrobin (100% active ingredient; Syngenta Crop Protection, Greensboro, NC), picoxystrobin (98.0% active ingredient; DuPont Crop Protection, Newark, DE), or famoxadone (97.8% active ingredient; DuPont Crop Protection, Newark, DE) dissolved in acetone, to reach final concentrations of 0.01, 0.1, 1, 10, 100 µg/ml. A no-fungicide control was included and all acetone concentrations in the fungicide amended-media were 0.1% by volume. Salicylhydroxamic acid (SHAM) was added at 100 µg/ml to the media to prevent the *Alternaria* spp. isolates from overcoming the activity of the QoI fungicides through an alternative oxidative pathway (Pasche et al., 2004). Once the isolates were grown, a sterile glass rod and sterile distilled H_2O were used to scrape conidia from the agar surface. The conidial concentration was diluted with distilled water and adjusted to 1×10^4 conidia/ml using a hemocytometer. The conidial suspension (100 µl) was

added to the surface of each fungicide amended media plate and spread using a sterile glass rod. Plate were incubated at 25 ± 2°C in the dark for 16h. The spore germination percentage (fifty conidia for each treatment) was estimated using a compound microscope at 100x magnification. A conidium was classified as germinated if one germ tube was at least the length of the conidium, or if multiple germ tubes developed from a single conidium (Pasche et al., 2004).

In vitro sensitivity of small-spored *Alternaria* spp. to SDHI fungicides

In vitro sensitivity of small-spored *Alternaria* spp. baseline and non-baseline isolates was determined using a conidial germination inhibition assay, as previously described (Pasche et al., 2004). Twenty *A. alternata*, seven *A. arborescens*, and ten *A. tenuissima* baseline isolates collected from 1999 to 2004 with no exposure to SDHI fungicides were obtained from long-term cryogenic storage. Twenty-nine *A. alternata*, ten *A. arborescens*, and twenty-two *A. tenuissima* isolates collected from 2011, 2013, 2015, and 2017 were evaluated to determine the non-baseline in vitro sensitivity to SDHI fungicides. The 99 isolates of small-spored *Alternaria* spp. were assayed in 15 trials, with five to eight isolates included in each trial. This study was performed twice with two replicates per trial.

Water agar media were amended with individual technical formulation of boscalid (99% active ingredient; BASF Corporation, Research Triangle Park, NC), fluopyram (97.78% active ingredient; Bayer CropScience, Raleigh, NC), solatenol (97% active ingredient; Syngenta Crop Protection, Greensboro, NC), or adepidyn (98.3% active ingredient; Syngenta Crop Protection, Greensboro, NC) dissolved in acetone to reach final concentrations of 0.01, 0.1, 1, 10, 100 µg/ml. A no-fungicide control was included and all acetone concentrations in the fungicide amended-media were 0.1% by volume. SHAM was added at 100 µg/ml to the media to prevent

the *Alternaria* spp. from overcoming the activity of the SDHI fungicides through an alternative oxidative pathway.

In vivo fungicide efficacy of boscalid, fluopyram, solatenol, and adepidyn to *Alternaria* spp.

Isolates of small-spored *Alternaria* spp. were selected for in vivo sensitivity based on the solatenol and adepidyn in vitro sensitivity (Table 2.1). The in vivo sensitivity assay is a 24-h preventative test (Pasche et al., 2004). The Orange Pixie tomato cultivar (Tomato Growers Supply Company, Fort Myers, FL) was chosen because of its susceptibility to leaf spot diseases, its compact size when compared to potato plants, and the resistance of leaves to dehisce when severely infected. Three tomato seeds were sown in 10 cm^3 plastic pots containing Sunshine Mix LC1 (Sun Gro Horticulture Inc., Bellevue, WA). After emergence, plants were thinned to acquire two uniformly sized plants per pot. When the plants reached a height of 15 to 20 cm and the first three leaves were fully expanded, that were treated with a commercial formulation of boscalid (Endura ®, BASF Corporation, Research Triangle Park, NC), fluopyram (Luna ® Privilege, Bayer CropScience, Raleigh, NC), solatenol (Aprovia ®, Syngenta Crop Protection, Greensboro, NC) or adepidyn (Miravis ™, Syngenta Crop Protection, Greensboro, NC).The concentrations of formulated fungicides used were 0, 0.1, 1, 10, and 100 µg/ml of active ingredient and were applied to the plants in order to obtain a dose-response curve. The fungicide was applied using a Generation II Research Sprayer (Devries Manufacturing, Hollandale, MN) at approximately 400 kPa. A 50 ml conidial suspension at a concentration of 2.0 x 10^5 conidia/ml was prepared from 10- to 12-day old cultures of the small-spored *Alternaria* spp. grown on CV-8 medium under 24h fluorescent light at 22 ± 2°C. The conidial suspension was applied on the plants using a Preval paint-spray gun (Preval Sprayer Division, Prevision Valve Corporation, Yonkers, NY). The

inoculated plants were placed in individual humidity chambers (Phytotronic Inc.; 1626D) set at >95% RH at 22 ± 2 ℃ for 24h.

Table 2.1. Species, isolate, state of origin, and collection year of small-spored *Alternaria* spp. isolates chosen for in vivo experiment

Species	Isolate	State of origin	Collection Year
A. alternata	1716-1	Texas	2017
A. alternata	1714-3	Texas	2017
A. alternata	1715-7	Texas	2017
A. alternata	Aa 3-1	Indiana	2015
A. alternata	125-1	New Mexico	1999
A. alternata	Aa 7-1	Indiana	2015
A. arborescens	1294-3	New Mexico	2013
A. arborescens	1298-2	New Mexico	2013
A. arborescens	1713-1	Colorado	2017
A. arborescens	1713-3	Colorado	2017
A. arborescens	1713-6	Colorado	2017
A. arborescens	Ar 1-1	Indiana	2015
A. tenuissima	At 8-2	Idaho	2015
A. tenuissima	At 9-2	Idaho	2015
A. tenuissima	1714-1	Texas	2017
A. tenuissima	At 13-1	Indiana	2015
A. tenuissima	1317-9	Nebraska	2013
A. tenuissima	1702-5	Colorado	2017

The plants were transferred to confinement chambers (plastic chambers with an open ceiling) on the greenhouse benches to avoid any cross-contamination. Greenhouse temperature was maintained at 25 ± 2 ℃ with daily water applications. Disease severity was visually rated at 6-, 9- and 12-days post-inoculation. Foliar disease elevations were done by estimating the percentage of infected leaf area on the first three true leaves and recorded as percentage diseased tissue. This in vivo experiment was performed twice with two samples (two plants per pot) and three replicates (three pots) for each isolate at each fungicide concentration.

Statistical analyses

The effective concentration where fungal germination is inhibited by 50% (EC_{50} value) was calculated using the percentage reduction in germination relative to the non-fungicide-amended controls and regressed against the $\log_{10}$ fungicide concentration using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Isolates with EC_{50} values of <0.01 and >100 were analyzed as 0.01 and 100 µg/ml, respectively. The experiments were analyzed with an F-test to determine homogeneity of variance among experiments (Fonseka and Gudmestad, 2016). In the small-spored *Alternaria* spp. experiments, internal control isolates (125-1, a QoI sensitive *A. alternata* isolate, and 1702-5, a QoI reduced-sensitive *A. tenuissima* isolate) were used in each trial to determine assay reproducibility (Wong and Wilcox, 2002). Data from individual trials were used in the final analysis if the internal control EC_{50} values were within the 95% confidence interval (Appendix H). Mean separation was calculated using Fisher's protected least significant difference (LSD) test (P = 0.05). Pearson correlation coefficients were calculated to compare the in vitro fungicide EC_{50} values for baseline and non-baseline small-spored *Alternaria* spp..

In vivo experiments were arranged as split-plot randomized complete block designs with small-spored *Alternaria* spp. isolates as the main plot (isolate) and fungicide concentrations as split-plots. For every isolate at all fungicide concentrations (0, 0.1, 1, 10, and 100 µg/ml), disease severity data was transformed to percentage disease control using the formula: [(1 – (% diseased tissue / % diseased tissue in non-treated plants)) x 100] (Gudmestad et al., 2013; Pasche et al., 2004). Disease control data was utilized for further statistical analyses and the Levene's test was used to determine the homogeneity of variance between two independent experiments (Milliken and Johnson, 1992). Analysis of variance (ANOVA) was conducted for isolate x fungicide at each fungicide concentration using SAS (Appendix I). F-tests were used on the combined data to

detect differences at each fungicide concentration. Area under the dose-response curve (AUDRC) (also known as the area under the disease progress curve across all doses of fungicide) was calculated to determine if there were significant differences in early blight control provided by boscalid, fluopyram, solatenol, and adepidyn in managing the three small-spored *Alternaria* spp. (Appendix J).

Results

In vitro sensitivity of small-spored *Alternaria* spp. to QoI fungicides

Independent analysis of variance of in vitro fungicide sensitivity experiments for azoxystrobin, famoxadone, and picoxystrobin determined that variances were homogenous (P = 0.05) and the experiments were combined for further analysis. EC_{50} values of *A. alternata* baseline isolates for sensitivity to azoxystrobin, famoxadone, and picoxystrobin ranged from <0.01 to 0.15, <0.01 to 0.16, and <0.01 to 0.06 µg/ml, respectively. The variation in sensitivity among *A. alternata* baseline isolates were 15-, 16-, and a 6-fold difference in sensitivity to azoxystrobin, famoxadone, and picoxystrobin from most to least sensitive isolates, respectively. The EC_{50} values of the sixteen *A. alternata* baseline isolates are significantly more sensitive to picoxystrobin (0.02 µg/ml) when compared to famoxadone (0.03 µg/ml) or azoxystrobin (0.06 µg/ml) (Table 2.2). However, they were significantly more sensitive to famoxadone than to azoxystrobin.

The sensitivity of baseline *A. arborescens* isolates to azoxystrobin, famoxadone, and picoxystrobin ranged from 0.03 to 0.29, <0.01 to 0.03, and 0.02 to 0.25 µg/ml, respectively. The *A. arborescens* baseline isolates had a 10-, 3-, and a 13-fold variation in sensitivity to azoxystrobin, famoxadone, and picoxystrobin from most to least QoI sensitive isolates, respectively. The EC_{50} values of the three *A. arborescens* baseline isolates are significantly more

sensitive to famoxadone (0.02 µg/ml) than to picoxystrobin (0.14 µg/ml) or azoxystrobin (0.20 µg/ml) (Table 2.2). However, they are significantly more sensitive to picoxystrobin than to azoxystrobin.

The EC_{50} values of *A. tenuissima* baseline isolates for sensitivity to azoxystrobin, famoxadone, and picoxystrobin ranged from 0.02 to 14.61, 0.03 to 0.74, and <0.01 to 8.30 µg/ml, respectively. The *A. tenuissima* baseline isolates had a 731-, 25-, and an 830-fold variation in sensitivity to azoxystrobin, famoxadone, and picoxystrobin from most to least QoI sensitive isolates, respectively. The EC_{50} value of the seven baseline *A. tenuissima* isolates were significantly more sensitive to famoxadone (0.20 µg/ml) than to azoxystrobin (2.17 µg/ml) but is not significantly different from picoxystrobin (1.21 µg/ml) (Table 2.2). Overall, the mean EC_{50} values of *A. arborescens* and *A. alternata* baseline isolates are significantly more sensitive to azoxystrobin, famoxadone, and picoxystrobin than *A. tenuissima* isolates. The mean EC_{50} value of *A. alternata* is also significantly higher than that of *A. arborescens*.

Table 2.2. In vitro fungicide sensitivity of small-spored *Alternaria* spp. baseline isolates to azoxystrobin, famoxadone, and picoxystrobin

Species	Mean fungicide EC_{50} values (µg/ml)			
	Azoxystrobin	Famoxadone	Picoxystrobin	$LSD_{P=0.05}$ [y]
Alternaria alternata	0.06 b	0.03 b	0.02 b	0.009
(n= 16)	A	B	C	
Alternaria arborescens	0.20 b	0.02 c	0.14 b	0.049
(n= 3)	A	C	B	
Alternaria tenuissima	2.17 a	0.20 a	1.21 a	1.159
(n= 7)	A	B	AB	
$LSD_{P=0.05}$	0.235	0.007	0.141	

[y] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed among fungicides. Columns containing the same lowercase letter indicate no significant differences exist between species, within each fungicide

Table 2.3. Relationship between the EC_{50} values of small-spored *Alternaria* spp. calculated within species and groups between QoI and SDHI fungicides based on Pearson correlation analyses

	Alternaria alternata		*Alternaria arborescens*		*Alternaria tenuissima*	
	Baseline r[z]	Non-baseline r	Baseline r	Non-baseline r	Baseline r	Non-baseline r
QoI fungicides[x]						
Azo vs Fam	0.4652	-0.4044*	ND	ND	0.9524	ND
Azo vs Pic	0.3919	0.1605	ND	ND	0.9995	ND
Fam vs Pic	-0.1545	0.2894	ND	ND	0.9512	ND
SDHI fungicides[y]						
Adep vs Bos	-0.3194	0.8656***	0.9342***	0.9916***	0.7513*	0
Adep vs Flu	-0.2690	0.7829***	0.8334*	0.1094	0.6609*	0
Adep vs Sol	-0.0214	0.3610	0.5683	0.2588	0.8975***	0
Bos vs Flu	0.3983	0.9159	0.7294	0.1640	0.5119	0.7450***
Bos vs Sol	0.4145	0.4692**	0.6325	0.3114	0.7725**	0.1264
Flu vs sol	0.9960***	0.5680**	0.8475*	0.9748***	0.3961	-0.1254

[x] Azo=Azoxystrobin; Fam=Famoxadone; Pic=Picoxystrobin

[y] Adep=Adepidyn; Bos=Boscalid; Flu=Fluopyram; Sol=Solatenol

[z] ND indicates undetermined r, due to a low number of isolates (<10 isolates), one asterisk (*) indicates P value < 0.05, two (**) P value < 0.01, three (***) P value < 0.0001

EC_{50} values of *A. alternata* non-baseline isolates for sensitivity to azoxystrobin, famoxadone, and picoxystrobin ranged from 0.05 to >100, 0.04 to >100, and 0.02 to >100 µg/ml, respectively. The *A. alternata* non-baseline isolates had a 2000-, 2500-, and a 5000-fold difference in sensitivity to azoxystrobin, famoxadone, and picoxystrobin from most to least QoI sensitive isolates, respectively. The EC_{50} values of the 28 *A. alternata* non-baseline isolates was significantly more sensitive to azoxystrobin (22.06 µg/ml) than to famoxadone (80.53 µg/ml) or picoxystrobin (40.22 µg/ml) (Table 2.4). However, *A. alternata* non-baseline isolates were significantly more sensitive to picoxystrobin than to famoxadone. The Rfs for *A. alternata* non-baseline: baseline isolates in response to azoxystrobin, famoxadone, and picoxystrobin were 362-, 2368-, and 1749-fold, respectively. Among the 40 non-baseline *A. alternata* isolates evaluated, 39, 37 and 39 fell outside of the baseline range for azoxystrobin, famoxadone, and picoxystrobin, respectively (Figure 2.1).

The EC_{50} value of the *A. arborescens* non-baseline isolate was significantly more sensitive to famoxadone (0.21 µg/ml) than to picoxystrobin (28.55 µg/ml) or azoxystrobin (>100 µg/ml) (Table 2.4). However, the mean EC_{50} value of picoxystrobin was significantly more sensitive than azoxystrobin. The shift in sensitivity of non-baseline *A. arborescens* isolates to azoxystrobin, famoxadone, and picoxystrobin gave Rf of 508-, 6-, and 204-fold, respectively. The single *A. arborescens* non-baseline isolate falls outside of the *A. arborescens* baseline range for all three fungicides (Figure 2.2).

The EC_{50} value of the single *A. tenuissima* non-baseline isolate was more sensitive to picoxystrobin (13.40 µg/ml) and azoxystrobin (11.35 µg/ml) than to famoxadone (33.64 µg/ml) (Table 2.4). The Rfs calculated for *A. tenuissima* isolates on azoxystrobin, famoxadone, and picoxystrobin were 5-, 168-, and 11-fold, respectively. The single *A. tenuissima* non-baseline

isolate fell outside of the *A. tenuissima* baseline range for azoxystrobin and famoxadone but not picoxystrobin (Figure 2.3).

There were no trends observed in sensitivity of the three *Alternaria* spp. to the three QoI fungicides. The mean EC_{50} value of the *A. tenuissima* non-baseline isolate was significantly more sensitive to azoxystrobin than *A. alternata* and *A. arborescens,* but *A. alternata* isolates were more sensitive than the *A. arborescens* isolate (Table 2.4). The *A. arborescens* non-baseline isolate was more sensitive to famoxadone than *A. alternata* and *A. tenuissima,* but the *A. tenuissima* isolate was more sensitive than the *A. alternata* isolates (Table 2.4). The EC_{50} value of the *A. tenuissima* non-baseline isolate was more sensitive to picoxystrobin than *A. alternata* and *A. arborescens,* but the *A. arborescens* isolate was more sensitive than the *A. alternata* isolates (Table 2.4). Overall, the mean fungicide sensitivities of the *A. alternata* baseline were significantly more sensitive than the non-baseline isolates within the QoI fungicides.

Table 2.4. In vitro fungicide sensitivity of small-spored *Alternaria* spp. non-baseline isolates to azoxystrobin, famoxadone, and picoxystrobin

Species	Mean fungicide EC_{50} values (µg/ml)			
	Azoxystrobin	Famoxadone	Picoxystrobin	LSD $_{P=0.05}$ [y]
Alternaria alternata	22.06 b	80.53 a	40.22 a	6.795
(n= 28)	C	A	B	
Alternaria arborescens	>100 a	0.12 c	28.55 b	0.921
(n= 1)	A	C	B	
Alternaria tenuissima	11.35 c	33.64 b	13.40 c	15.873
(n= 1)	B	A	B	
LSD $_{P=0.05}$	2.714	3.238	2.886	

[y] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed between the fungicides. Columns containing the same lowercase letter indicate no significant differences exist between the species within the fungicide

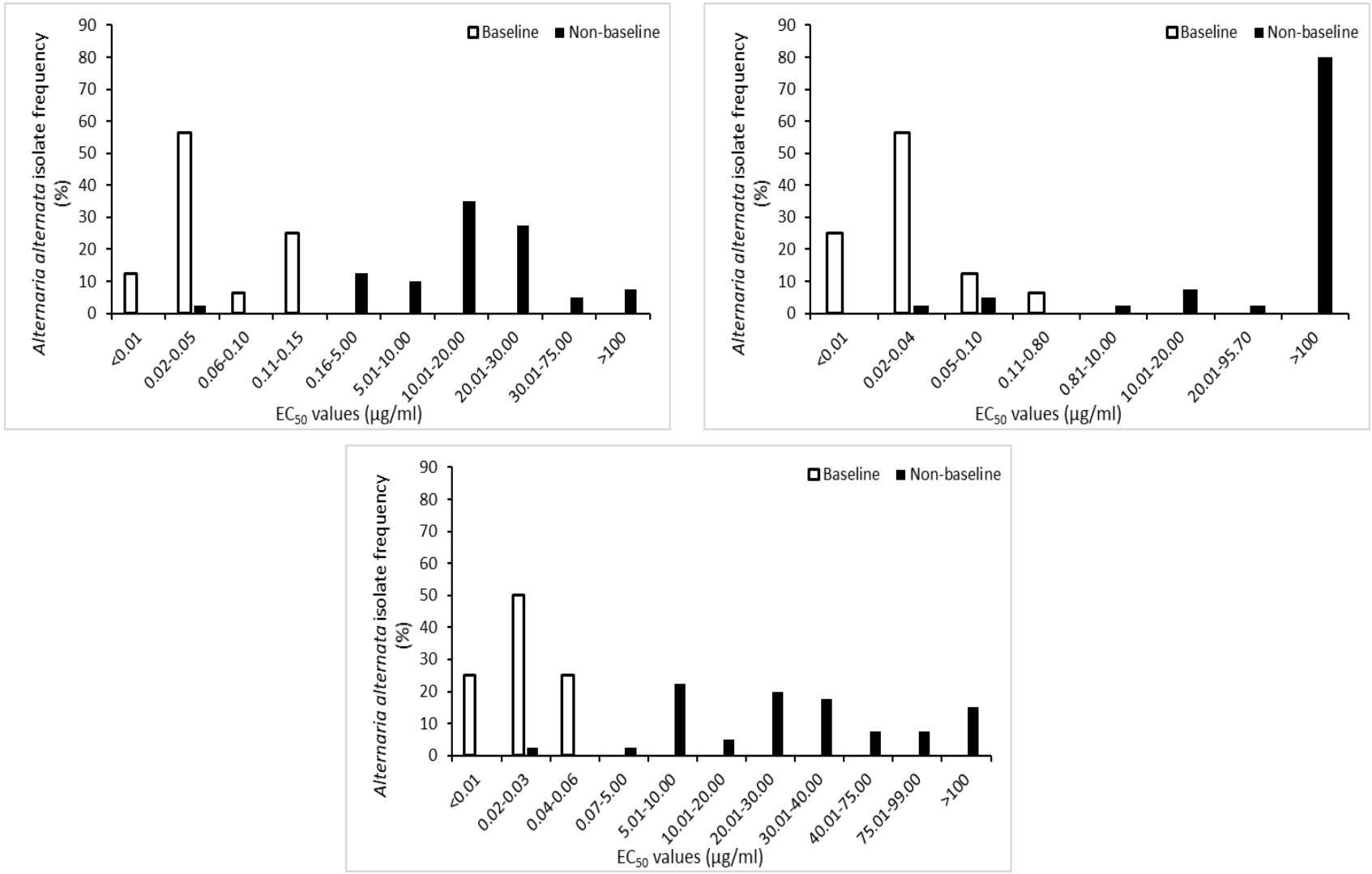

Figure 2.1. Frequency distribution of in vitro sensitivity of 16 baseline and 40 non-baseline *Alternaria alternata* isolates to the QoI fungicides (A) azoxystrobin, (B) famoxadone, and (C) picoxystrobin. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

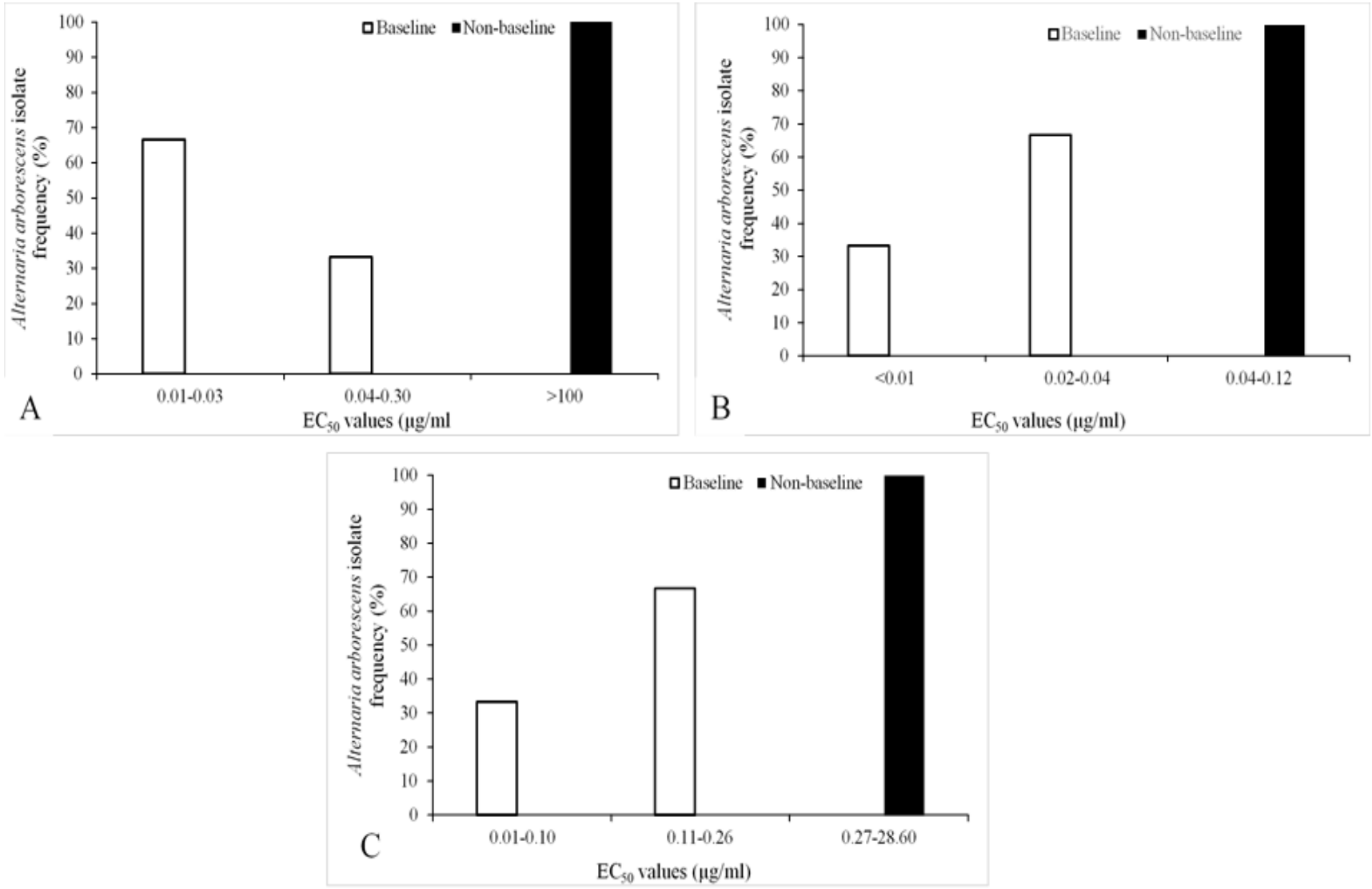

Figure 2.2. Frequency distribution of in vitro sensitivity of three baseline and one non-baseline *Alternaria arborescens* isolates to the QoI fungicides (A) azoxystrobin, (B) famoxadone, and (C) picoxystrobin. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

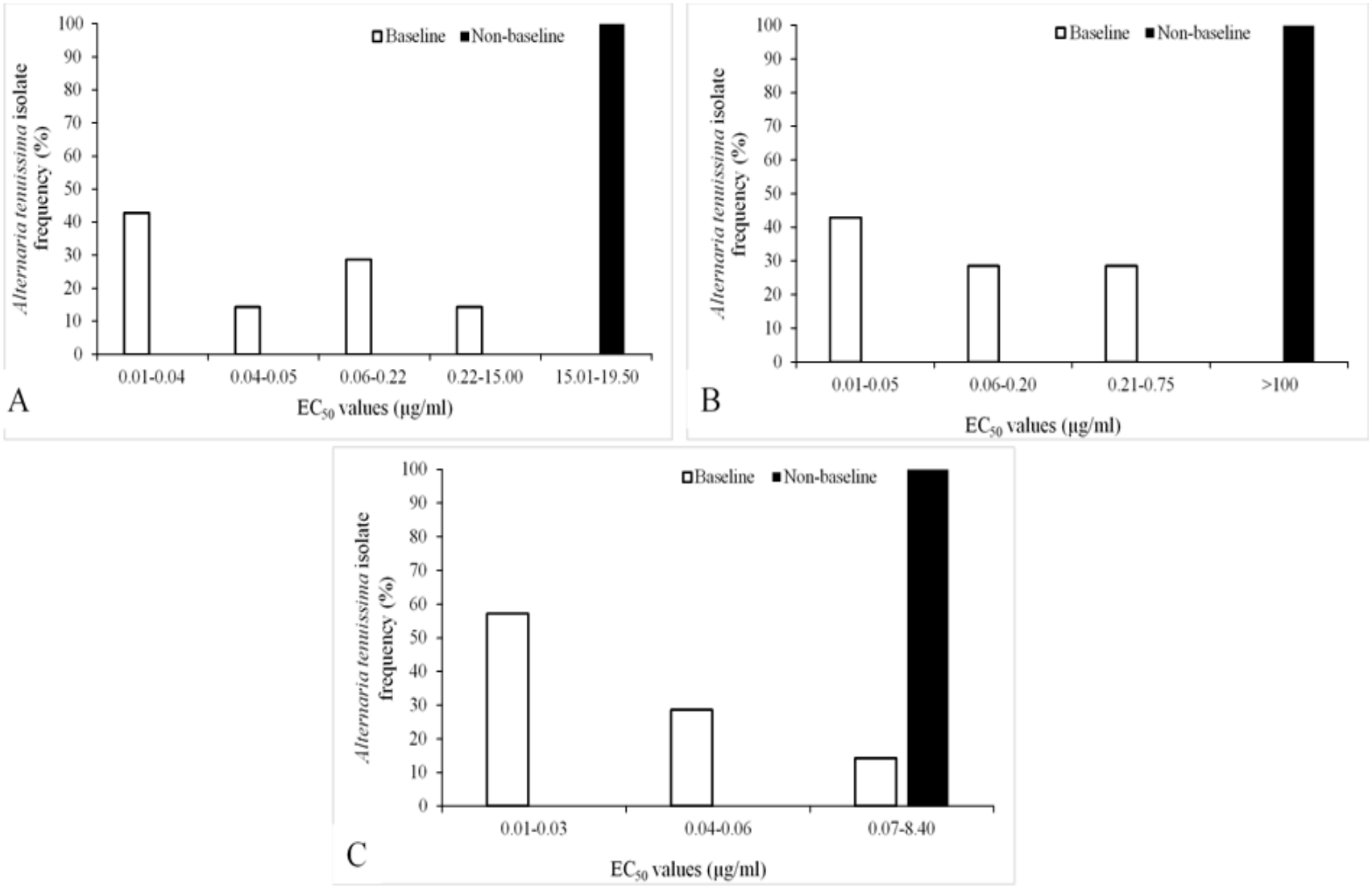

Figure 2.3. Frequency distribution of in vitro sensitivity of seven baseline and one non-baseline *Alternaria tenuissima* isolates to the QoI fungicides (A) azoxystrobin, (B) famoxadone, and (C) picoxystrobin. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

There were no significant correlations among *A. alternata* baseline isolates to the QoI fungicides (Table 2.3). A significant and strong correlation in the sensitivity of the *A. tenuissima* baseline isolates to famoxadone and picoxystrobin was detected (r = 0.9512, P = 0.0098). A significant and strong correlation in the sensitivity of the *A. tenuissima* baseline isolates was detected between famoxadone and azoxystrobin (r = 0.9524, P = 0.00093). In the *A. tenuissima* baseline isolates, a significant and strong correlation was detected between azoxystrobin and picoxystrobin (r = 0.9995, P = <0.00001). A statistically significant but weak negative association was detected among non-baseline *A. alternata* isolates sensitivity to famoxadone and azoxystrobin (r = -0.4044, P = 0.0302).

In vitro sensitivity of small-spored *Alternaria* spp. to SDHI fungicides

Independent analysis of variance of in vitro fungicide sensitivity experiments for boscalid, fluopyram, solatenol, and adepidyn determined that variances were homogenous (P = 0.05) and the experiments were combined for further analysis. EC_{50} values of the 20 *A. alternata* baseline isolates for sensitivity to boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 0.89, <0.01 to 1.13, <0.01 to 1.14, and <0.01 to 0.02 µg/ml, respectively. The *A. alternata* baseline isolates had a 89-, 113-, 114-, and a 2-fold difference in sensitivity to boscalid, fluopyram, solatenol, and adepidyn from most to least SDHI sensitive isolates, respectively. The EC_{50} values of the 20 *A. alternata* baseline isolates to adepidyn (0.01 µg/ml) were significantly more sensitive than to solatenol (0.10 µg/ml), fluopyram (0.11 µg/ml), and boscalid (0.11 µg/ml) (Table 2.5).

The sensitivity of the baseline *A. arborescens* isolates to boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 0.03, <0.01 to 0.02, <0.01 to 0.05, and <0.01 µg/ml, respectively. The *A. arborescens* baseline isolates had a 3-, 2-, and a 5-fold difference in

sensitivity to boscalid, fluopyram, and solatenol from most to least SDHI sensitive isolates, respectively. The EC_{50} values of the seven *A. arborescens* baseline isolates to adepidyn (0.01 µg/ml) were significantly more sensitive than to solatenol (0.03 µg/ml), fluopyram (0.02 µg/ml), and boscalid (0.02 µg/ml) (Table 2.5). However, *A. arborescens* baseline isolates to fluopyram were more sensitive than to solatenol, but not more sentitive than boscalid (Table 2.5).

Table 2.5. In vitro fungicide sensitivity of small-spored *Alternaria* spp. baseline isolates to boscalid, fluopyram, solatenol, and adepidyn

Species	Mean fungicide EC_{50} values (µg/ml)				
	Boscalid	Fluopyram	Solatenol	Adepidyn	$LSD_{P=0.05}$ [y]
Alternaria alternata	0.113 a	0.108 a	0.097 a	0.013 a	0.054
(n= 20)	A	A	A	B	
Alternaria arborescens	0.023 b	0.019 b	0.026 b	0.011 b	0.005
(n= 7)	AB	B	A	C	
Alternaria tenuissima	0.030 b	0.020 b	0.013 b	0.013 a	0.005
(n= 10)	A	B	C	C	
$LSD_{P=0.05}$	0.055	0.021	0.051	0.001	

[y] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed between the fungicides. Columns containing the same lowercase letter indicate no significant differences exist between the species within the fungicide

The sensitivity of baseline *A. tenuissima* isolates to boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 0.06, <0.01 to 0.05, <0.01 to 0.02, and <0.01 to 0.02 µg/ml, respectively. The *A. tenuissima* baseline isolates had a 6-, 5-, 2-, and a 2-fold difference in sensitivity to boscalid, fluopyram, solatenol, and adepidyn from most to least SDHI sensitive isolates, respectively. The EC_{50} values of the *A. tenuissima* baseline isolates to adepidyn (0.01 µg/ml) and solatenol (0.01 µg/ml) were significantly more sensitive than to fluopyram (0.02 µg/ml) and boscalid (0.03 µg/ml) (Table 2.5). However, *A. tenuissima* baseline isolates to fluopyram were more sensitive than boscalid.

In the boscalid, fluopyram, and solatenol fungicides, the EC_{50} values of the *A. arborescens* and *A. tenuissima* baseline isolates were more sensitive than *A. alternata* (Table

2.5). In the adepidyn fungicide, *A. alternata* and *A. tenuissima* baseline isolates were more sensitive than *A. arborescens.*

EC_{50} values of *A. alternata* non-baseline isolates for sensitivity to boscalid, fluopyram, solatenol, and adepidyn ranged from <0.01 to 3.85, <0.01 to 3.83, <0.01 to 1.87, and <0.01 to 0.04 µg/ml, respectively. The *A. alternata* non-baseline isolates had a 385-, 383-, 187-, and a 4-fold difference in sensitivity to boscalid, fluopyram, solatenol, and adepidyn from most to least SDHI sensitive isolates, respectively. The shift in sensitivity between the mean EC_{50} values of the baseline and non-baseline (Rf) *A. alternata* isolates for boscalid, fluopyram, and solatenol were 6-, 6-, and 7-fold, respectively. The EC_{50} values of the 29 *Alternaria alternata* non-baseline isolates are significantly more sensitive to adepidyn (0.01 µg/ml) than to solatenol (0.67 µg/ml), fluopyram (0.61 µg/ml), and boscalid (0.64 µg/ml) (Table 2.6). In the boscalid fungicide, six out of 29 *A. alternata* non-baseline isolates fall outside of the *A. alternata* baseline range (Figure 2.4). In the fluopyram fungicide, three out of 29 *A. alternata* non-baseline isolates fall outside of the *A. alternata* baseline range. In the solatenol fungicide, seven out of 29 *A. alternata* non-baseline isolates fall outside of the *A. alternata* baseline range. In the adepidyn fungicide, five out of 29 *A. alternata* non-baseline isolates fall outside of the *A. alternata* baseline range.

The sensitivity of non-baseline *A. arborescens* isolates to boscalid, fluopyram, solatenol, and adepidyn ranged from 0.05 to 3.80, <0.01 to 2.03, <0.01 to 2.19, and <0.01 to 0.14 µg/ml, respectively. The *A. arborescens* non-baseline isolates had a 76-, 203-, 219-, and a 14-fold difference in sensitivity to boscalid, fluopyram, solatenol, and adepidyn from most to least SDHI sensitive isolates, respectively. The Rfs in the *A. arborescens* isolates for boscalid, fluopyram, solatenol, and adepidyn were 29-, 29-, 25-, and 2-fold, respectively.

Table 2.6. In vitro fungicide sensitivity of small-spored *Alternaria* spp. non-baseline isolates to boscalid, fluopyram, solatenol, and adepidyn

Species	Mean fungicide EC_{50} values (µg/ml)				
	Boscalid	Fluopyram	Solatenol	Adepidyn	LSD $_{P=0.05}$ [y]
Alternaria alternata (n= 29)	0.636 a A	0.610 a A	0.666 a A	0.014 b B	0.126
Alternaria arborescens (n= 10)	0.674 a A	0.545 a A	0.652 a A	0.027 a B	0.267
Alternaria tenuissima (n= 22)	0.268 b A	0.125 b B	0.012 b C	0.010 c C	0.005
LSD $_{P=0.05}$	0.088	0.021	0.051	0.001	

[y] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed between the fungicides. Columns containing the same lowercase letter indicate no significant differences exist between the species within the fungicide

The mean EC_{50} values of the ten *A. arborescens* non-baseline isolates are significantly more sensitive to adepidyn (0.03 µg/ml) than to solatenol (0.65 µg/ml), fluopyram (0.55 µg/ml), and boscalid (0.67 µg/ml) (Table 2.6). In the boscalid fungicide, all ten of the *A. arborescens* non-baseline isolates fall outside of the *A. arborescens* baseline range (Figure 2.5). In the fluopyram fungicide, seven out of ten *A. arborescens* non-baseline isolates fall outside of the *A. arborescens* baseline range. In the solatenol fungicide, five out of ten *A. arborescens* non-baseline isolates fall outside of the *A. arborescens* baseline range. In the adepidyn fungicide, three out of ten *A. arborescens* non-baseline isolates fall outside of the *A. arborescens* baseline range.

The sensitivity of non-baseline *A. tenuissima* isolates to boscalid, fluopyram, and solatenol, ranged from <0.01 to 2.66, <0.01 to 1.31, and <0.01 to 0.03 µg/ml, respectively. The *A. tenuissima* non-baseline isolates had a 266-, 131-, 3-fold difference in sensitivity to boscalid, fluopyram, and solatenol from most to least SDHI sensitive isolates, respectively. The mean EC_{50} values of the 22 *A. tenuissima* non-baseline isolates to adepidyn (<0.01 µg/ml) and

solatenol (0.01 µg/ml) were significantly more sensitive than fluopyram (0.13 µg/ml) and boscalid (0.27 µg/ml), but fluopyram EC_{50} values are more sensitive than boscalid (Table 2.6). The sensitivity shift in the *A. tenuissima* isolates for boscalid and fluopyram were 9- and 6-fold respectively. There was no sensitivity shift observed in *A. tenuissima* isolates for adepidyn or solatenol, therefore, no Rfs were calculated. In the boscalid fungicide, 12 out of 22 *A. tenuissima* non-baseline isolates fall outside of the *A. tenuissima* baseline range (Figure 2.6). In the fluopyram fungicide, 3 out of 22 *A. tenuissima* non-baseline isolates fall outside of the *A. tenuissima* baseline range. In the solatenol fungicide, 1 out of 22 *A. tenuissima* non-baseline isolates fall outside of the *A. tenuissima* baseline range. In the adepidyn fungicide, none of 22 *A. tenuissima* non-baseline isolates fall outside of the *A. tenuissima* baseline range.

For the boscalid fungicide, mean EC_{50} values of *A. tenuissima* non-baseline isolates were more sensitive than *A. alternata* and *A. arborescens* (Table 2.6). For the fluopyram fungicide, the EC_{50} values of the *A. tenuissima* non-baseline isolates were more sensitive than *A. alternata* and *A. arborescens* (Table 2.6). For the solatenol fungicide, the EC_{50} values of the *A. tenuissima* non-baseline isolates were more sensitive than *A. alternata* and *A. arborescens* (Table 2.6). For the adepidyn fungicide, *A. tenuissima* non-baseline isolates were more sensitive than *A. arborescens* and *A. alternata*, but *A. alternata* was significantly more sensitive than *A. arborescens* (Table 2.6).

Overall, the mean fungicide sensitivities of the small-spored *Alternaria* spp. baseline isolates were significantly more sensitive than the non-baseline isolates within the boscalid, fluopyram, and solatenol fungicides. The mean fungicide sensitivities of the small-spored *Alternaria* spp. to adepidyn baseline and non-baseline isolates were not significantly different.

A strong and significant correlation in the sensitivity of the baseline isolates of *A. alternata* was detected in sensitivity to fluopyram and solatenol ($r = 0.996$, $P < 0.00001$) (Table 2.3). A strong and significant correlation in the sensitivity of the baseline isolates of *A. arborescens* was detected in sensitivity to adepidyn and boscalid ($r = 0.9342$, $P = 0.0021$). Similarily, a significant and strong correlation was detected in the sensitivity of baseline isolates to adepidyn and fluopyram ($r = 0.8334$, $P = 0.0198$), and fluopyram and solatenol ($r = 0.8475$, $P = 0.0160$). There was a strong and significant correlation detected in the sensitivity of *A. tenuissima* baseline isolates to adepidyn and boscalid ($r = 0.7513$, $P = 0.0122$), adepidyn and fluopyram ($r = 0.6609$, $P = 0.0375$), adepidyn and solatenol ($r = 0.8975$, $P = 0.0004$), and boscalid and solatenol ($r = 0.7725$, $P = 0.0088$).

A strong and significant correlation in the sensitivity of the non-baseline isolates of *A. alternata* was detected to adepidyn and boscalid ($r = 0.8656$, $P < 0.00001$), adepidyn and fluopyram ($r = 0.7829$, $P < 0.00001$), and boscalid and fluopyram ($r = 0.9159$, $P < 0.00001$). Similarly, a significant and moderately strong correlation was detected in sensitivity to boscalid and solatenol ($r = 0.4692$, $P = 0.0122$), and fluopyram and solatenol ($r = 0.5680$, $P = 0.0013$). There was a significant and strong correlation in the fungicide sensitivity of non-baseline *A. arborescens* isolates to adepidyn and boscalid ($r = 0.9916$, $P < 0.00001$). Similarly, there was a significant and strong correlation in the fungicide sensitivity of non-baseline *A. arborescens* isolates was detected to fluopyram and solatenol ($r = 0.9748$, $P < 0.00001$). A strong and significant correlation in the sensitivity of the non-baseline isolates of *A. tenuissima* was detected to boscalid and fluopyram ($r = 0.7450$, $P = 0.00007$).

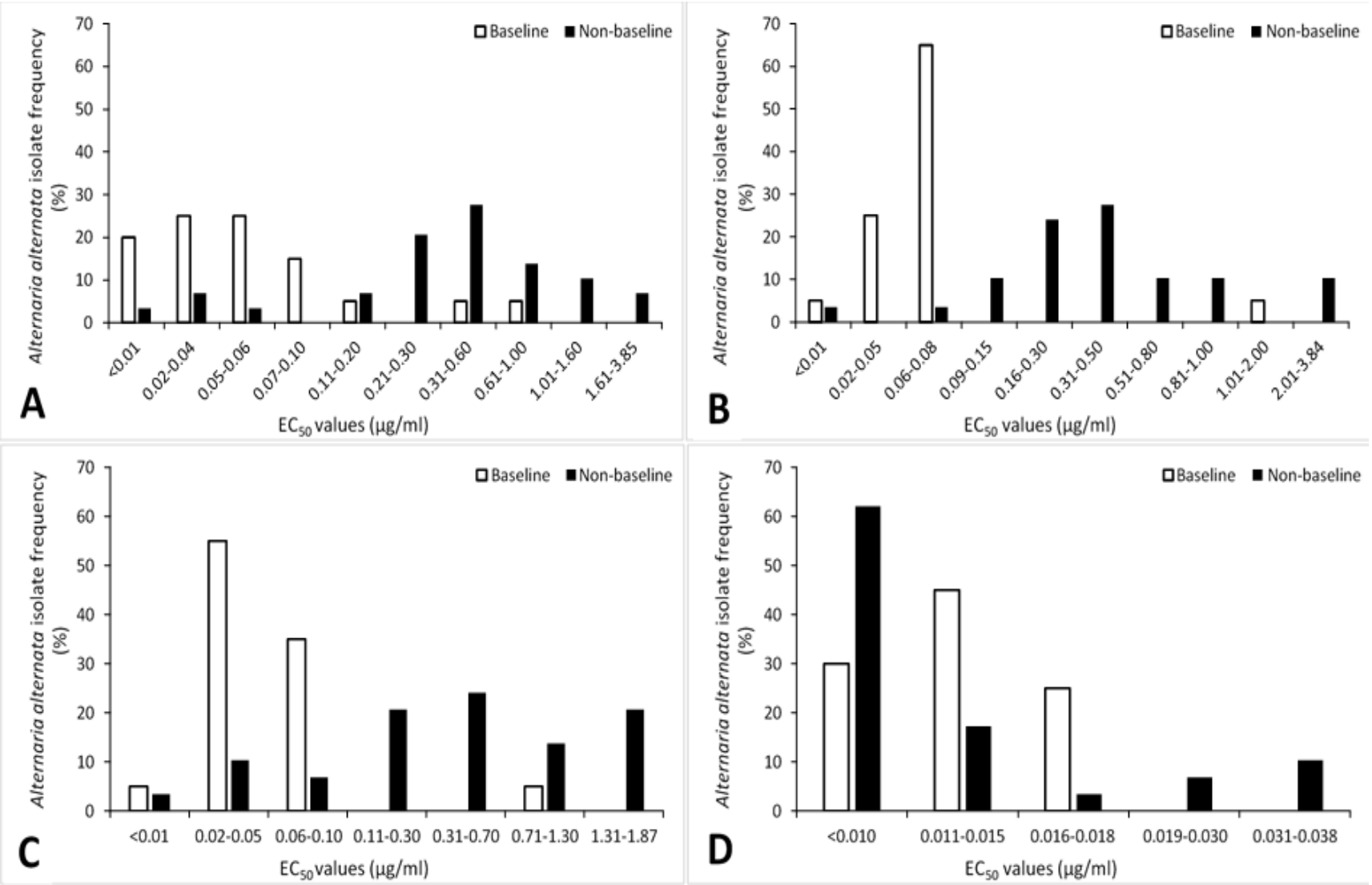

Figure 2.4. Frequency distribution of sensitivity of 20 baseline and 29 non-baseline *Alternaria alternata* isolates to the SDHI fungicides (A) boscalid, (B) fluopyram, (C) solatenol, and (D) adepidyn. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

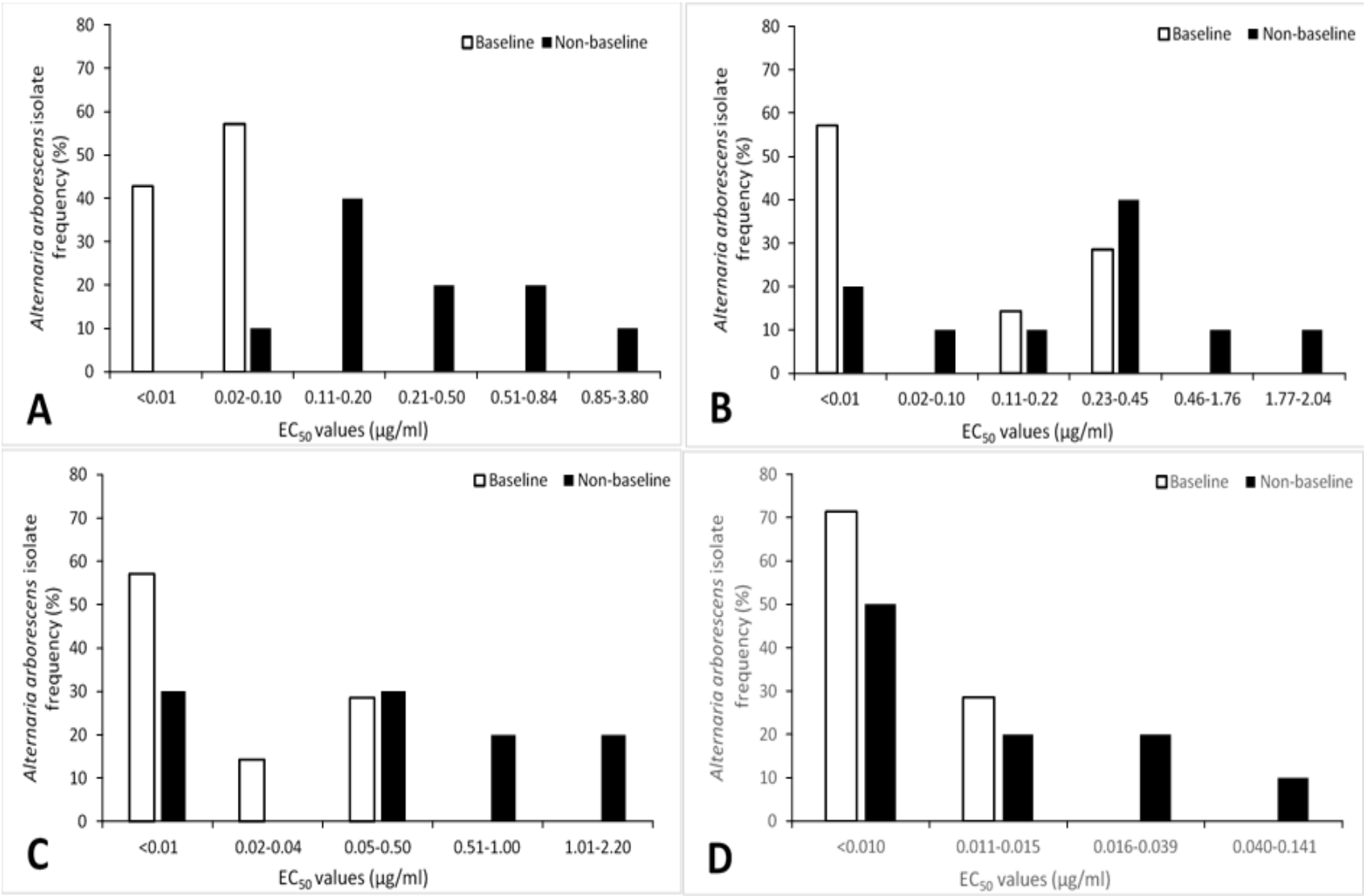

Figure 2.5. Frequency distribution of sensitivity of seven baseline and ten non-baseline *Alternaria arborescens* isolates to the SDHI fungicides (A) boscalid, (B) fluopyram, (C) solatenol, and (D) adepidyn. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

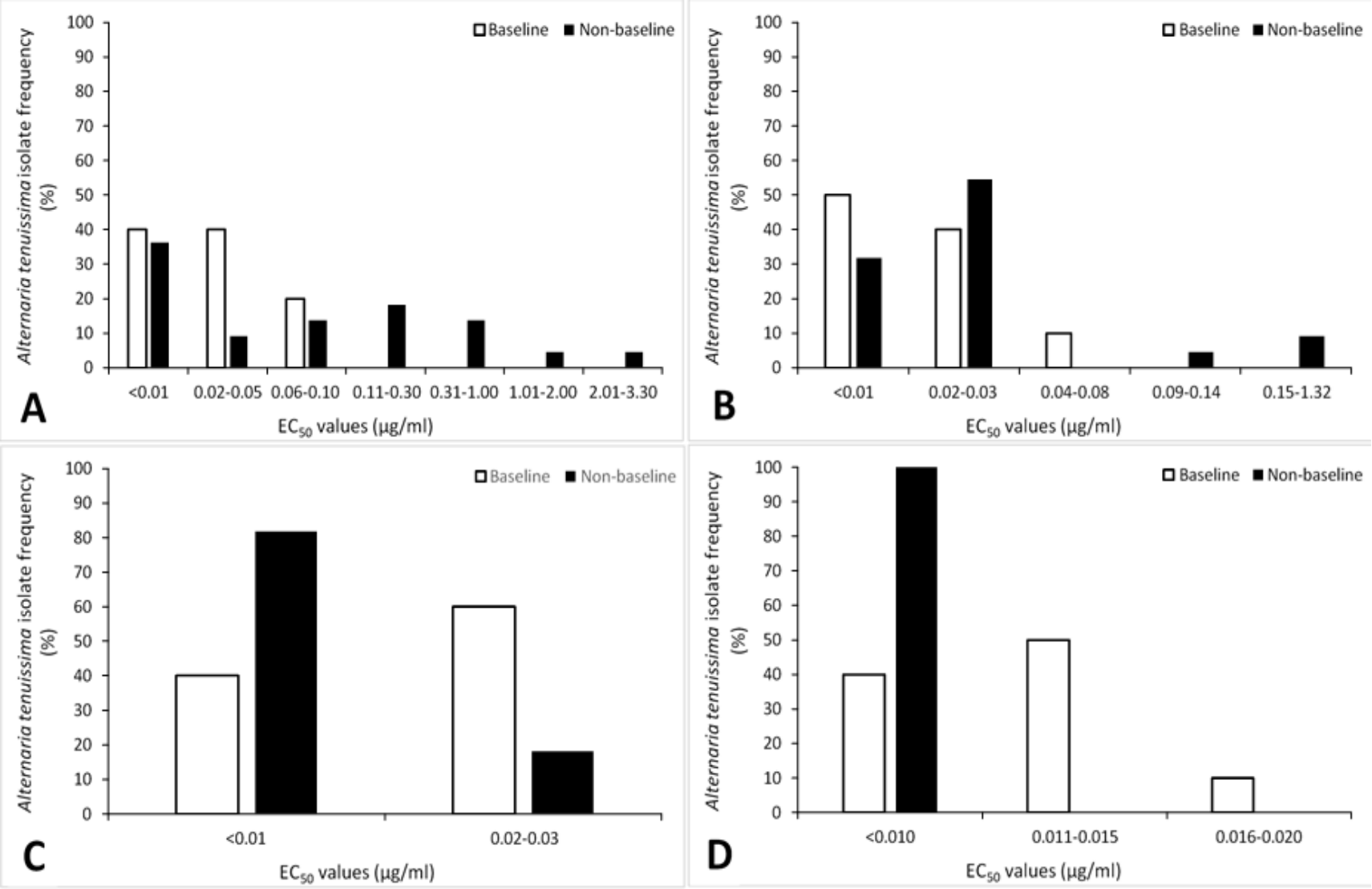

Figure 2.6. Frequency distribution of sensitivity of ten baseline and 22 non-baseline *Alternaria tenuissima* isolates to the SDHI fungicides (A) boscalid, (B) fluopyram, (C) solatenol, and (D) adepidyn. The sensitivity is determined based on the effective concentration which inhibits the spore germination growth by 50% compared to the non-treated control (EC_{50} µg/ml).

Disease control of small-spored *Alternaria* spp. isolates to boscalid, fluopyram, solatenol, and adepidyn

A significant interaction was observed between the main plot and subplot (isolate and fungicide concentration, respectively) for percentage disease control in adepidyn and solatenol ($P<0.0001$). Significantly lower levels of disease control, as measured by AUDRC, were only observed in *A. tenuissima* when compared to *A. alternata* and *A. arborescens* isolates when plants were treated with solatenol (Table 2.7). This indicates that solatenol provides better disease control of *A. alternata* and *A. arborescens* than to *A. tenuissima*. There was no significant difference in disease control provided by adepidyn and fluopyram of the isolates of small-spored *Alternaria* spp. evaluated here. Significantly higher disease control was observed for adepidyn and solatenol compared to boscalid and fluopyram when plants were inoculated with *A. alternata* isolates. When plants were inoculated with isolates of *A. arborescens* or *A. tenuissima*, boscalid provided significantly less disease control compared to fluopyram, solatenol, and adepidyn.

Table 2.7. Mean area under the dose response curve (AUDRC) for small-spored *Alternaria* spp. among the four SDHI fungicides

Species	Mean AUDRC[x]				LSD $_{P=0.05}$ [x]
	Boscalid	Fluopyram	Solatenol	Adepidyn	
Alternaria alternata (n = 6)	9602.4 a B	9709.3 a B	9920.1 a A	9963.7 a A	198.4
Alternaria arborescens (n = 6)	9441.8 a B	9887.1 a A	9883.9 a A	9947.1 a A	193.6
Alternaria tenuissima (n = 6)	9250.6 a B	9853.9 a A	9795.3 b A	9948.7 a A	364.9
LSD $_{P=0.05}$	394.5	190.0	78.7	21.6	

[x] Fisher's protected least significant difference at the P = 0.05 level, rows containing the same uppercase letter indicate no significant differences existed between the fungicides. Columns containing the same lowercase letter indicate no significant differences exist between the species within the fungicide.

Discussion

An important component of resistance management is the establishment of baseline sensitivity and the monitoring of pathogen populations over time (Fonseka and Gudmestad, 2016). These two facets of resistance management can aid in the detection of pathogen sensitivity shifts, predicting the efficacy of fungicide application programs, and recommend necessary resistance management tactics (Thomas et al., 2012).

This is the first report of monitoring sensitivity levels of *A. alternata, A. arborescens,* and *A. tenuissima* populations to QoI and SDHI fungicides across multiple years and potato production areas. The response of small-spored *Alternaria* isolates to QoI and SDHI fungicides were highly variable. Variation in baseline isolates of *A. alternata* in response to azoxystrobin (15-fold) and famoxadone (16-fold) were wider than the six-fold response to picoxystrobin. Variation in baseline isolates of *A. arborescens* in response to famoxadone (three-fold) was narrower than the responses observed to azoxystrobin (10-fold) and picoxystrobin (13-fold). In

contrast, within the *A. tenuissima* baseline isolates, the response to azoxystrobin (731-fold), famoxadone (25-fold), and picoxystrobin (830-fold) was highly variable. Similarly, this high variability was observed also in *A. alternata* non-baseline isolates in response to azoxystrobin (2000-fold), famoxadone (2500-fold), and picoxystrobin (5000-fold). QoI variation among the non-baseline *A. arborescens* and *A. tenuissima* isolates were not calculated since only one isolate was assayed. A narrow distribution range was reported in baseline isolates of *A. solani* (Pasche et al., 2004; Pasche et al., 2005) and *A. rabiei* (Wise et al., 2008) in sensitivity to azoxystrobin. However, a wide variation was observed in baseline isolates of *C. zeae-maydis* in response to azoxystrobin (Bradley et al., 2011). Additionally, wide distributions were also observed in baseline, and non-baseline *A. solani* isolates in response to famoxadone (Pasche et al., 2005) which is similar to results reported here with baseline and non-baseline isolates of *A. alternata* and baseline isolates of *A. tenuissima* in response to famoxadone.

One objective of this study was to compare the sensitivity of a small-spored *Alternaria* spp. baseline population to non-baseline isolates to the QoI fungicides. Results of the in vitro QoI fungicide sensitivity assays demonstrate a substantial shift in sensitivity between the mean EC_{50} values of baseline and non-baseline isolates (Rf > 10-fold). The baseline and non-baseline assessments of the small-spored *Alternaria* spp. determined that significant differences exist between the mean EC_{50} values within the species to the QoI fungicides and within the fungicides to the species. Based on these results, the current *A. alternata* population is not likely to be managed by the application of QoI fungicides alone. Unfortunately, due to the low number of isolates representing the baseline of *A. arborescens* and *A. tenuissima* (three and seven, respectively) and non-baseline (one and one, respectively), an accurate determination of the shift in sensitivity among the QoI fungicides cannot be concluded. A similar azoxystrobin sensitivity

study was conducted with *A. alternata*, *A. arborescens*, and *A. tenuissima* isolates from pistachio (Ma et al., 2003). In that study, a 4348-fold Rf in azoxystrobin sensitivity was observed between the baseline and non-baseline isolates using a combination of all three species. While the study did not report EC_{50} values for individual species, the shift of sensitivity of these *Alternaria* spp. between baseline and non-baseline is still evident.

It is interesting to note that, *A. tenuissima* possessed the highest EC_{50} values in the QoI baseline among the three small-spored *Alternaria* spp., while in the non-baseline population, the highest EC_{50} values were observed in *A. alternata* to picoxystrobin and famoxadone, and *A. arborescens* to azoxystrobin. The increase of EC_{50} values across *Alternaria* spp. can be explained by the presence of the G143A mutation, which has been identified in multiple studies, and has no known fitness penalties (Avenot et al., 2007; Avenot et al., 2009; Ding et al., 2019; Karaoglanidis et al., 2011; Tymon et al., 2016b).

No cross-sensitivity was detected in the baseline and non-baseline isolates of *A. alternata* or *A. arborescens* to the QoI fungicides and little to no cross-sensitivity was observed among the *A. alternata* isolates. Strong and significant correlations were detected in the baseline *A. tenuissima* isolates. This suggests that there is, perhaps, biological relevance among *Alternaria* spp. in causing brown leaf spot of potato and that accurate identification could aid in disease management.

In the Pacific Northwest, *A. arborescens* was the most frequently isolated species from potato and pathogenicity assays demonstrated it was more aggressive than *A. alternata* (Tymon et al., 2016b). It has been suggested that accurate identification of *Alternaria* spp. is critical for disease management (Ding et al., 2019; Tymon et al., 2016a). However, the study reported here is the first evidence that supports the importance of accurate identification of small-spored

Alternaria spp. for effective disease management given the differing sensitivity to SDHI fungicides among the three species.

It is common among Midwestern US potato growers to incorporate single-site 'specialty' fungicides in rotation with multi-site 'standard' fungicides for early blight management (Yellareddygari et al., 2016). In that study, no significant difference was found in disease severity or yield response between the group 1 (QoIs in rotation with anilinopyrimidines) and group 2 (SDHIs in rotation with QoIs or triazoles) specialty fungicides. Traditionally in the Midwest, small-spored *Alternaria* spp. have been considered a minor pathogen compared to *A. solani* on potato (Stevenson et al., 2001). However, with the increased recovery of small-spored *Alternaria* spp. in Wisconsin, it is important to investigate other single-site fungicides that could be effective in managing all *Alternaria* spp. detected in midwestern potato fields (Ding et al., 2019).

The next objective of this study was to compare the sensitivity of a small-spored *Alternaria* spp. baseline to a non-baseline population to the SDHI fungicides. The mean EC_{50} values of *A. alternata* and *A. arborescens* baseline and non-baseline isolates were significantly more sensitive to adepidyn than it is to solatenol, fluopyram, and boscalid. However, in *A. tenuissima* the mean EC_{50} values in the baseline and non-baseline isolates were significantly more sensitive to adepidyn and solatenol than it was to fluopyram and boscalid. Among the *Alternaria* spp. evaluated for sensitivity to the SDHI fungicides, *A. alternata* baseline isolates had the highest mean EC_{50} values compared to *A. arborescens* and *A. tenuissima*. However, in the non-baseline isolates, *A. alternata* had the highest mean EC_{50} values to fluopyram and solatenol, but *A. arborescens* had the highest mean EC_{50} values to adepidyn and boscalid compared to the other *Alternaria* spp.. This could be due to the *Alternaria* spp. isolates

possessing a mutation in the Sdh complex, however it is unknown if *A. arborescens* or *A. tenuissima* can also develop similar mutations (Avenot et al., 2008).

The final objective of this study was to determine the disease control of the small-spored *Alternaria* spp. using the SDHI fungicides in the greenhouse. The high intrinsic activity detected among the small-spored *Alternaria* spp. to SDHI fungicides may not translate into observed disease control. Previous studies demonstrated that no loss of disease control occurred with two- and three-fold sensitivity shifts in response to famoxadone and fenamidone, respectively, (Pasche et al., 2005). Greenhouse results demonstrated that adepidyn, solatenol, and fluopyram provided significantly higher disease control of the *A. arborescens* and *A. tenuissima* isolates than boscalid. Adepidyn and solatenol provided significantly better disease control than fluopyram and boscalid in plants inoculated with *A. alternata* isolates.

It is possible that the fungicide disease control variation is due to isolates possession of SDH mutations. Methods to detect SDH mutations have been developed in *A. alternata*; however, it is unknown whether these primers can accurately detect SDH mutations among all three *Alternaria* spp. (Avenot et al., 2009). In addition, the development of a more accurate small-spored *Alternaria* spp. identification assay will aid in our understanding of how the pathogen interacts with new and existing fungicides.

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CHAPTER THREE: SENSITIVITY OF *ALTERNARIA* SPP. FROM POTATO TO PYRIMETHANIL, CYPRODINIL, AND FLUDIOXONIL

Abstract

Early blight caused by *Alternaria solani* and brown leaf spot caused by the small-spored *Alternaria* spp., *Alternaria alternata, Alternaria arborescens,* and *Alternaria tenuissima,* are observed annually in midwestern potato production areas. *Alternaria* spp. have developed reduced-sensitivity and/or resistance to many single-site fungicides such as quinone outside inhibitor (QoI), succinate dehydrogenase inhibitor (SDHI), and anilinopyrimidines (AP) in recent years. Isolates of these *Alternaria* spp. were evaluated using the mycelial growth method. In vivo evaluation of *A. solani* baseline isolates in response to pyrimethanil, cyprodinil, and fludioxonil was performed to determine the fungicide efficacy. Mean baseline sensitivity EC_{50} values (effective concentration where fungal growth is inhibited by 50%) of *A. alternata* (n = 15), *A. arborescens* (n = 3), *A. tenuissima* (n = 5), and *A. solani* (n = 60) in response to the AP fungicide pyrimethanil were, 1.70, 0.69, 1.81, and 1.17 µg/ml, respectively, and 0.57, 0.35, 1.61, and 0.57 µg/ml, respectively for cyprodinil. The baseline sensitivity of *A. alternata, A. arborescens, A. tenuissima,* and *A. solani* isolates to the PP fungicide fludioxonil were 0.69, 0.18, 0.45, and 0.44 µg/ml, respectively. A statistically significant and strong correlation was observed between pyrimethanil and cyprodinil EC_{50} values among *A. alternata* isolates, but no relationship was observed in other *Alternaria* spp.. Fludioxonil and cyprodinil exhibited a higher level of disease control for *A. solani* compared to pyrimethanil in greenhouse evaluations. Results from this study indicate that fludioxonil and cyprodinil are potentially good additions into fungicide rotation programs for early blight and brown leaf spot management.

Introduction

Early blight and brown leaf spot are two foliar diseases that are a constant problem in potato production areas. *Alternaria solani* Sorauer, which causes the disease early blight, is the dominant pathogen, compared to the three small-spored *Alternaria* spp., *Alternaria alternata* (Fr.) Keissler, *Alternaria arborescens* E.G. Simmons, and *Alternaria tenuissima* (Kunze) which causes the disease brown leaf spot. Early blight and brown leaf spot can cause potato yield damage up to 30 and 18%, respectively, if conditions are favorable (Christ and Maczuga, 1989; Droby et al., 1984; Shtienberg et al., 1990).

A recent study reported that specialty fungicides such as quinone outside inhibitors (QoI) and succinate dehydrogenase inhibitors (SDHI) utilized in rotation with standard protectant fungicides such as mancozeb or chlorothalonil, can increase early blight disease control and potato yield (Yellareddygari et al., 2019). While SDHI and QoI fungicides are useful additions to potato disease management programs, they are regarded as high resistance-risk fungicides due to single-site modes of action. However, specialty fungicides including demethylation inhibitors (DMI, FRAC group 3), anilinopyrimidines (AP, FRAC group 9), and phenylpyrroles (PP, FRAC group 12) have provided a high level of disease control and are all classified as medium-risk fungicides.

While a low frequency of reduced-sensitivity and/or resistance to the AP and PP fungicides has appeared in numerous pathogens, these chemistries are still effective in disease management (Avenot and Michailides, 2015; Fairchild et al., 2013; Fonseka and Gudmestad, 2016; Kanetis et al., 2008). A recent study determined that two DMI fungicides, difenoconazole, and metconazole, demonstrated high intrinsic activity against *A. solani* and *A. alternata* (Fonseka and Gudmestad, 2016). In that study, some isolates of *A. solani* exhibited some reduced-

sensitivity to the AP fungicide, pyrimethanil, in vitro conditions and exhibited significantly lower disease control than the sensitive isolates in the greenhouse evaluations. Other studies have demonstrated the AP fungicide, cyprodinil, and the PP fungicide, fludioxonil, effectively controlled *A. alternata* (Avenot and Michailides, 2015). In that study, a few isolates displayed resistance to fludioxonil and/or cyprodinil with no observed fitness penalties. Pyrimethanil has been utilized in potato grower management programs for over a decade and reduced-sensitivity has only been detected in a small number of *A. solani* isolates in Colorado, Idaho, Minnesota, and Texas (Fairchild et al., 2013; Fonseka and Gudmestad, 2016).

AP and PP fungicides are frequently used in combination with other fungicide chemistries in pre-packaged mixtures. These mixtures have been highly effective in disease management of *Botrytis cinerea* (Chapeland et al., 1999; Hilber and Schüepp, 1996;), *A. alternata* (Avenot and Michailides, 2015), and *Penicillium digitatum* (Kanetis et al., 2008). Fludioxonil is used primarily as a seed treatment fungicide registered on numerous crops, and as a post-harvest fungicide used on several tree fruit crops. Fludioxonil is also used in a pre-packaged mixture with cyprodinil for foliar disease control in pulse crops, and numerous vegetable and fruit crops. In potato, fludioxonil is used as a seed treatment for seed-borne tuber black scurf (*Rhizoctonia solani*) and, more recently, has been mixed with other chemistries to manage potato storage diseases such as *Fusarium* dry rot (*Fusarium* spp.), and silver scurf of potato (*Helimthosporium solani)*. However, the early blight and brown leaf spot disease control provided by cyprodinil and fludioxonil is still unknown and these chemistries are not registered for foliar use on potato.

Previous studies have determined baseline (isolates collected before fungicide was registered) for both *A. alternata* and *A. solani* (Fonseka and Gudmestad, 2016). However, a

recently published study determined that potato brown spot can be caused by three small-spored *Alternaria* spp. (*A. alternata, A. arborescens,* and *A. tenuissima*) (Tymon et al., 2016). A previous analysis of our small-spored *Alternaria* spp. collection determined that our collection included all three species (Budde-Rodriguez et al. Plant Disease in review). While the previously determined baselines were excellent insights into the fungicide sensitivities of pyrimethanil, it was compiled of the all three small-spored *Alternaria* spp. and warranted reexamination (Fonseka and Gudmestad, 2016). A baseline for the *Alternaria* spp. in response to cyprodinil and fludioxonil has not been established. Analyzing baseline isolates in response to new or existing fungicides aids in determining the fungicide risk factors. *Alternaria solani* and *A. alternata* are classified as medium- and high-risk pathogens, respectively (FRAC, 2019). However, it is still unknown where *A. arborescens* and *A. tenuissima* rank as pathogen risks (FRAC, 2019).

The objectives of this study were to (i) determine the baseline sensitivity of four *Alternaria* spp. to anilinopyrimidine and phenylpyrrole fungicides, and to (ii) determine the disease control of the *A. solani* isolates provided by pyrimethanil, cyprodinil, and fludioxonil.

Materials and Methods

Collection and maintenance of isolates

Isolates of *A. solani* and three small-spored *Alternaria* spp. were recovered from foliage submitted to the Gudmestad Laboratory from potato growing regions across the United States. Fifteen *A. alternata,* five *A. tenuissima,* and three *A. arborescens* baseline isolates collected in 1999 to 2002 and 60 *A. solani* baseline isolates assayed on pyrimethanil, cyprodinil, and fludioxonil, collected in 1998 to 2013, were obtained from long-term cryogenic storage (Appendix K). The 23 small-spored *Alternaria* spp. isolates and the 60 *A. solani* isolates were the

same isolates used in the previous pyrimethanil and DMI fungicide sensitivity study (Fonseka and Gudmestad, 2016).

Foliar sections with lesions characteristic of early blight and brown spot were surface sterilized in a 10% bleach solution for one min. and rinsed in sterile, distilled water. The lesions were aseptically excised from the foliar surface using a sterile scalpel blade and transferred to a 1.5% unamended agar media (water agar) and incubated at room temperature (22 ± 2°C) for three to four days until conidia were produced. Purification of the isolates was performed by transferring a single conidium from the plate using a sterile glass needle to a clarified V8 (CV-8) (Campbell's V8 juice, 100 ml; $CaCO_3$, 1.5g; agar, 15g; and distilled water 900 ml) medium amended with 50 mg/ml ampicillin. Isolates were incubated under 24h fluorescent light at room temperature (22 ± 2°C) for 7 days and examined for the presence of *Alternaria* spp. For long-term cryogenic storage, a 4-mm diameter sterilized cork borer was used to remove plugs of media with fungal conidia and mycelia and placed into screw-top centrifuge tubes. The caps were loosely screwed on to the tubes, labeled, and placed in a closed container with silica gel for two to three days to remove excess moisture. After drying, the tubes were capped tightly, sealed with Parafilm, and stored in an ultra-freezer set at -80°C. Herbarium specimens were also made for each tissue sample where *Alternaria* spp. isolates were obtained. The leaf specimens were labeled and pressed between cardboard and placed into a leaf press to be stored at ambient room temperature.

In vitro sensitivity of baseline *Alternaria* spp. isolates to pyrimethanil, cyprodinil, and fludioxonil

A study was performed to determine the in vitro sensitivity of isolates of the four *Alternaria* spp. with no exposure to pyrimethanil, cyprodinil, and fludioxonil. Twenty-three

isolates of small-spored *Alternaria* spp. were assayed in three trials with eight isolates included in each trial. Sixty *A. solani* isolates were assayed in ten trials with five to seven isolates included in each trial. This study was performed twice with two replications per trial.

Fungicide sensitivity for pyrimethanil and cyprodinil was determined using a mycelial growth assay on a solid synthetic media containing L-asparagine (asp-agar) (Fonseka and Gudmestad, 2016; Hilber and Schüepp, 1996). The asp-agar procedure was developed for the evaluation of *B. cinerea* sensitivity to anilinopyrimidine fungicides because complex media such as malt-agars were not appropriate for the in vitro assays due to the high nutrient-rich medium that would allow the pathogen to overcome the fungicide activity (Hilber and Schüepp, 1996; Stevenson et al., 2019). Asp-agar media was amended with either technical grade pyrimethanil (95.0% active ingredient; Bayer CropScience, Raleigh, NC), or cyprodinil (98.0% active ingredient; Syngenta Crop Protection, Greensboro, NC) dissolved in acetone to reach final concentrations of 0.1, 1, 10, and 100 µg/ml (Fonseka and Gudmestad, 2016). Fludioxonil does not require the synthetic media, but rather a technical grade fludioxonil (98.0% active ingredient; Syngenta Crop Protection, Greensboro, NC) was dissolved in acetone to reach final concentrations of 0.1, 1, 10, and 100 µg/ml in quarter-strength potato dextrose agar (PDA) media (5g Potato Dextrose broth, 15g agar, 1L H_2O) (Avenot and Michailides, 2015). A no-fungicide control was included and all acetone concentrations in the fungicide amended-media were 0.1% by volume. A five-mm mycelial plug excised from the edge of a seven-day old small-spored *Alternaria* spp. or *A. solani* culture was inverted onto the center of the fungicide-amended media so that fungal growth was in contact with the media surface. The plates were incubated at 24 ± 2°C in the dark for seven days. After the incubation, mycelial growth diameter of each isolate

was measured in two perpendicular directions, with the original five-mm diameter mycelial plug subtracted from the final measurement.

In vivo fungicide efficacy of pyrimethanil, cyprodinil, and fludioxonil to *Alternaria solani*

Alternaria solani isolates expressing the highest in vitro EC_{50} values to either cyprodinil and/or fludioxonil were evaluated under greenhouse conditions in addition to four baseline isolates evaluated in a previous study (Fonseka and Gudmestad, 2016) (Table 3.1). The in vivo sensitivity assay is a twenty-four-hour preventative test. The Orange Pixie tomato cultivar was chosen because of its susceptibility to early blight, its compact size compared to potato plants, and the resistance of leaves to dehisce once severely infected. Three tomato seeds were sown in 10 cm^3 plastic pots containing Sunshine Mix LC1 (Sun Gro Horticulture Inc., Bellevue, WA). After emergence, plants were thinned to acquire two uniformly sized plants per pot. When the plants reached a height of 15 to 20 cm and the first three leaves were fully expanded, the plants were treated with the commercial formulation of pyrimethanil (Scala ® SC, Bayer CropScience LP, St. Louis, MO), fludioxonil (Scholar ®, Syngenta Crop Protection, Greensboro, NC), or cyprodinil (Vangard ® WG, Syngenta Crop Protection, Greensboro, NC). Formulated fungicide concentrations of 0, 0.1, 1, 10, and 100 µg/ml were applied to the plants to obtain a dose-response curve. The fungicide was applied using a Generation II Research Sprayer (Devries Manufacturing, Hollandale, MN) at approximately 400 kPa.

Table 3.1. *Alternaria solani* isolates assayed for in vivo sensitivity to pyrimethanil, cyprodinil, and fludioxonil

Isolate	State of origin	Collection Year
13-1	Nebraska	1998
22-1	Minnesota	1998
38-4	Nebraska	1998
88-1	Wisconsin	1998
1168-3	Idaho	2010
1179-3	North Dakota	2010
1184-14	Colorado	2011
1332-6	Texas	2013

A 50 ml conidial suspension at a concentration of 2.0 x 10^5 conidia/ml was prepared from 10 to 12-day old cultures of *A. solani* on CV-8 medium grown under 24h fluorescent light at 22 ± 2°C. The conidial suspension was applied to the plants using a Preval paint-spray gun (Preval Sprayer Division, Prevision Valve Corporation, Yonkers, NY). The inoculated plants were placed in individual humidity chambers (Phytotronic Inc.; 1626D) set at >95% RH at 22 ± 2 °C for 24 hours. The plants were transferred to confinement chambers (plastic chambers with an open ceiling) on the greenhouse benches to avoid any cross-contamination from other isolates. The greenhouse temperature was maintained at 25 ± 2 °C with daily water applications. Disease severity evaluations were visually rated at 6, 9- and 12-days post-inoculation. Foliar disease evaluations were conducted by estimating the percentage of infected leaf area on the first three true leaves and recorded as diseased tissue percentage. This in vivo study was performed twice with two samples (two plants per pot) and three replicates (three pots) for each isolate at each fungicide concentration.

Statistical analysis

All in vitro experiments were performed twice using a completely random design with two replicates for each fungicide concentration. The effective concentration where fungal germination is inhibited by 50% (EC_{50} value) was calculated using the percentage reduction in mycelial growth relative to the non-fungicide-amended controls and regressed against the $\log_{10}$ fungicide concentration. Using the Statistical Analysis System (SAS Institute Inc., Cary, NC), the concentration determined to reduce mycelial growth by half compared to the 0 µg/ml concentration was deduced from the 50% intercept (EC_{50} value). Isolates with EC_{50} values of <0.01 and >100 were analyzed as 0.01 and 100 µg/ml, respectively. Internal control isolates for *A. solani* (13-1, a wild-type isolate, and 526-3, a QoI reduced-sensitive isolate) and small-spored *Alternaria* spp. (125-1, an *A. alternata* QoI sensitive isolate, and 1702-5, an *A. tenuissima* QoI reduced-sensitive isolate) were used in each trial to determine assay reproducibility (Wong and Wilcox, 2002). Trials were included in the final analysis if the internal control EC_{50} values were within the 95% confidence interval (Wong and Wilcox, 2002). Experiments were analyzed with an F-test to determine the homogeneity of variance among the experiments. Mean separation was calculated using Fisher's protected least significant difference (LSD) test (P = 0.05). Pearson correlation coefficients were calculated to compare the in vitro fungicide EC_{50} values for the baseline *Alternaria* spp.

In vivo experiments were arranged as split-plot randomized complete block designs with *A. solani* isolates as the main plot and fungicide concentrations as split-plots. For every isolate at all fungicide concentrations (0, 0.1, 1, 10, and 100 µg/ml), disease severity data was transformed to percentage disease control using the formula: [(1 – (% diseased tissue / % diseased tissue in non-treated plants)) x 100] (Gudmestad et al., 2013; Pasche et al., 2004). Disease control data

was utilized for further statistical analyses and the Levene's test was used to determine the homogeneity of variance between two independent experiments (Milliken and Johnson, 1992). Analysis of variance (ANOVA) was conducted for isolate x fungicide at each fungicide concentration using SAS. F-tests were used on the combined data to detect differences at each fungicide concentration. Area under the dose-response curve (AUDRC) (also known as the area under the disease progress curve across all doses of fungicide) values were calculated to determine significant differences in disease control provided by pyrimethanil, cyprodinil, and fludioxonil in managing the *A. solani* isolates (Shaner and Finney, 1977).

Results

In vitro sensitivity of baseline *Alternaria spp.* to pyrimethanil, cyprodinil, and fludioxonil

Independent analysis of variance of in vitro fungicide sensitivity experiments for pyrimethanil, cyprodinil, and fludioxonil determined that variances were homogenous for *A. alternata* (P = 0.9583), *A. arborescens* (P = 0. 6915), *A. tenuissima* (P = 0. 7334), and *A. solani* (P = 0. 6813), and the experiments were combined for further analysis (Appendix L). EC_{50} values of *A. alternata* baseline isolates for sensitivity to pyrimethanil, cyprodinil, and fludioxonil ranged from <0.1 to 5.85, <0.1 to 1.55, and <0.1 to 3.36 µg/ml, respectively. The sensitivity variation among *A. alternata* baseline isolates to pyrimethanil, cyprodinil, and fludioxonil from the most to the least sensitive was 56-, 16-, and 34-fold, respectively. The mean fungicide sensitivity of the fifteen baseline *A. alternata* baseline isolates were significantly less sensitive to pyrimethanil (1.70 µg/ml) than to cyprodinil (0.57 µg/ml) and fludioxonil (0.69 µg/ml) (Figure 3.1).

The sensitivity of baseline *A. arborescens* isolates to pyrimethanil, cyprodinil, and fludioxonil ranged from <0.1 to 1.14, <0.1 to 0.84, and <0.1 to 0.35 µg/ml, respectively. The

sensitivity variation among *A. arborescens* baseline isolates to pyrimethanil, cyprodinil, and fludioxonil from the most to least sensitive was 11-, 8-, and 4-fold, respectively. The mean fungicide sensitivities of the three baseline *A. arborescens* isolates to pyrimethanil, cyprodinil, and fludioxonil was 0.69, 0.35, and 0.18 µg/ml, respectively, and these *A. arborescens* isolates were significantly less sensitive to pyrimethanil compared to cyprodinil and fludioxonil (Figure 3.1).

The EC_{50} values of *A. tenuissima* baseline isolates for sensitivity to pyrimethanil, cyprodinil, and fludioxonil ranged from 1.08 to 4.23, <0.1 to 4.12, and <0.1 to 1.34 µg/ml, respectively. The *A. tenuissima* baseline isolates had a 4-, 41-, and 13-fold variation in sensitivity from the least to most sensitive isolate in response to pyrimethanil, cyprodinil, and fludioxonil, respectively. The mean fungicide sensitivity of the five baseline *A. tenuissima* isolates to pyrimethanil, cyprodinil, and fludioxonil was 1.81, 1.61, and 0.45 µg/ml, respectively (Figure 3.1). In contrast to the previous *Alternaria* spp., *A. tenuissima* baseline isolates were significantly more sensitive to fludioxonil compared to pyrimethanil and cyprodinil (Figure 3.1).

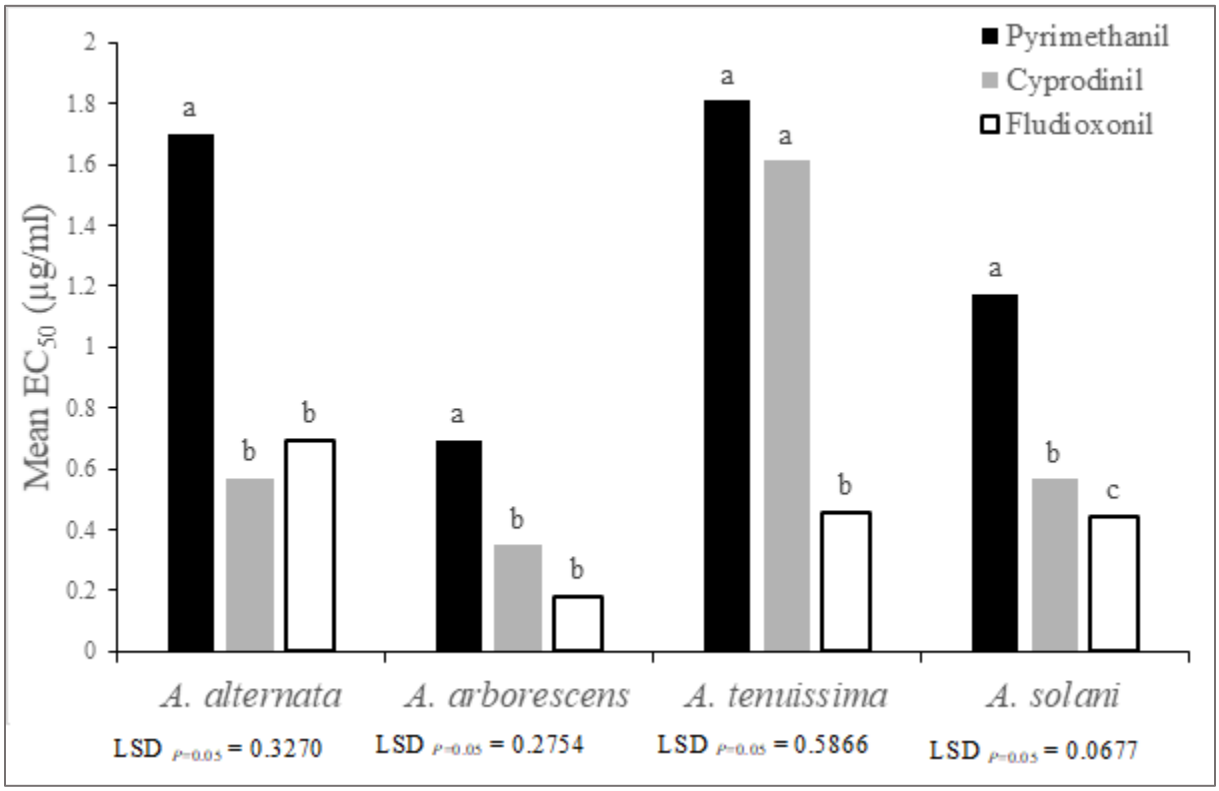

Figure 3.1. Mean EC_{50} values for in vitro isolate sensitivity of *Alternaria* spp. to anilinopyrimidine and phenylpyrrole fungicides. Within species, columns with the same letter are not significantly different based on Fisher's protected least significant difference at the P = 0.05 level.

The EC_{50} values of *A. solani* baseline isolates test for sensitivity to pyrimethanil, cyprodinil, and fludioxonil ranged from <0.1 to 2.45, <0.1 to 1.85, and <0.1 to 1.25 µg/ml, respectively. The sensitivity variation among *A. solani* baseline isolates from the most to the least sensitive isolates for pyrimethanil, cyprodinil, and fludioxonil were 25-, 19-, and 13-fold, respectively. The mean fungicide sensitivity of the 60 *A. solani* baseline isolates were significantly more sensitive to fludioxonil (0.44 µg/ml) than to either cyprodinil (0.57 µg/ml) or pyrimethanil (1.17 µg/ml) (Figure 3.1). However, among the two AP fungicides, *A. solani* is significantly more sensitive to cyprodinil than pyrimethanil.

A significant interaction was observed among the *Alternaria* spp. and fungicide ($P < 0.0001$) for mean EC_{50}. *Alternaria arborescens* has significantly lower EC_{50} values among the

three fungicides assayed compared to *A. solani, A. alternata,* and *A. tenuissima* (Figure 3.1). *Alternaria solani* was significantly more sensitive to pyrimethanil, cyprodinil, and fludioxonil compared to *A. alternata* and *A. tenuissima,* and *A. alternata* has significantly lower EC_{50} values among all three fungicides assayed compared to *A. tenuissima* (Figure 3.1).

There was a significant and strong correlation in the fungicide sensitivity among baseline *A. alternata* isolates to pyrimethanil and cyprodinil (r = 0.7679, P = 0.0008) (Table 3.2). Among the *A. arborescens* baseline isolates, no significant correlation was observed between the three fungicides. There was a significant and strong correlation in the sensitivity of the *A. tenuissima* baseline isolates for pyrimethanil and fludioxonil (r = 0.9819, P = 0.0005). Among the *A. solani* baseline isolates there was no significant correlation observed between any of the fungicides.

Table 3.2. Relationship between the of EC_{50} values of small-spored *Alternaria* spp. and *Alternaria solani* isolates in response to anilinopyrimidine and phenylpyrrole fungicides

	Alternaria alternata	*Alternaria arborescens*	*Alternaria tenuissima*	*Alternaria solani*
	r[z]	r	r	r
Pyr vs Cyp[y]	0.7679***	ND	-0.0920	0.1351
Pyr vs Flu	0.2992	ND	0.9819***	ND
Cyp vs Flu	0.0186	ND	-0.2250	ND

[y] Pyr=Pyrimethanil; Cyp=Cyprodinil; Flu=Fludioxonil
[z] ND indicates undetermined r, due to a low number of isolates (<10 isolates), one asterisk (*) indicates P value < 0.05, two (**) P value < 0.01, three (***) P value < 0.0001

Disease control of *A. solani* isolates to pyrimethanil, cyprodinil, and fludioxonil

Independent analysis of in vivo disease control experiments for pyrimethanil, cyprodinil, and fludioxonil determined that variances were homogenous for *A. solani* (P = 0.5070), and experiments were combined for further analysis (Appendix M). A significant interaction was observed between isolate and fungicide concentration for percentage disease control in pyrimethanil, cyprodinil, and fludioxonil (P < 0.0001). Generally, significantly lower levels of disease control, based on mean AUDRC, were observed in the for *A. solani* isolates collected

after 2010 compared to the 1998 isolates when plants were treated with pyrimethanil (Table 3.3). Early blight disease control with cyprodinil in isolates collected in 1998 and 2010 was significantly higher than isolates collected in 2011 and 2013, but not those collected in 2010. Early blight disease control with fludioxonil was significantly higher in isolates collected after 2010 compared to isolates collected in 1998. Overall, numerically higher levels of early blight disease control were observed when plants were treated with fludioxonil compared to cyprodinil and pyrimethanil.

Table 3.3. Mean in vivo percentage early blight disease control and area under the dose response curve (AUDRC) of *Alternaria solani* isolates by pyrimethanil, cyprodinil, and fludioxonil as determined in greenhouse assays

Fungicide	*Alternaria solani* Isolate	Year collected	In vitro EC_{50}[x] (µg/ml)	Fungicide concentration (µg/ml)[y]				AUDRC
				0.1	1	10	100	
Pyrimethanil	13-1	1998	1.10 c	11.7 e	91.5 ab	100.0 a	100.0 a	9908.0 a
	22-1	1998	1.60 a	53.1 bc	88.1 b	93.7 ab	100.0 a	9598.1 ab
	38-4	1998	1.31 b	60.6 ab	98.3 a	100.0 a	100.0 a	9964.1 a
	88-1	1998	1.19 bc	72.6 a	98.1 a	100.0 a	100.0 a	9968.1 a
	1168-3	2010	1.55 a	40.7 cd	75.9 c	85.9 b	100.0 a	9147.0 c
	1179-3	2010	1.15 bc	33.7d	75.9 c	90.6 b	100.0 a	9373.9 bc
	1184-14	2011	1.12 c	32.2 d	44.5 d	54.0 d	100.0 a	7406.3 e
	1332-6	2013	1.22 bc	40.2 cd	69.4 c	75.7 c	100.0 a	8609.3 d
	$LSD_{P=0.05}$		0.17	15.3	9.0	9.2	0.0	374.5
Cyprodinil	13-1	1998	0.10 d	76.7 a	100.0 a	100.0 a	100.0 a	9979.5 a
	22-1	1998	1.46 b	35.2 b	91.7 b	100.0 a	100.0 a	9919.6 a
	38-4	1998	0.10 d	24.4 b	100.0 a	100.0 a	100.0 a	9956.0 a
	88-1	1998	1.70 a	4.9 b	100.0 a	100.0 a	100.0 a	9947.2 a
	1168-3	2010	1.24 c	84.4 a	100.0 a	100.0 a	100.0 a	9983.0 a
	1179-3	2010	0.10 d	88.9 a	97.0 ab	100.0 a	100.0 a	9970.2 a
	1184-14	2011	0.10 d	0.0 b	54.6 d	68.2 c	91.7 b	7766.9 c
	1332-6	2013	0.10 d	23.4 b	63.5 c	79.4 b	92.1 b	8401.6 b
	$LSD_{P=0.05}$		0.16	37.2	8.2	4.2	2.6	326.8
Fludioxonil	13-1	1998	0.10 c	88.1 a	100.0 a	100.0 a	100.0 a	9984.6 a
	22-1	1998	1.18 ab	76.5 b	92.6 b	100.0 a	100.0 a	9942.9 b
	38-4	1998	1.24 a	89.4 a	100.0 a	100.0 a	100.0 a	9985.2 a
	88-1	1998	1.13 b	85.0 ab	99.4 a	100.0 a	100.0 a	9980.4 a
	1168-3	2010	0.10 c	65.8 c	91.4 b	100.0 a	100.0 a	9932.1 b
	1179-3	2010	0.10 c	79.1 b	88.8 b	100.0 a	100.0 a	9925.4 b
	1184-14	2011	0.10 c	35.3 d	91.5 b	100.0 a	100.0 a	9918.8 b
	1332-6	2013	0.10 c	30.5 d	63.4 c	100.0 a	100.0 a	9777.6 c
	$LSD_{P=0.05}$		0.10	8.8	6.4	0.0	0.0	31.6

[X] EC_{50} (effective concentration where the fungal growth is inhibited by 50%) values from in vitro experiments.

[Y] Columns containing the same letters, within each fungicide, indicate that no significant differences exist between the fungicides based on Fisher's protected least significant difference at the P = 0.05 level.

Discussion

Small-spored *Alternaria* spp. and *A. solani* have rapidly developed resistance and/or reduced-sensitivity to multiple fungicide classes in a short amount of time (Avenot and Michailides, 2007; Avenot and Michailides, 2015; Fairchild et al., 2013; Malandrakis et al., 2015; Miles et al., 2014; Pasche et al., 2004). Reduced-sensitive isolates to pyrimethanil have been identified in *A. alternata* (Fairchild et al., 2013), *A. solani* (Fonseka and Gudmestad, 2016), *B. cinerea* (Amiri et al., 2013), and *Penicillium expansum* (Xiao et al., 2011). Resistance to pyrimethanil of small-spored *Alternaria* spp. and *A. solani* was first identified in 2010 Idaho field isolates (Fairchild et al., 2013). In that study, it was reported that one of nine *A. alternata* and four of 21 *A. solani* isolates were resistant to pyrimethanil. A later study classified six of 245 *A. solani* isolates to have reduced-sensitivity to pyrimethanil, but reduced sensitivity was not observed among 109 *A. alternata* isolates (Fonseka and Gudmestad, 2016). Monitoring and examining the response of *Alternaria* spp. to multiple fungicide classes with different modes of action will be valuable in fungicide rotation programs to safeguard the development of resistance in high-risk chemistries.

Pyrimethanil was registered for use on potato in 2005 and has been utilized for early blight and brown leaf spot management in North Dakota as a foliar fungicide in potato for over a decade (Fonseka and Gudmestad, 2016). Pyrimethanil is a pre-packaged mixture partner with the single-site SDHI fungicide fluopyram or is used as a stand-alone fungicide in rotation with a standard protectant such as mancozeb or chlorothalonil. Baseline sensitivity studies have been established for both *A. solani* and small-spored *Alternaria* spp. in response to pyrimethanil, difenoconazole, and metconazole (Fonseka and Gudmestad, 2016). In that study, the 50 small-spored *Alternaria* spp. were all classified as *A. alternata.* However, it was determined using

next-generation sequencing methods that 32% of those isolates were *A. alternata*, 14% were *A. tenuissima*, 6% were *A. arborescens*, and 48% of the isolate's classification were inconclusive (Budde-Rodriguez et al. Plant Disease in review). Therefore, in the current study pyrimethanil sensitivity was again determined for these baseline isolates to detect differences among the three small-spored *Alternaria* spp. and to determine if cross-sensitivity exists between the AP and PP fungicides. The *A. solani* baseline isolates from the previously mentioned study were also assayed for sensitivity to AP fungicides, pyrimethanil and cyprodinil in the current study. A PP fungicide, fludioxonil, was also evaluated to determine its intrinsic activity on *Alternaria* spp. and potential use for early blight and brown leaf spot disease management.

The response of the *Alternaria* spp. to the AP and PP fungicides were highly variable. Variation in baseline isolates of *A. alternata, A. arborescens, A. tenuissima,* and *A. solani* in response to pyrimethanil were wider than previously reported pyrimethanil variations (Fonseka and Gudmestad, 2016). The variation between mean EC_{50} values in previously reported pyrimethanil experiments and the current study's experiment can be explained by the inter-experimenter variability. *Alternaria solani* baseline isolates were less sensitive to pyrimethanil compared to the results reported previously (Fonseka and Gudmestad, 2016). This is most likely due to the increased number of isolates assayed and the high variability within the *Alternaria* spp..

This is the first report establishing baseline sensitivities of *A. solani, A. alternata, A. arborescens*, and *A. tenuissima* populations to pyrimethanil, cyprodinil, and fludioxonil collected across multiple potato production areas. *Alternaria alternata* was significantly less sensitive to pyrimethanil than to cyprodinil and fludioxonil, based on the mean EC_{50} values. Interestingly, significant and strong cross-sensitivity was detected between pyrimethanil and cyprodinil, but

only in *A. alternata* isolates and not among the other *Alternaria* spp. evaluated. Strong cross-sensitivity between pyrimethanil and cyprodinil is quite common and it has been detected in *B. cinerea* (Amiri et al., 2013; Fernández-Ortuño et al., 2013; Myresiotis et al., 2007), and *B. fuckeliana* (Hilber and Schüepp, 1996). Pyrimethanil and cyprodinil belong to the same chemical group, indicating that the cross-sensitivity risk is higher due to the similar chemical structure. Among *A. arborescens* baseline isolates, no correlations in EC_{50} values were observed between pyrimethanil, cyprodinil, and fludioxonil probably because only three isolates were used in this study. Obtaining a larger sample size of all three small-spored *Alternaria* spp. that cause brown leaf spot on potato will aid in the understanding of how these pathogens respond to the different fungicide chemistries.

One of the objectives of this study was to determine the baseline of the small-spored *Alternaria* spp. and *A. solani* population to the anilinopyrimidine and phenylpyrrole fungicides. *Alternaria tenuissima* isolates were significantly more sensitive to fludioxonil than to pyrimethanil and cyprodinil. Among *A. tenuissima* isolates there was no correlation detected between pyrimethanil and cyprodinil or pyrimethanil and fludioxonil. However, a strong and significant correlation was observed in the fungicide sensitivity of *A. tenuissima* isolates between cyprodinil and fludioxonil. These results contrast cyprodinil and fludioxonil correlations calculated in *A. alternata* isolates collected from pistachio (Avenot and Michailides, 2015). To the best of our knowledge, this is the first report of cross-sensitivity between cyprodinil and fludioxonil in *A. tenuissima* isolates. It is likely that this is an artifact created by the low number of isolates used in the study. Only five *A. tenuissima* isolates were assayed, more isolates should be evaluated to corroborate the cyprodinil and fludioxonil correlation.

Alternaria solani is significantly more sensitive to fludioxonil than to cyprodinil or pyrimethanil based on in vitro results. However, among the two AP fungicides, *A. solani* is significantly more sensitive to cyprodinil than to pyrimethanil. The intrinsic activity of pyrimethanil, cyprodinil, and fludioxonil was high in all *Alternaria* spp. with fludioxonil being the most consistent. With the observed increase in *A. solani* in vitro sensitivity values, greenhouse trials were conducted to evaluate disease control.

Early blight disease control with pyrimethanil was higher in the 1998 collected isolates compared to the 2010-2013 isolates. Similarly, *A. solani* disease control with cyprodinil was higher in isolates collected in 1998-2010. Similar disease control between cyprodinil and pyrimethanil is possible because of the similar chemical structure. Higher disease control of *A. solani* isolates with fludioxonil was observed in isolates collected before 2010. Overall, disease control provided by fludioxonil was higher among most assessed isolates compared to cyprodinil and pyrimethanil. These results suggest that pyrimethanil and cyprodinil are less effective at managing the current *A. solani* populations compared to fludioxonil. This may be due to the previous use of pyrimethanil to manage early blight and the ability for cross-resistance to develop among similar chemical classes. However, very few studies have examined the cross-resistance development in AP fungicides using *Alternaria* spp..

Investigations into AP resistance and cross-resistance within the AP fungicides have been researched extensively using *B. cinerea* and several studies have proposed suggestions for what type of resistance pathogens can develop against these fungicides (Amiri et al., 2013; Fernández-Ortuño et al., 2013; Hilber and Schüepp, 1996; Kanetis et al., 2008). Qualitative resistance is believed to be the cause of *B. cinerea* AP resistance; however, this indicates that resistance should be rapidly detected unless a fitness penalty is detected (Amiri et al., 2013; Fernández-

Ortuño et al., 2013). However, high frequency of AP resistant isolates has not been reported. In contrast, three multidrug-resistant (MDR) phenotypes have been identified in *B. cinerea*: MDR1h, expresses a strong resistance in response to cyprodinil and fludioxonil, MDR2 are less sensitive to cyprodinil, and MDR3 which is an MDR1xMDR2 recombinant (Chapeland et al., 1999; Kretschmer et al., 2009). This suggests that AP and PP resistance in *B. cinerea* may be quantitative as opposed to qualitative due to the multiple phenotypes with varying resistance responses identified. Further evidence of qualitative resistance was observed in *A. alternata* isolates and were separated into two main sensitivity groups: fludioxonil-sensitive/cyprodinil-resistant, and fludioxonil-resistant/cyprodinil-sensitive (Avenot and Michailides, 2015). In that study, most of the 126 isolates were sensitive to both or either fungicide, but two isolates with fludioxonil-resistant/cyprodinil-resistant phenotypes were identified. Results also concluded that AP and PP fungicides were still effective in *A. alternata* isolates that possessed both a QoI and SDH mutation (Avenot and Michailides, 2015). Based on those results it suggests that the AP and PP resistance in *A. alternata* isolates may be qualitative.

In Midwest potato production areas, brown leaf spot and early blight can be devastating if left untreated. While early blight has been regarded as more important than brown leaf spot, isolation frequency of the small-spored *Alternaria* spp. has been increasing (Ding et al., 2019). In this study, the high intrinsic activity in all *Alternaria* spp. combined with the high early blight disease control exhibited, it is clear that both cyprodinil and fludioxonil could be useful in early blight and brown leaf spot management programs. However, further investigations on the cross-sensitivity and disease control of the AP and PP fungicides should be done with a larger sample of small-spored *Alternaria* spp. isolates. Continual monitoring of the current *Alternaria* spp. populations to new and utilized chemistries is important in safeguarding the effective

chemistries. Determining the non-baseline sensitivities of current *Alternaria* spp. to the AP and PP fungicides should be examined.

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GENERAL CONCLUSIONS

Early blight caused *by Alternaria solani* and brown spot caused by the small-spored *Alternaria* spp., *Alternaria alternata, Alternaria arborescens,* and *Alternaria tenuissima* are frequently observed in midwestern potato production areas. The use of foliar fungicides remains a primary management strategy. However, *Alternaria* spp. have developed reduced-sensitivity and/or resistance to many single-site fungicides such as quinone outside inhibitor (QoI), succinate dehydrogenase inhibitor (SDHI), and anilinopyrimidines (AP) fungicides in recent years.

The QoI fungicide azoxystrobin was introduced in United States potato fields in 1999 (Pasche et al., 2004). However, reduced efficacy was reported two years later in Nebraska due to the high selective pressure caused by the extensive usage (Pasche et al., 2004). Boscalid, fluopyram, solatenol, and adepidyn are EPA-registered SDHI fungicides, which have been used commercially on a variety of crops including potato. In *A. solani,* there are five known mutations that convey reduced sensitivity to SDHI, the H278R and H278Y mutations which were the predominate in 2012, the H133R, H134R and D123E mutations (Mallik et al., 2014). *A. solani* isolates with the D123E mutation exhibited significantly reduced disease control when boscalid and fluopyram were applied compared to the other SDHI mutations (Bauske et al., 2018). Anilinopyrimidine (AP) (pyrimethanil and cyprodinil) and phenylpyrrole (PP) (fludioxonil) had demonstrated high intrinsic activity and disease control in *A. alternata* (Avenot and Michailides, 2015). Determining the effectiveness of these fungicides on *Alternaria* spp. is important to the potato industry.

There were several objectives in this research, including (i) determining the impact of 'new' SDHI fungicides, solatenol and adepidyn, on the development of the D123E mutation in

A. solani; (ii) to determine the in vitro and in vivo sensitivity of the small-spored *Alternaria* spp. to QoI and SDHI fungicides; and (iii) to determine the in vitro and in vivo sensitivity of *A. solani* and the small-spored *Alternaria* spp. to anilinopyrimidine and phenylpyrrole fungicides.

Comparing the 'new' SDHI fungicides, solatenol and adepidyn, and the 'older' SDHI fungicides, fluopyram and solatenol, in laboratory experiments determined that fluopyram, solatenol, and adepidyn have higher intrinsic activity in *A. solani* than boscalid. Greenhouse experiments also determined that boscalid and fluopyram are less effective at managing *A. solani* than solatenol and adepidyn. Field experiments determined that disease severity was significantly lower in all treatments in both locations compared to the non-treated control. However, at the Larimore location, the yield from the grower's standard with adepidyn and fludioxonil treatment was significantly higher than the non-treated plot while at the Inkster location, the yield from the mancozeb treatment was significantly higher than the non-treated plot. Molecular characterization of *A. solani* field isolates determined that the frequency of the D123E- and H134R-mutations are increasing in response to more recently developed SDHI fungicides. In contrast, the H278R/Y- and H133R-mutations are decreasing to the point of being nearly absent in these field experiments.

Using the same SDHI fungicides in the previous experiment, in all the small-spored *Alternaria* spp. high intrinsic activity was observed in boscalid, fluopyram, and solatenol while the highest intrinsic activity was observed in adepidyn. The small-spored *Alternaria* spp. were evaluated among the QoI fungicides, azoxystrobin, famoxadone, and picoxystrobin. Low intrinsic activity was observed among the QoI fungicides assayed to the small-spored *Alternaria* spp. In greenhouse experiments, 18 isolates (six from each small-spored *Alternaria* spp.) were selected for further evaluation on the SDHI fungicides. Adepidyn, solatenol, and fluopyram

provided better disease control to *A. arborescens* and *A. tenuissima* than boscalid. While adepidyn and solatenol provided better disease control for *A. alternata* than fluopyram and boscalid. These results determined that knowing the exact *Alternaria* spp. that causes brown leaf spot is important for disease management of potato.

Anilinopyrimidine fungicides, pyrimethanil and cyprodinil, and phenylpyrrole fungicide, fludioxonil, were assessed in laboratory and greenhouse conditions for *A. solani* and the small-spored *Alternaria* spp.. High intrinsic activity was observed among all species and all fungicides with pyrimethanil providing the lowest level of intrinsic activity to all four species. *Alternaria solani* isolates were moved into the greenhouse to determine the disease control for the three fungicides. All three fungicides provided high levels of disease control but fludioxonil provided the highest level of disease control to the eight *A. solani* isolates assayed. This suggests that fludioxonil would be a strong mixing partner to have in an early blight management application program.

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APPENDIX A. COLLECTION DATA OF *ALTERNARIA SOLANI* ISOLATES COLLECTED FROM FIELDS FROM 1998-2017: SDHI ASSAY

Year	Isolate	SDH mutation	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
1998	1-1	NA	Dalhart, TX	0.11	0.01	0.03	0.01
1998	3-1	NA	Minden, NE	0.10	0.07	0.02	0.01
1998	6-1	NA	Park Rapids, MN	0.04	0.02	0.01	0.01
1998	11-1	NA	Columbus, NE	0.06	0.02	0.01	0.01
1998	12-1	NA	Minden, NE	0.02	0.17	0.02	0.01
1998	12-3	NA	Minden, NE	0.03	0.06	0.02	0.01
1998	13-1	NA	O'Neill, NE	0.60	0.29	0.03	0.01
1998	14-1	NA	Minden, NE	0.12	0.09	0.03	0.02
1998	14-3	NA	Minden, NE	0.22	0.17	0.01	0.01
1998	17-1	NA	Hastings, MN	0.01	0.01	0.01	0.01
1998	22-1	NA	Staples, MN	0.01	0.01	0.01	0.01
1998	30-1	NA	Buxton, ND	0.97	0.06	0.10	0.01
1998	31-1	NA	O'Neill, NE	0.12	1.31	0.05	0.02
1998	31-4	NA	O'Neill, NE	0.01	0.01	0.02	0.01
1998	32-1	NA	Park Rapids, MN	0.16	0.03	0.01	0.01
1998	33-1	NA	Park Rapids, MN	0.01	0.01	0.01	0.01
1998	37-1	NA	O'Neill, NE	0.11	0.15	0.01	0.01
1998	37-4	NA	O'Neill, NE	0.01	0.28	0.01	0.01
1998	38-1	NA	Minden, NE	0.04	0.26	0.02	0.01
1998	38-4	NA	Minden, NE	0.06	0.09	0.02	0.01
1998	40-1	NA	Watertown, SD	0.02	0.01	0.01	0.01
1998	68-1	NA	Shelley, ID	0.05	0.02	0.01	0.03
1998	83-1	NA	Hamer, ID	0.05	0.02	0.01	0.01
1998	88-1	NA	Hancock, WI	0.05	0.09	0.03	0.01
2001	521-1	NA	O'Neill, NE	0.02	0.01	0.01	0.01
2001	526-3	NA	O'Neill, NE	0.18	0.36	0.07	0.01
2001	528-2	NA	O'Neill, NE	0.31	0.01	0.01	0.01
2001	528-3	NA	O'Neill, NE	0.31	0.02	0.01	0.01
2001	532-2	NA	O'Neill, NE	0.04	0.03	0.01	0.01
2001	535-2	NA	O'Neill, NE	0.23	0.08	0.01	0.01
2001	538-2	NA	O'Neill, NE	0.82	0.48	0.05	0.01
2001	547-1	NA	Dawson, ND	0.06	0.02	0.02	0.01
2001	547-2	NA	Dawson, ND	0.14	0.02	0.02	0.02
2001	547-4	NA	Dawson, ND	0.03	0.01	0.01	0.02
2001	549-1	NA	Dawson, ND	0.42	0.53	0.01	0.01
2001	549-2	NA	Dawson, ND	0.02	0.01	0.01	0.01
2001	549-3	NA	Dawson, ND	0.13	0.02	0.03	0.01
2001	572-1	NA	Minden, NE	0.07	0.06	0.01	0.01

Year	Isolate	SDH mutation	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2001	574-1	NA	Columbus, NE	0.01	0.01	0.01	0.01
2001	574-3	NA	Columbus, NE	0.02	0.01	0.01	0.01
2001	577-1	NA	Minden, NE	0.54	0.01	0.01	0.01
2001	578-1	NA	Minden, NE	0.43	0.01	0.02	0.01
2001	580-4	NA	Columbus, NE	0.02	0.06	0.03	0.01
2001	583-2	NA	Dawson, ND	0.26	0.04	0.18	0.01
2001	583-3	NA	Dawson, ND	0.01	0.05	0.02	0.01
2001	584-6	NA	Dawson, ND	0.03	0.08	0.01	0.01
2001	585-1	NA	Dawson, ND	0.32	0.24	0.09	0.02
2001	586-1	NA	Browerville, MN	0.15	0.15	0.01	0.01
2001	586-2	NA	Browerville, MN	0.29	0.17	0.01	0.01
2001	587-3	NA	Browerville, MN	0.60	0.31	0.05	0.01
2001	587-4	NA	Browerville, MN	0.17	0.18	0.01	0.01
2001	587-5	NA	Browerville, MN	0.68	0.12	0.01	0.01
2001	588-1	NA	Kearney, NE	0.21	0.09	0.03	0.02
2001	588-2	NA	Kearney, NE	0.18	0.09	0.14	0.03
2001	589-1	NA	Kearney, NE	0.50	0.17	0.02	0.01
2001	589-2	NA	Kearney, NE	0.45	0.26	0.23	0.08
2001	590-1	NA	Pettibone, ND	0.34	0.40	0.19	0.01
2010	1172-6	H278R	Acequia, ID	0.71	0.01	0.01	0.01
2013	1309-5	H278Y	Bath, IL	0.27	0.01	0.01	0.01
2013	1317-6	H278Y	Columbus, NE	0.23	0.01	0.08	0.04
2013	1317-13	H133R	Columbus, NE	11.15	1.63	0.26	0.03
2013	1318-13	H278R	Farmington, NM	0.57	0.03	0.01	0.01
2013	1321-7	H278R	Plover, WI	2.46	0.09	0.02	0.01
2013	1322-7	H278R	Plover, WI	0.18	0.02	0.01	0.01
2013	1326-6	H278R	Wray, CO	0.09	0.03	0.02	0.01
2013	1327-1	H278R	Wray, CO	0.10	0.08	0.01	0.02
2013	1329-4	H278R	Wray, CO	1.41	0.59	0.02	0.01
2013	1330-5	H134R	Wray, CO	5.25	2.77	0.04	0.17
2013	1332-11	D123E	Dalhart, TX	0.01	0.36	0.30	0.03
2013	1339-1	H278R	Inkster, ND	0.61	0.03	0.02	0.01
2013	1339-5	H278Y	Inkster, ND	0.96	0.09	0.06	0.03
2013	1340-8	H134R	Minden, NE	11.26	0.74	0.02	0.01
2013	1341-3	H278Y	Columbus, NE	23.95	0.75	0.01	0.02
2013	1341-6	H278R	Columbus, NE	0.09	0.02	0.02	0.01
2013	1342-6	D123E	Columbus, NE	1.90	0.36	0.16	0.04
2013	1342-7	H134R	Columbus, NE	1.63	0.41	0.01	0.02
2013	1342-10	D123E	Columbus, NE	0.48	1.72	0.23	0.07
2013	1344-8	H278R	Karlsruhe, ND	5.92	0.03	0.01	0.01
2013	1345-2	H278R	Oakes, ND	2.76	1.29	0.02	0.02
2013	1346-2	D123E	Oakes, ND	0.06	0.15	0.06	0.06

Year	Isolate	SDH mutation	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2013	1346-6	H278R	Oakes, ND	4.59	0.01	0.01	0.02
2013	1347-2	H134R	Oakes, ND	3.21	2.22	0.03	0.03
2013	1350-4	H134R	Lisbon, ND	0.11	0.04	0.04	0.01
2013	1356-7	H133R	Browerville, MN	0.04	0.05	0.03	0.01
2013	1356-8	H133R	Browerville, MN	0.01	0.01	0.01	0.01
2013	1357-3	H133R	Browerville, MN	0.11	0.16	0.08	0.01
2013	1363-1	H133R	Columbus, NE	2.11	0.72	0.15	0.03
2013	1363-2	D123E	Columbus, NE	10.16	0.82	3.43	0.07
2013	1367-1	H133R	Three Rivers, MI	0.34	2.17	0.06	0.37
2013	1367-3	D123E	Three Rivers, MI	17.44	0.10	0.05	0.05
2013	1367-12	H133R	Three Rivers, MI	9.77	0.49	0.14	0.02
2013	1370-8	H133R	Wray, CO	23.67	0.08	0.06	0.11
2013	1381-6	H134R	Inkster, ND	2.52	0.06	0.78	0.09
2013	1385-6	H278Y	Inkster, ND	0.04	1.58	0.22	0.02
2013	1385-10	H278R	Inkster, ND	1.42	0.11	0.02	0.02
2013	1389-3	H134R	Wadena, MN	0.99	1.34	0.02	0.02
2013	1393-10	H278R	Inkster, ND	65.03	0.04	0.04	0.02
2013	1393-12	H278R	Inkster, ND	0.01	0.01	0.01	0.01
2013	1393-18	D123E	Inkster, ND	20.15	0.08	0.13	0.03
2013	1394-15	H278Y	Inkster, ND	3.79	0.02	0.21	0.03
2013	1397-17	D123E	Inkster, ND	0.11	0.02	0.03	0.01
2013	1401-17	H134R	Inkster, ND	1.63	0.04	0.12	0.24
2015	1528-1	H133R	Minden, NE	2.97	2.56	0.12	0.23
2015	1528-9	H133R	Minden, NE	0.20	0.38	0.24	0.03
2015	1528-17	H134R	Minden, NE	0.36	0.12	0.14	0.02
2015	1528-21	H134R	Minden, NE	4.32	0.45	1.40	0.02
2015	1531-10	H134R	Dawson, ND	0.06	3.49	0.05	0.21
2015	1531-11	H134R	Dawson, ND	0.03	0.01	0.01	0.01
2015	1531-13	H134R	Dawson, ND	0.10	0.04	0.04	0.01
2015	1532-3	H278Y	Dawson, ND	0.40	2.60	0.10	0.01
2015	1532-7	D123E	Dawson, ND	0.80	0.06	0.02	0.03
2015	1533-2	H278Y	Dawson, ND	1.05	1.30	0.07	0.06
2015	1534-4	H134R	Dawson, ND	2.53	0.07	0.37	0.01
2015	1535-23	D123E	Ponsford, MN	0.04	0.20	0.14	0.04
2015	1536-2	H278Y	East Hubbard, MN	1.26	1.26	2.33	0.18
2015	1536-4	H278Y	East Hubbard, MN	0.13	1.73	0.37	0.02
2015	1536-8	D123E	East Hubbard, MN	0.05	0.59	0.01	0.01
2015	1536-9	H133R	East Hubbard, MN	8.66	2.30	0.15	0.04
2015	1536-10	H133R	East Hubbard, MN	18.55	0.46	0.04	0.01
2015	1536-12	H133R	East Hubbard, MN	0.22	1.43	0.15	0.17
2015	1537-1	H278Y	West Hubbard, MN	0.14	0.15	0.01	0.01
2015	1537-8	H133R	West Hubbard, MN	29.29	1.51	0.01	0.03

Year	Isolate	SDH mutation	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2015	1538-9	D123E	Park Rapids, MN	2.41	0.11	0.09	0.04
2015	1539-2	H133R	Park Rapids, MN	12.86	1.42	0.23	0.03
2015	1539-6	D123E	Park Rapids, MN	3.09	0.11	0.28	0.06
2015	1539-11	H278Y	Park Rapids, MN	0.07	1.56	0.02	0.02
2015	1540-4	D123E	Park Rapids, MN	0.39	0.07	0.10	0.05
2015	1540-12	D123E	Park Rapids, MN	2.46	2.30	0.27	0.09
2015	1541-4	H133R	Columbus, NE	0.01	0.01	0.01	0.01
2015	1541-7	H133R	Columbus, NE	0.03	0.94	0.05	0.01
2015	1542-4	H134R	Columbus, NE	0.03	0.04	0.01	0.01
2015	1544-1	H278Y	Dalhart, TX	16.71	1.23	1.53	0.07
2015	1544-3	H278Y	Dalhart, TX	2.11	0.19	0.16	0.02
2015	1544-7	H278R	Dalhart, TX	1.00	0.02	0.07	0.01
2015	1545-1	D123E	Alliance, NE	4.14	3.18	0.43	0.13
2015	1545-5	H278Y	Alliance, NE	8.46	0.02	1.49	0.69
2015	1545-8	D123E	Alliance, NE	0.62	0.08	0.26	0.05
2015	1547-2	H278Y	Bridgeport, NE	0.21	0.15	0.03	0.01
2015	1547-3	D123E	Bridgeport, NE	60.41	0.01	0.01	0.02
2015	1549-3	H278R	Ft. Morgan, CO	0.07	0.17	0.02	0.02
2015	1550-4	H278R	Ft. Morgan, CO	3.41	1.24	0.02	0.01
2015	1551-7	H134R	Dalhart, TX	0.30	0.01	0.01	0.01
2015	1551-12	H278Y	Dalhart, TX	0.19	1.98	0.01	0.21
2015	1551-20	D123E	Dalhart, TX	8.09	0.05	0.11	0.01
2015	1553-3	Sensitive	Dalhart, TX	0.58	0.22	0.22	0.03
2015	1554-3	H134R	Dalhart, TX	0.23	0.48	0.07	0.10
2015	1555-1	H278Y	Dalhart, TX	1.27	1.58	0.05	0.01
2015	1557-5	H134R	Dalhart, TX	0.61	0.02	0.01	0.01
2015	1557-7	D123E	Dalhart, TX	2.45	0.79	0.20	0.01
2015	1563-9	D123E	O'Neill, NE	5.33	0.08	0.25	0.06
2015	1563-10	D123E	O'Neill, NE	2.03	8.64	0.25	0.04
2015	1566-1	H278R	Antigo, WI	0.03	0.28	0.01	0.02
2015	1566-2	H278Y	Antigo, WI	2.48	1.18	0.45	0.03
2015	1566-12	H278R	Antigo, WI	0.16	1.38	0.02	0.14
2015	1566-14	H278R	Antigo, WI	0.19	0.30	0.02	0.18
2015	1567-4	H134R	Sebeka, MN	0.59	0.51	0.02	0.02
2015	1574-2	H133R	Becker, MN	0.64	0.11	0.14	0.01
2015	1576-5	H134R	Becker, MN	1.85	0.02	0.09	0.01
2015	1577-3	Sensitive	Perham, MN	0.07	0.42	0.12	0.03
2015	1578-5	H278Y	Perham, MN	4.74	1.20	0.30	0.04
2015	1579-4	H278Y	Tappen, ND	0.59	0.34	0.02	0.02
2015	1580-5	D123E	Tappen, ND	1.57	0.05	0.05	0.02
2015	1580-8	H134R	Tappen, ND	20.45	0.28	0.04	0.01
2015	1582-2	H133R	Perham, MN	3.38	1.68	0.26	0.02

Year	Isolate	SDH mutation	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	**Fluopyram**	**Solatenol**	**Adepidyn**
2015	1582-3	H133R	Perham, MN	11.96	1.36	0.29	0.02
2015	1582-7	H134R	Perham, MN	1.56	1.50	0.05	0.02
2015	1583-9	H133R	Perham, MN	1.39	1.54	0.35	0.06

APPENDIX B. FUNGICIDE TREATMENT, USE RATES, AND APPLICATION SCHEDULE OF TREATMENTS EVALUATED IN FIELD TRIALS CONDUCTED IN 2018 IN INKSTER AND LARIMORE, ND.

	Treatment	Rate[x]	Schedule[y]	Interval[z]
Non-trt	Non-treated	-	-	-
Chloro	Chlorothalonil	2.5 l/ha	Full season	7-day
Manco	Mancozeb	2.25 kg/ha	Full season	7-day
GrwStd/ Adep+Flud	Azoxystrobin +	0.5 l/ha	1,3	7-day
	Chlorothalonil +	0.1% v/v +		
	Mancozeb	2.25 kg/ha		
	Chlorothalonil	1.5 l/ha	2	
	Chlorothalonil	2.5 l/ha	4,6,8-10	
	Adepidyn/fludioxonil	0.7 l/ha	5	
	Mancozeb	2.25 kg/ha		
	difenoconazole +	0.5 l/ha	7	
	Mancozeb	2.25 kg/ha		
Sol-IF	Solatenol	1.4 l/ha	In-Furrow	At Planting
Sol + BsubL-IF	Solatenol +	1.4 l/ha	In-Furrow	At Planting
	Bacillus subtilis	4.7 l/ha		
Sol + BsubH-IF	Solatenol +	1.4 l/ha	In-Furrow	At Planting
	Bacillus subtilis	9.4 l/ha		
Flu-IF	Fluopyram	0.6 l/ha	In-Furrow	At Planting
Flu + BsubH-IF	Fluopyram +	0.6 l/ha	In-Furrow	At Planting
	Bacillus subtilis	9.4 l/ha		
GrwStd	Fenamidone +	0.5 l/ha	1,3	7-day
	Chlorothalonil +	0.1% v/v +		
	Mancozeb	2.25 kg/ha		
	Chlorothalonil	1.5 l/ha	2	
	Chlorothalonil	2.5 l/ha	4,6,8-10	
	Fluopyram/Pyrimethanil	11 oz/ha	5	
	Mancozeb	2.25 kg/ha		
	Pyrimethanil +	0.5 l/ha	7	
	Mancozeb	2.25 kg/ha		

[x] l/ha = liter per hectare; kg/ha = kilogram per hectare

[y] week(s) fungicide was applied

[z] days between subsequent applications of treatments

APPENDIX C. SUMMARY OF STATISTICAL ANALYSIS FOR *ALTERNARIA SOLANI* IN VITRO SDHI SENSITIVITY ASSAYS

Table C1. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria solani* baseline EC_{50} values for boscalid, fluopyram, solatenol, and adepidyn

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.004	0.10	0.7506
Fungicide	3	5.525	51.09	<0.0001
Trial x fungicide	3	0.009	0.08	0.9700

Table C2. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria solani* non-baseline EC_{50} values for boscalid, fluopyram, solatenol, and adepidyn

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.053	0.00	0.9666
Fungicide	3	1763.958	58.52	<0.0001
Trial x fungicide	3	0.013	0.00	1.0000

APPENDIX D. SUMMARY OF STATISTICAL ANALYSIS FOR *ALTERNARIA SOLANI* IN VIVO SENSITIVITY ASSAYS: SDHI FUNGICIDES

Table D1. Combined analysis of variance of in vivo percentage disease control of *A. solani* isolates provided by boscalid.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	33.133	0.05	0.8158
Rep	2	67.997	0.11	0.8945
Fungicide concentration	3	89027.300	146.06	<0.0001
Rep x fungicide concentration	6	18.914	0.03	0.9999
Trial x fungicide concentration	3	24.598	0.04	0.9892
Trial x rep	2	17.119	0.03	0.9723
Trial x rep x fungicide concentration	6	7.824	0.01	1.0000

Table D2. Combined analysis of variance of in vivo percentage disease control of *A. solani* isolates provided by fluopyram.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	173.395	0.27	0.6016
Rep	2	377.787	0.59	0.5521
Fungicide concentration	3	122512.600	192.91	<0.0001
Rep x fungicide concentration	6	80.215	0.13	0.9931
Trial x fungicide concentration	3	293.583	0.46	0.7088
Trial x rep	2	79.369	0.12	0.8826
Trial x rep x fungicide concentration	6	68.420	0.11	0.9955

Table D3. Combined analysis of variance of in vivo percentage disease control of *A. solani* isolates provided by solatenol.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	0.130	0.00	0.9868
Rep	2	3.142	0.01	0.9934
Fungicide concentration	3	82818.450	174.58	<0.0001
Rep x fungicide concentration	6	34.632	0.07	0.9985
Trial x fungicide concentration	3	52.339	0.11	0.9540
Trial x rep	2	37.448	0.08	0.9241
Trial x rep x fungicide concentration	6	37.477	0.08	0.9981

Table D4. Combined analysis of variance of in vivo percentage disease control of *A. solani* isolates provided by adepidyn.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	8.779	0.03	0.8698
Rep	2	278.510	0.85	0.4268
Fungicide concentration	3	26241.840	80.39	<0.0001
Rep x fungicide concentration	6	122.543	0.38	0.8946
Trial x fungicide concentration	3	2.590	0.01	0.9990
Trial x rep	2	33.517	0.10	0.9024
Trial x rep x fungicide concentration	6	17.360	0.05	0.9994

APPENDIX E. EARLY BLIGHT DISEASE SEVERITY RATINGS

Table E1. Early blight severity ratings observed during the 2018 season at the Larimore, ND site. Treatments are shown with their corresponding numbers, the list of chemicals applied for each treatment, the rate of treatment, schedule and the interval of the schedule. Foliar disease severity was expressed as a percentage for the listed dates starting from July 4th, 2018 (7/4)—September 19th, 2018 (9/19). Area under the disease progress curve values (AUDPC) and relative area under the disease progress curve values (RAUDPC) are also shown on the table and the least significant differences (LSD; P=0.05) were calculated between the different treatments.

2018 Early Blight - Larimore																		
Treatment		Rate	Schedule	Interval	Foliar Disease (% Severity)												AUDPC	RAUDPC
					7/4	7/11	7/18	7/25	8/1	8/7	8/15	8/22	8/29	9/5	9/12	9/19		
1401	Non-treated	-	-	-	0.03	0.16	0.13	0.49	1.19	5.50	12.19	13.75	16.88	31.88	58.79	86.25	1293.78 a	0.185 a
1402	Echo Zn	2.125 pt/a	Full Season	7 day	0.01	0.05	0.14	0.31	0.38	2.75	4.63	5.44	4.44	7.31	11.00	22.25	335.15 ef	0.050 ef
1403	Manzate	2.0 lb	Full Season	7 day	0.03	0.15	0.07	0.41	0.38	3.63	4.38	3.56	4.69	7.06	12.19	31.25	369.58 ef	0.053 ef
1404	Quadris +	6.8 fl oz +	1,3															
	Bond +	0.1 % v/v +																
	Manzate	2.0 lb																
	Echo ZN	1.3 pt	2															
	Echo ZN	2.125 pt	4,6,8-10	7 day	0.14	0.09	0.13	0.33	0.91	2.44	4.94	3.50	2.44	2.81	3.56	11.88	192.13 f	0.028 f
	Miravis +	9.6 fl oz	5															
	Manzate	2 lb																
	Inspire +	6.8 fl oz	7															
	Manzate	2 lb																
1405	Elatus	7.26 oz/a	In-furrow	-	0.10	0.44	0.07	0.48	0.97	4.88	3.81	6.75	11.63	22.81	46.56	78.13	961.21 b	0.137 b
1406	Elatus +	7.26 oz/a +	In-furrow	-	0.04	0.04	0.08	0.19	0.72	3.00	4.44	5.19	6.31	13.75	7.19	51.88	679.92 bcd	0.097 bcd
	Serenade ASO	64 fl oz																
1407	Elatus +	7.26 oz/a +	In-furrow	-	0.14	0.09	0.07	0.74	0.93	4.44	5.19	7.94	10.44	19.44	39.69	53.44	812.38 bc	0.116 bc
	Serenade ASO	128 fl oz																
1408	Velum Prime	6.84 fl oz	In-furrow	-	0.22	0.03	0.12	0.27	0.43	3.00	4.38	2.56	4.44	8.63	21.25	45.00	475.89 def	0.068 def
1409	Velum Prime +	6.84 fl oz	In-furrow	-	0.08	0.10	0.13	0.44	0.86	1.26	3.81	3.50	4.38	7.25	31.25	51.25	551.92 cde	0.079 cde
	Serenade ASO	128 fl oz																
1410	Reason	6.8 fl oz +	1,3															
	Bond +	0.1% v/v +																
	Manzate	2 lb																
	Echo ZN	1.3 pt	2															
	Echo ZN	2.125 pt	4,6,8-10	7 day	0.16	0.11	0.05	0.25	1.28	4.06	3.56	5.88	6.13	6.13	10.94	19.38	338.15 ef	0.048 ef
	Luna Tranquility	11 fl oz +	5															
	Manzate	2 lb																
	Scala	6.8 fl oz +	7															
	Manzate	2 lb																
$LSD_{P=0.05}$					0.17	0.15	0.12	0.39	0.73	1.77	2.06	2.14	3.21	5.85	8.94	11.98	301.32	0.043

Table E2. Early blight severity ratings observed during the 2018 season at the Inkster, ND site. Treatments are shown with their corresponding numbers, the list of chemicals applied for each treatment, the rate of treatment, schedule and the interval of the schedule. Foliar disease severity was expressed as a percentage for the listed dates starting from July 4th, 2018 (7/4)—September 19th, 2018 (9/19). Area under the disease progress curve values (AUDPC) and relative area under the disease progress curve values (RAUDPC) are also shown on the table and the least significant differences (LSD; P=0.05) were calculated between the different treatments.

2018 Early Blight - Inkster																		
Treatment		Rate	Schedule	Interval	Foliar Disease (% Severity)												AUDPC	RAUDPC
					7/4	7/11	7/18	7/25	8/1	8/7	8/15	8/22	8/29	9/5	9/12	9/19		
1501	Non-treated	-	-	-	0.02	0.04	0.19	0.54	0.80	1.58	8.19	13.13	8.50	20.00	44.38	78.13	958.56 a	0.137 a
1502	Echo Zn	2.125 pt/a	Full Season	7 day	0.03	0.04	0.29	0.34	0.74	0.66	3.56	4.38	3.63	4.81	5.50	20.31	240.18 de	0.034 de
1503	Manzate	2.0 lb	Full Season	7 day	0.16	0.04	0.12	0.72	0.33	1.08	3.31	1.69	2.25	3.38	5.06	20.63	221.01 e	0.032 e
1504	Quadris + Bond + Manzate Echo ZN Echo ZN + Miravis + Manzate Inspire + Manzate	6.8 fl oz + 0.1 % v/v + 2.0 lb 1.3 pt 2.125 pt + 9.6 fl oz + 2 lb 6.8 fl oz + 2 lb	1,3 2 4,6,8-10 5 7	7 day	0.00	0.04	0.11	0.31	0.43	0.80	4.31	2.50	3.50	1.19	3.19	7.88	144.09 e	0.021 e
1505	Elatus	7.26 oz/a	In-furrow	-	0.03	0.11	0.26	0.40	0.20	1.13	4.13	4.44	3.56	13.25	22.50	53.75	539.98 b	0.077 b
1506	Elatus + Serenade ASO	7.26 oz/a + 64 fl oz	In-furrow	-	0.01	0.18	0.23	0.29	0.63	1.04	3.88	3.56	3.88	10.63	21.56	53.13	508.58 b	0.073 b
1507	Elatus + Serenade ASO	7.26 oz/a + 128 fl oz	In-furrow	-	0.03	0.11	0.06	0.23	0.32	0.89	3.75	6.13	3.25	11.88	22.81	55.63	542.53 b	0.078 b
1508	Velum Prime	6.84 fl oz	In-furrow	-	0.09	0.05	0.18	0.30	0.60	0.79	4.13	4.06	3.25	4.63	9.44	43.75	347.10 cd	0.050 cd
1509	Velum Prime + Serenade ASO	6.84 fl oz 128 fl oz	In-furrow	-	0.01	0.23	0.06	0.48	0.30	0.21	4.38	4.06	2.50	4.94	15.00	42.50	375.84 c	0.054 c
1510	Reason + Bond + Manzate Echo ZN Echo ZN + Luna Tranquility + Manzate Scala + Manzate	6.8 fl oz + 0.1% v/v + 2 lb 1.3 pt 2.125 pt + 11 fl oz + 2 lb 6.8 fl oz + 2 lb	1,3 2 4,6,8-10 5 7	7 day	0.14	0.06	0.18	0.41	0.31	0.46	2.94	4.69	2.50	2.50	4.38	9.44	163.84 e	0.023 e
$LSD_{P=0.05}$					0.11	0.14	0.21	0.40	0.52	0.77	2.15	2.16	1.87	3.19	4.78	8.71	108.67	0.016

APPENDIX F. COLLECTION DATA OF SMALL-SPORED *ALTERNARIA* SPP. ISOLATES COLLECTED FROM FIELDS FROM 1999-2015: QOI ASSAY

Year	Isolate	Species	QoI Mutation	Location	QoI EC_{50} (µg/ml)		
					Azoxystrobin	Famoxadone	Picoxystrobin
1999	122-3	*Alternaria tenuissima*	Sensitive	Clovis, NM	0.03	0.14	0.02
1999	123-2	*Alternaria alternata*	Sensitive	Dalhart, TX	0.01	0.03	0.01
1999	123-6	*Alternaria tenuissima*	NA	Dalhart, TX	0.02	0.18	0.02
1999	125-1	*Alternaria alternata*	Sensitive	Clovis, NM	14.61	0.74	8.31
1999	128-1	*Alternaria tenuissima*	Sensitive	Dalhart, TX	0.22	0.23	0.03
1999	147-1B	*Alternaria alternata*	Sensitive	Dalhart, TX	0.01	0.01	0.03
1999	147-3	*Alternaria alternata*	Sensitive	Dalhart, TX	0.04	0.04	0.03
1999	154-1	*Alternaria tenuissima*	Sensitive	Rexburg, ID	0.19	0.03	0.03
1999	178-2	*Alternaria tenuissima*	Sensitive	Dalhart, TX	0.05	0.03	0.01
1999	187-2	*Alternaria alternata*	Sensitive	Park Rapids, MN	0.04	0.05	0.09
1999	209-4	*Alternaria alternata*	Sensitive	Alamosa, CO	0.03	0.01	0.15
2000	245-1	*Alternaria alternata*	Sensitive	Olton, TX	0.02	0.01	0.04
2000	247-8	*Alternaria alternata*	Sensitive	Dalhart, TX	0.16	0.01	0.13
2000	302-1	*Alternaria tenuissima*	Sensitive	Dawson, ND	0.04	0.04	0.05
2000	306-1	*Alternaria alternata*	Sensitive	Dawson, ND	0.06	0.01	0.04
2000	310	*Alternaria alternata*	Sensitive	Rupert, ID	0.14	0.03	0.06
2000	314	*Alternaria arborescens*	Sensitive	Rupert, ID	0.29	0.03	0.25
2000	336-1	*Alternaria alternata*	Sensitive	St. Thomas, ND	0.10	0.03	0.04
2000	342-3	*Alternaria arborescens*	Sensitive	Dawson, ND	0.03	0.01	0.02
2000	355-4	*Alternaria alternata*	Sensitive	Dawson, ND	0.03	0.01	0.02
2000	364-1	*Alternaria alternata*	Sensitive	Dawson, ND	0.03	0.04	0.01

Year	Isolate	Species	QoI Mutation	Location	QoI EC_{50} (µg/ml)		
					Azoxystrobin	Famoxadone	Picoxystrobin
2000	371	*Alternaria alternata*	Sensitive	Oakes, ND	0.05	0.03	0.02
2000	444	*Alternaria alternata*	Sensitive	Larimore, ND	0.03	0.03	0.01
2000	479-4	*Alternaria alternata*	Sensitive	Dawson, ND	0.03	0.01	0.01
2001	527-1A	*Alternaria arborescens*	Sensitive	Dawson, ND	0.28	0.01	0.15
2002	604-1	*Alternaria alternata*	Sensitive	Columbus, NE	0.03	0.01	0.02
2015	Aa 1-1	*Alternaria arborescens*	G143A	Shelbyville, IN	100.00	100.00	28.54
2015	Aa 2-1	*Alternaria alternata*	G143A	Shelbyville, IN	19.86	17.67	17.67
2015	Aa 3-1	*Alternaria alternata*	G143A	Shelbyville, IN	100.00	0.79	31.25
2015	Aa 3-2	*Alternaria alternata*	G143A	Shelbyville, IN	16.64	100.00	100.00
2015	Aa 4-1	*Alternaria alternata*	G143A	Shelbyville, IN	19.54	100.00	68.50
2015	Aa 5-1	*Alternaria alternata*	G143A	Shelbyville, IN	7.76	100.00	35.84
2015	Aa 7-1	*Alternaria alternata*	G143A	Shelbyville, IN	25.49	100.00	95.03
2015	Aa 7-2	*Alternaria alternata*	G143A	Shelbyville, IN	26.05	100.00	100.00
2015	Aa 10-1	*Alternaria alternata*	G143A	Petersburg, MI	22.34	100.00	100.00
2015	Aa 11-1	*Alternaria alternata*	Sensitive	Galveston, IN	0.05	0.036	0.02
2015	Aa 12-1	*Alternaria alternata*	G143A	Galveston, IN	28.31	100.00	100.00
2015	Aa 13-1	*Alternaria tenuissima*	G143A	Estes, IN	19.43	100.00	7.57
2015	Aa 15-1	*Alternaria alternata*	G143A	Kokomo, IN	17.37	100.00	34.22
2015	Aa 16-1	*Alternaria alternata*	G143A	Kokomo, IN	4.86	0.07	31.25
2015	Aa 17-1	*Alternaria alternata*	G143A	Kokomo, IN	29.15	100.00	100.00
2015	Aa 18-1	*Alternaria alternata*	G143A	Findley, OH	26.82	100.00	20.07
2015	Aa 18-3	*Alternaria alternata*	G143A	Findley, OH	17.26	100.00	24.23
2015	Aa 19-2	*Alternaria alternata*	NA	Rollersville, OH	20.08	100.00	98.67

Year	Isolate	Species	QoI Mutation	Location	QoI EC_{50} (µg/ml)		
					Azoxystrobin	Famoxadone	Picoxystrobin
2015	Aa 20-1	*Alternaria alternata*	G143A	Blissfield, MI	20.82	17.73	8.98
2015	Aa 21-1	*Alternaria alternata*	G143A	Deersfield, MI	100.00	18.45	34.99
2015	Aa 22-2	*Alternaria alternata*	G143A	Deersfield, MI	8.19	100.00	20.09
2015	Aa 23-1	*Alternaria alternata*	G143A	Deersfield, MI	12.57	100.00	13.41
2015	Aa 23-2	*Alternaria alternata*	G143A	Deersfield, MI	13.40	100.00	23.20
2015	Aa 24-1	*Alternaria alternata*	NA	Monroe, MI	5.73	100.00	6.91
2015	Aa 24-3	*Alternaria alternata*	NA	Monroe, MI	19.44	100.00	20.08
2015	Aa 24-6	*Alternaria alternata*	NA	Monroe, MI	4.29	100.00	6.57
2015	Aa 24-7	*Alternaria alternata*	NA	Monroe, MI	4.59	100.00	8.22
2015	Aa 24-9	*Alternaria alternata*	NA	Monroe, MI	15.47	100.00	9.54
2015	Aa 24-10	*Alternaria alternata*	NA	Monroe, MI	13.44	100.00	24.94

APPENDIX G. COLLECTION DATA OF SMALL-SPORED *ALTERNARIA* SPP. ISOLATES COLLECTED FROM FIELDS FROM 1999-2017: SDHI ASSAY

Year	Isolate	Species	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
1999	122-3	*Alternaria tenuissima*	Clovis, NM	0.04	0.05	0.01	0.01
1999	123-2	*Alternaria alternata*	Dalhart, TX	0.03	0.06	0.03	0.02
1999	123-6	*Alternaria tenuissima*	Dalhart, TX	0.02	0.01	0.02	0.01
1999	125-1	*Alternaria alternata*	Clovis, NM	0.05	0.06	0.01	0.02
1999	128-1	*Alternaria tenuissima*	Dalhart, TX	0.05	0.02	0.02	0.02
1999	147-1B	*Alternaria alternata*	Dalhart, TX	0.05	0.03	0.03	0.01
1999	147-3	*Alternaria alternata*	Dalhart, TX	0.05	0.06	0.05	0.02
1999	154-1	*Alternaria tenuissima*	Rexburg, ID	0.06	0.03	0.01	0.01
1999	178-2	*Alternaria tenuissima*	Dalhart, TX	0.06	0.01	0.02	0.01
1999	187-2	*Alternaria alternata*	Park Rapids, MN	0.07	0.05	0.05	0.02
1999	209-4	*Alternaria alternata*	Alamosa, CO	0.06	0.06	0.08	0.01
2000	245-1	*Alternaria alternata*	Olton, TX	0.05	0.07	0.04	0.02
2000	247-8	*Alternaria alternata*	Dalhart, TX	0.04	0.07	0.04	0.01
2000	302-1	*Alternaria tenuissima*	Dawson, ND	0.04	0.03	0.01	0.01
2000	306-1	*Alternaria alternata*	Dawson, ND	0.01	0.07	0.05	0.01
2000	310	*Alternaria alternata*	Rupert, ID	0.06	0.05	0.07	0.01
2000	314	*Alternaria arborescens*	Rupert, ID	0.06	0.03	0.03	0.01
2000	336-1	*Alternaria alternata*	St. Thomas, ND	0.01	0.08	0.06	0.01
2000	342-3	*Alternaria arborescens*	Dawson, ND	0.04	0.04	0.06	0.01
2000	355-4	*Alternaria alternata*	Dawson, ND	0.05	0.08	0.02	0.01
2000	364-1	*Alternaria alternata*	Dawson, ND	0.07	0.05	0.06	0.02

Year	Isolate	Species	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2000	371	*Alternaria alternata*	Oakes, ND	0.01	0.02	0.04	0.01
2000	444	*Alternaria alternata*	Larimore, ND	0.05	0.06	0.04	0.01
2000	479-4	*Alternaria alternata*	Dawson, ND	0.01	0.06	0.05	0.02
2001	527-1A	*Alternaria arborescens*	Dawson, ND	0.03	0.02	0.05	0.01
2002	604-1	*Alternaria alternata*	Columbus, NE	0.05	0.06	0.03	0.01
2003	801-1	*Alternaria arborescens*	Tappan, ND	0.01	0.01	0.01	0.01
2003	802-2	*Alternaria tenuissima*	Tappan, ND	0.01	0.01	0.01	0.01
2003	803-1	*Alternaria tenuissima*	Tappan, ND	0.01	0.01	0.01	0.01
2003	807-1	*Alternaria arborescens*	Tappan, ND	0.01	0.01	0.01	0.01
2003	853-1	*Alternaria tenuissima*	Tappan, ND	0.01	0.01	0.01	0.01
2003	854-2	*Alternaria tenuissima*	Tappan, ND	0.01	0.01	0.01	0.01
2003	855-3	*Alternaria arborescens*	Tappan, ND	0.01	0.01	0.01	0.01
2003	857-3	*Alternaria alternata*	Tappan, ND	0.14	0.01	0.01	0.01
2003	858-1	*Alternaria alternata*	Tappan, ND	0.89	0.01	0.01	0.01
2003	860-2	*Alternaria arborescens*	Tappan, ND	0.01	0.01	0.01	0.01
2004	912-1	*Alternaria alternata*	Cummings, ND	0.51	1.13	1.14	0.01
2011	1209-2	*Alternaria alternata*	Inkster, ND	0.21	0.28	0.60	0.01
2011	1253-1	*Alternaria arborescens*	Acequia, ID	0.80	1.76	1.82	0.03
2013	1285-9	*Alternaria alternata*	Brawley, CA	0.22	0.16	0.10	0.01
2013	1290-1	*Alternaria alternata*	Savanna, IL	0.54	0.43	0.96	0.01
2013	1294-3	*Alternaria arborescens*	Farmington, NM	0.84	2.03	2.19	0.02
2013	1298-2	*Alternaria arborescens*	Farmington, NM	3.80	0.42	0.86	0.14
2013	1299-2	*Alternaria alternata*	Dalhart, TX	0.96	0.68	1.51	0.01

Year	Isolate	Species	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2013	1306-3	*Alternaria alternata*	Erie, IL	0.04	0.08	0.03	0.01
2013	1309-10	*Alternaria alternata*	Bath, IL	0.06	0.11	0.04	0.01
2013	1311-1	*Alternaria alternata*	Olton, TX	0.04	0.11	0.04	0.01
2013	1314-1	*Alternaria alternata*	Dawson, ND	0.01	0.01	0.01	0.01
2013	1317-9	*Alternaria tenuissima*	Columbus, NE	0.10	0.01	0.01	0.01
2014	14-392	*Alternaria tenuissima*	DuPont, IN	0.01	0.01	0.01	0.01
2014	14-397	*Alternaria alternata*	DuPont, IN	0.21	0.16	0.08	0.01
2015	Ar 1-1	*Alternaria arborescens*	Shelbyville, IN	0.05	0.01	0.01	0.01
2015	Aa 3-1	*Alternaria alternata*	Shelbyville, IN	1.35	0.83	1.29	0.02
2015	Aa 4-1	*Alternaria alternata*	Shelbyville, IN	0.18	0.30	0.19	0.01
2015	Aa 5-1	*Alternaria alternata*	Shelbyville, IN	0.11	0.26	0.20	0.01
2015	Aa 7-1	*Alternaria alternata*	Shelbyville, IN	0.35	0.21	0.25	0.01
2015	Aa 7-2	*Alternaria alternata*	Shelbyville, IN	0.35	0.30	1.56	0.01
2015	Aa 8-1	*Alternaria tenuissima*	Malad City, ID	0.01	0.01	0.01	0.01
2015	Aa 8-2	*Alternaria tenuissima*	Malad City, ID	1.53	0.02	0.02	0.01
2015	Aa 9-1	*Alternaria tenuissima*	Malad City, ID	0.01	0.10	0.01	0.01
2015	Aa 9-2	*Alternaria tenuissima*	Malad City, ID	0.01	0.01	0.01	0.01
2015	Aa 10-1	*Alternaria alternata*	Petersburg, MI	0.33	0.33	0.36	0.01
2015	Aa 13-1	*Alternaria tenuissima*	Estes, IN	0.01	0.01	0.01	0.01
2015	Aa 18-1	*Alternaria alternata*	Findley, OH	0.76	0.76	0.29	0.01
2015	Aa 18-3	*Alternaria alternata*	Findley, OH	0.57	0.10	0.19	0.01
2015	Aa 19-2	*Alternaria alternata*	Rollersville, OH	0.29	0.31	0.65	0.01
2015	Aa 22-2	*Alternaria alternata*	Deersfield, MI	0.34	0.47	1.14	0.01

Year	Isolate	Species	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2015	Aa 23-1	*Alternaria alternata*	Deersfield, MI	0.47	0.40	0.41	0.01
2015	Aa 24-1	*Alternaria alternata*	Monroe, MI	0.24	0.34	0.59	0.01
2015	Aa 24-6	*Alternaria alternata*	Monroe, MI	0.85	0.82	1.64	0.02
2017	1701-3	*Alternaria tenuissima*	O'Neil, NE	2.66	1.31	0.01	0.01
2017	1701-4	*Alternaria tenuissima*	O'Neil, NE	0.48	0.96	0.01	0.01
2017	1701-5	*Alternaria tenuissima*	O'Neil, NE	0.30	0.02	0.01	0.01
2017	1701-6	*Alternaria tenuissima*	O'Neil, NE	0.05	0.01	0.01	0.01
2017	1701-7	*Alternaria tenuissima*	O'Neil, NE	0.08	0.02	0.01	0.01
2017	1701-8	*Alternaria tenuissima*	O'Neil, NE	0.04	0.01	0.01	0.01
2017	1701-9	*Alternaria tenuissima*	O'Neil, NE	0.36	0.03	0.01	0.01
2017	1702-5	*Alternaria tenuissima*	Greeley, CO	0.11	0.13	0.01	0.01
2017	1703-4	*Alternaria tenuissima*	Three Rivers, MI	0.07	0.02	0.01	0.01
2017	1703-5	*Alternaria tenuissima*	Three Rivers, MI	0.20	0.01	0.01	0.01
2017	1703-8	*Alternaria alternata*	Three Rivers, MI	0.27	0.22	0.28	0.01
2017	1703-9	*Alternaria alternata*	Three Rivers, MI	0.37	0.26	0.31	0.01
2017	1704-1	*Alternaria tenuissima*	Colorado City, CO	0.01	0.02	0.03	0.01
2017	1708-16	*Alternaria alternata*	Dalhart, TX	1.02	2.19	1.87	0.02
2017	1710-15	*Alternaria arborescens*	Dalhart, TX	0.12	0.41	0.48	0.01
2017	1710-16	*Alternaria tenuissima*	Dalhart, TX	0.17	0.02	0.02	0.01
2017	1710-17	*Alternaria alternata*	Dalhart, TX	1.57	0.62	0.44	0.03
2017	1712-4	*Alternaria tenuissima*	Colorado City, CO	0.14	0.03	0.01	0.01
2017	1713-1	*Alternaria arborescens*	Colorado City, CO	0.26	0.12	0.50	0.01
2017	1713-2	*Alternaria arborescens*	Colorado City, CO	0.17	0.35	0.28	0.01

Year	Isolate	Species	Location	SDHI EC_{50} values (µg/ml)			
				Boscalid	Fluopyram	Solatenol	Adepidyn
2017	1713-3	*Alternaria arborescens*	Colorado City, CO	0.11	0.32	0.34	0.01
2017	1713-6	*Alternaria arborescens*	Colorado City, CO	0.12	0.01	0.01	0.01
2017	1713-7	*Alternaria arborescens*	Colorado City, CO	0.49	0.02	0.01	0.01
2017	1714-1	*Alternaria tenuissima*	Dalhart, TX	0.01	0.02	0.01	0.01
2017	1714-3	*Alternaria alternata*	Dalhart, TX	2.20	2.11	1.33	0.04
2017	1714-6	*Alternaria tenuissima*	Dalhart, TX	0.01	0.01	0.01	0.01
2017	1715-7	*Alternaria alternata*	Dalhart, TX	0.69	0.99	1.82	0.01
2017	1716-1	*Alternaria alternata*	Dalhart, TX	3.85	3.83	1.13	0.03

APPENDIX H. SUMMARY OF STATISTICAL ANALYSIS FOR *ALTERNARIA* SPP. IN VITRO SENSITIVITY ASSAYS

Table H1. Combined analysis of variance for in vitro fungicide sensitivity of the small-spored *Alternaria* spp. baseline isolate EC_{50} values for QoI fungicides: azoxystrobin, famoxadone, and picoxystrobin.

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.002	0.00	0.9833
Fungicide	2	5.977	1.63	0.1983
Species	2	1.794	0.49	0.6141
Trial x fungicide	2	0.002	0.00	0.9995
Trial x species	2	0.027	0.01	0.9927
Fungicide x species	4	1.924	0.52	0.7184
Trial x fungicide x species	4	0.041	0.01	0.9997

Table H2. Combined analysis of variance for in vitro fungicide sensitivity of the small-spored *Alternaria* spp. non-baseline isolate EC_{50} values for QoI fungicides: azoxystrobin, famoxadone, and picoxystrobin.

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	2.678	0.00	0.9600
Fungicide	2	12895.000	12.14	<0.0001
Species	2	1958.808	1.84	0.1596
Trial x fungicide	2	4.702	0.00	0.9956
Trial x species	2	5.152	0.00	0.9952
Fungicide x species	4	13468.000	12.68	<0.0001
Trial x fungicide x species	4	4.851	0.00	1.0000

Table H3. Combined analysis of variance for in vitro fungicide sensitivity of the small-spored *Alternaria* spp. baseline isolate EC_{50} values for SDHI fungicides: boscalid, fluopyram, solatenol, and adepidyn.

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.003	0.11	0.7409
Fungicide	2	0.045	1.61	0.1859
Species	3	0.295	10.44	<0.0001
Trial x fungicide	1	0.001	0.03	0.9943
Trial x species	3	0.004	0.14	0.8713
Fungicide x species	6	0.032	1.14	0.3371
Trial x fungicide x species	6	0.001	0.03	0.9999

Table H4. Combined analysis of variance for in vitro fungicide sensitivity of the small-spored *Alternaria* spp. non-baseline isolate EC_{50} values for SDHI fungicides: boscalid, fluopyram, solatenol, and adepidyn

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.004	0.01	0.9183
Fungicide	2	10.755	30.43	<0.0001
species	3	17.220	48.72	<0.0001
Trial x fungicide	1	0.029	0.08	0.9894
Trial x species	3	0.039	0.11	0.8962
Fungicide x species	6	2.261	6.40	<0.0001
Trial x fungicide x species	6	0.029	0.08	0.9979

APPENDIX I. SUMMARY OF STATISTICAL ANALYSIS FOR SMALL-SPORED *ALTERNARIA* SPP. IN VIVO SENSITIVITY TESTS

Table I1. Combined analysis of variance of in vivo percentage disease control of small-spored *Alternaria* spp. isolates provided by boscalid.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	0.231	0.00	0.9683
Rep	2	32.764	0.22	0.7991
Fungicide concentration	3	20117.430	137.79	<0.0001
Rep x fungicide concentration	6	140.155	0.96	0.4521
Trial x fungicide concentration	3	7.412	0.05	0.9849
Trial x rep	2	3.517	0.02	0.9762
Trial x rep x fungicide concentration	6	6.627	0.05	0.9996

Table I2. Combined analysis of variance of in vivo percentage disease control of small-spored *Alternaria* spp. isolates provided by fluopyram.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	151.553	0.48	0.4870
Rep	2	496.987	1.59	0.2058
Fungicide concentration	3	25992.060	83.01	<0.0001
Rep x fungicide concentration	6	117.254	0.37	0.8952
Trial x fungicide concentration	3	52.024	0.17	0.9192
Trial x rep	2	68.804	0.22	0.8028
Trial x rep x fungicide concentration	6	26.445	0.08	0.9977

Table I3. Combined analysis of variance of in vivo percentage disease control of small-spored *Alternaria* spp. isolates provided by solatenol.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	253987.000	3.58	0.0643
Rep	2	1298.430	1.46	0.2342
Fungicide concentration	3	68070.210	76.38	<0.0001
Rep x fungicide concentration	6	583.289	0.65	0.6865
Trial x fungicide concentration	3	5132.836	5.76	0.0007
Trial x rep	2	1235.262	1.39	0.2513
Trial x rep x fungicide concentration	6	1789.152	2.01	0.0636

Table I4. Combined analysis of variance of in vivo percentage disease control of small-spored *Alternaria* spp. isolates provided by adepidyn.

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	165.689	0.73	0.3934
Rep	2	141.295	0.62	0.5371
Fungicide concentration	3	18985.930	83.64	<0.0001
Rep x fungicide concentration	6	161.406	0.71	0.6409
Trial x fungicide concentration	3	167.994	0.74	0.5286
Trial x rep	2	25.993	0.11	0.8921
Trial x rep x fungicide concentration	6	25.074	0.11	0.9952

APPENDIX J. SUMMARY OF SMALL-SPORED *ALTERNARIA* SPP. IN VIVO PERCENTAGE DISEASE CONTROL

Table J1. Mean in vivo percentage disease control of small-spored *Alternaria* spp. isolates by boscalid as determined in greenhouse assays

Species	Isolate	In vitro EC_{50}[x] (µg/ml)	Boscalid concentration (µg/ml)				AUDRC[y]	Species mean AUDRC
			0.1	1	10	100		
A. alternata	1716-1	3.85	63.4 efg	88.3 b	100.0 a	100.0 a	9915.6 ab	
A. alternata	1714-3	2.20	76.6 bcde	93.5 ab	98.2 ab	100.0 a	9858.0 abc	
A. alternata	1715-7	0.69	81.7 abcd	92.1 b	100.0 a	100.0 a	9942.8 ab	
A. alternata	Aa 3-1	1.35	43.2 hi	71.8 ef	96.5 bc	100.0 a	9649.8 def	9766.5 b
A. alternata	125-1	0.01	36.1 i	67.6 f	97.5 b	100.0 a	9675.1 cdef	
A. alternata	Aa 7-1	0.35	38.3 i	72.3 ef	94.6 c	100.0 a	9557.6 f	
A. arborescens	1294-3	0.84	93.1 a	100.0 a	100.0 a	100.0 a	9986.9 a	
A. arborescens	1298-2	3.8	82.8 abc	87.1 bc	98.2 ab	100.0 a	9830.3 abcd	
A. arborescens	1713-1	0.26	82.6 abc	100.0 a	100.0 a	100.0 a	9982.2 a	
A. arborescens	1713-3	0.11	86.3 ab	91.9 b	96.5 bc	100.0 a	9771.9 bcde	9905.4 a
A. arborescens	1713-6	0.12	81.9 abcd	92.8 b	100.0 a	100.0 a	9946.4 ab	
A. arborescens	Ar 1-1	0.05	57.4 fg	88.7 b	100.0 a	100.0 a	9914.7 ab	
A. tenuissima	At 8-2	0.48	68.8 def	80.6 cd	100.0 a	100.0 a	9879.7 ab	
A. tenuissima	At 9-2	2.66	52.8 gh	77.9 de	94.7 c	100.0 a	9599.4 ef	
A. tenuissima	1714-1	0.07	71.2 cde	89.2 b	98.3 ab	100.0 a	9841.7 abcd	
A. tenuissima	At 13-1	0.30	74.7 a	100.0 a	100.0 a	100.0 a	9978.6 a	9844.8 ab
A. tenuissima	1317-9	0.01	81.4 abcd	93.5 ab	97.4 b	100.0 a	9818.7 abcd	
A. tenuissima	1702-5	0.01	94.2 a	92.6 b	100.0 a	100.0 a	9950.7 ab	
LSD $_{P=0.05}$ [z]			13.7	7.1	2.5	0.0	200.0	

[X] EC_{50} (effective concentration where the fungal growth is inhibited by 50%) values from in vitro.
[Y] AUDRC = Area under the dose response curve.
[Z] Least significant difference at the P = 0.05 level.

Table J2. Mean in vivo percentage disease control of small-spored *Alternaria* spp. isolates by fluopyram as determined in greenhouse assays

Species	Isolate	In vitro EC_{50}[x] (µg/ml)	Fluopyram concentration (µg/ml)				AUDRC[y]	Species mean AUDRC
			0.1	1	10	100		
A. alternata	1716-1	3.83	31.8 gh	34.3 e	82.8 b	94.0 b	8513.2 b	
A. alternata	1714-3	2.11	96.5 a	100.0 a	100.0 a	100.0 a	9988.4 a	
A. alternata	1715-7	0.99	72.0 bcde	93.8 a	100.0 a	100.0 a	9946.5 a	9723.5 a
A. alternata	Aa 3-1	0.83	72.4 bcde	95.1 a	100.0 a	100.0 a	9953.4 a	
A. alternata	125-1	0.02	80.1 abcd	94.4 a	100.0 a	100.0 a	9953.5 a	
A. alternata	Aa 7-1	0.21	90.5 abc	100.0 a	100.0 a	100.0 a	9985.7 a	
A. arborescens	1294-3	2.03	59.3 def	89.4 ab	95.4 a	100.0 a	9689.8 a	
A. arborescens	1298-2	0.42	80.4 abcd	96.1 a	100.0 a	100.0 a	9961.7 a	
A. arborescens	1713-1	0.12	69.0 cde	100.0 a	100.0 a	100.0 a	9976.0 a	9883.8 a
A. arborescens	1713-3	0.32	45.0 fgh	93.3 a	98.5 a	100.0 a	9857.6 a	
A. arborescens	1713-6	0.01	28.5 h	74.3 cd	100.0 a	100.0 a	9830.6 a	
A. arborescens	Ar 1-1	0.01	93.4 ab	100.0 a	100.0 a	100.0 a	9987.0 a	
A. tenuissima	At 8-2	0.05	54.4 efg	75.6 cd	100.0 a	100.0 a	9848.6 a	
A. tenuissima	At 9-2	1.31	50.8 efgh	77.6 bc	94.9 a	100.0 a	9604.8 a	
A. tenuissima	1714-1	0.02	85.2 abc	95.1 a	100.0 a	100.0 a	9958.9 a	9849.8 a
A. tenuissima	At 13-1	0.03	52.7 efg	90.0 ab	100.0 a	100.0 a	9919.2 a	
A. tenuissima	1317-9	0.01	88.3 abc	100.0 a	100.0 a	100.0 a	9984.7 a	
A. tenuissima	1702-5	0.01	40.7 fgh	63.4 d	100.0 a	100.0 a	9782.3 a	
LSD $_{P=0.05}$[z]			22.7	13.4	6.6	2.3	465.4	190.0

[X] EC_{50} (effective concentration where the fungal growth is inhibited by 50%) values from in vitro.
[Y] AUDRC = Area under the dose response curve.
[Z] Least significant difference at the P = 0.05 level.

Table J3. Mean in vivo percentage disease control of small-spored *Alternaria* spp. isolates by solatenol as determined in greenhouse assays

Species	Isolate	In vitro EC_{50}[x] (µg/ml)	Solatenol concentration (µg/ml)				AUDRC[y]	Species mean AUDRC
			0.1	1	10	100		
A. alternata	1716-1	1.13	85.2 abcde	96.3 abc	100.0 a	100.0 a	9965.0 a	
A. alternata	1714-3	1.33	70.7 def	90.6 abcd	100.0 a	100.0 a	9930.4 a	
A. alternata	1715-7	1.82	77.3 bcdef	82.5 de	98.5 ab	100.0 a	9818.8 a	9932.0 a
A. alternata	Aa 3-1	1.29	75.0 bcdef	97.6 ab	100.0 a	100.0 a	9967.1 a	
A. alternata	125-1	0.02	84.0 abcde	87.5 bcd	100.0 a	100.0 a	9920.9 a	
A. alternata	Aa 7-1	0.25	100.0 a	100.0 a	100.0 a	100.0 a	9990.0 a	
A. arborescens	1294-3	2.19	79.4 bcdef	100.0 a	100.0 a	100.0 a	9980.7 a	
A. arborescens	1298-2	0.86	73.0 cdef	88.1 bcd	100.0 a	100.0 a	9918.8 a	
A. arborescens	1713-1	0.50	81.6 bcdef	100.0 a	100.0 a	100.0 a	9981.7 a	9907.3 a
A. arborescens	1713-3	0.34	90.6 abc	86.1 cd	93.7 c	100.0 a	9603.0 b	
A. arborescens	1713-6	0.01	87.3 abcd	100.0 a	100.0 a	100.0 a	9984.3 a	
A. arborescens	Ar 1-1	0.01	67.6 ef	100.0 a	100.0 a	100.0 a	9975.4 a	
A. tenuissima	At 8-2	0.02	73.1 cdef	93.1 abcd	100.0 a	100.0 a	9943.5 a	
A. tenuissima	At 9-2	0.03	93.1 ab	100.0 a	100.0 a	100.0 a	9986.9 a	
A. tenuissima	1714-1	0.01	78.8 bcdef	83.7 de	94.4 bc	100.0 a	9623.7 b	9808.4 b
A. tenuissima	At 13-1	0.01	42.5 g	72.6 e	100.0 a	100.0 a	9828.7 a	
A. tenuissima	1317-9	0.01	65.0 f	82.6 de	94.3 bc	97.4 b	9489.3 b	
A. tenuissima	1702-5	0.01	74.5 cdef	100.0 a	100.0 a	100.0 a	9978.5 a	
LSD $_{P=0.05}$ [z]			18.3	11.1	4.3	1.0	192.8	78.7

[X] EC_{50} (effective concentration where the fungal growth is inhibited by 50%) values from in vitro.
[Y] AUDRC = Area under the dose response curve.
[Z] Least significant difference at the P = 0.05 level.

Table J4. Mean in vivo percentage disease control of small-spored *Alternaria* spp. isolates by adepidyn as determined in greenhouse assays

Species	Isolate	In vitro EC_{50}[x] (µg/ml)	Adepidyn concentration (µg/ml)				AUDRC[y]	Species mean AUDRC
			0.1	1	10	100		
A. alternata	1716-1	0.03	97.2 a	100.0 a	100.0 a	100.0 a	9988.8 a	
A. alternata	1714-3	0.04	81.6 abcd	100.0 a	100.0 a	100.0 a	9981.7 a	
A. alternata	1715-7	0.01	39.4 g	84.7 bc	100.0 a	100.0 a	9887.1 cd	9963.7 a
A. alternata	Aa 3-1	0.02	62.3 ef	98.1 a	100.0 a	100.0 a	9963.7 ab	
A. alternata	125-1	0.01	74.5 bcde	100.0 a	100.0 a	100.0 a	9978.5 ab	
A. alternata	Aa 7-1	0.01	83.3 abcd	100.0 a	100.0 a	100.0 a	9982.5 a	
A. arborescens	1294-3	0.02	69.4 cdef	100.0 a	100.0 a	100.0 a	9976.3 ab	
A. arborescens	1298-2	0.14	68.7 cdef	100.0 a	100.0 a	100.0 a	9975.9 ab	
A. arborescens	1713-1	0.01	14.4 h	79.2 c	100.0 a	100.0 a	9848.3 d	9948.7 a
A. arborescens	1713-3	0.01	100.0 a	87.3 abc	100.0 a	100.0 a	9927.0 bc	
A. arborescens	1713-6	0.01	93.1 ab	100.0 a	100.0 a	100.0 a	9986.9 a	
A. arborescens	Ar 1-1	0.01	58.8 ef	99.3 a	100.0 a	100.0 a	9968.0 ab	
A. tenuissima	At 8-2	0.01	89.8 ab	100.0 a	100.0 a	100.0 a	9985.4 a	
A. tenuissima	At 9-2	0.01	87.0 abc	100.0 a	100.0 a	100.0 a	9984.2 a	
A. tenuissima	1714-1	0.01	67.7 def	97.4 ab	100.0 a	100.0 a	9962.5 ab	9947.1 a
A. tenuissima	At 13-1	0.01	58.7 ef	88.0 abc	99.8 b	100.0 a	9900.6 cd	
A. tenuissima	1317-9	0.01	100.0 a	100.0 a	100.0 a	100.0 a	9990.0 a	
A. tenuissima	1702-5	0.01	54.8 fg	79.8 c	100.0 a	100.0 a	9869.8 d	
$LSD_{P=0.05}$ [z]			18.9	13.3	0.2	0.0	52.8	21.6

[X] EC_{50} (effective concentration where the fungal growth is inhibited by 50%) values from in vitro.
[Y] AUDRC = Area under the dose response curve.
[Z] Least significant difference at the P = 0.05 level.

APPENDIX K. COLLECTION DATA OF *ALTERNARIA* SPP. ISOLATES COLLECTED FROM FIELDS FROM 1998-2017: AP AND PP ASSAYS

Year	Isolate	Species	Location	AP and PP EC_{50} (µg/ml)		
				Pyrimethanil	Cyprodinil	Fludioxonil
1999	125-1	*Alternaria alternata*	Clovis, NM	1.59	0.51	0.43
2017	1702-5	*Alternaria tenuissima*	Greeley, CO	1.19	4.12	0.10
1999	122-3	*Alternaria tenuissima*	Clovis, NM	4.23	1.22	1.34
1999	123-2	*Alternaria alternata*	Dalhart, TX	5.85	1.55	1.19
1999	123-6	*Alternaria tenuissima*	Dalhart, TX	3.55	1.15	1.21
1999	147-1B	*Alternaria alternata*	Dalhart, TX	4.43	1.25	1.30
1999	147-3	*Alternaria alternata*	Dalhart, TX	4.46	1.27	1.23
1999	178-2	*Alternaria tenuissima*	Dalhart, TX	1.10	0.10	0.33
1999	209-4	*Alternaria alternata*	Alamosa, CO	1.11	0.10	0.10
2000	245-1	*Alternaria alternata*	Olton, TX	1.24	1.08	0.10
2000	247-8	*Alternaria alternata*	Dalhart, TX	1.11	0.10	0.33
2000	302-1	*Alternaria tenuissima*	Dawson, ND	1.08	0.86	0.10
2000	306-1	*Alternaria alternata*	Dawson, ND	1.08	0.10	0.10
2000	310	*Alternaria alternata*	Rupert, ID	1.08	0.56	0.10
2000	314	*Alternaria arborescens*	Rupert, ID	1.14	0.84	0.35
2000	336-1	*Alternaria alternata*	St. Thomas, ND	1.10	0.36	0.10
2000	342-3	*Alternaria arborescens*	Dawson, ND	0.84	0.10	0.10
2000	355-4	*Alternaria alternata*	Dawson, ND	0.55	1.10	0.10
2000	364-1	*Alternaria alternata*	Dawson, ND	0.10	0.34	0.10
2000	371	*Alternaria alternata*	Oakes, ND	1.14	0.37	0.10
2000	444	*Alternaria alternata*	Larimore, ND	1.09	0.10	3.36
2000	479-4	*Alternaria alternata*	Dawson, ND	0.80	0.10	2.08
2001	527-1A	*Alternaria arborescens*	Dawson, ND	0.10	0.10	0.10
2002	604-1	*Alternaria alternata*	Columbus, NE	0.36	0.10	0.10
1998	13-1	*Alternaria solani*	Minden, NE	1.10	0.10	0.10
2001	526-3	*Alternaria solani*	O'Neil, NE	1.13	1.24	1.15
1998	1-1	*Alternaria solani*	Dalhart, TX	0.10	1.85	0.10
1998	3-1	*Alternaria solani*	Minden, NE	1.17	0.10	0.10
1998	6-1	*Alternaria solani*	Park Rapids, MN	0.10	0.10	0.10
1998	11-1	*Alternaria solani*	Columbus, NE	1.57	1.79	0.10
1998	12-1	*Alternaria solani*	Minden, NE	1.16	1.11	0.10
1998	12-3	*Alternaria solani*	Columbus, NE	1.43	1.46	0.10
1998	14-1	*Alternaria solani*	Minden, NE	0.10	0.10	0.10
1998	14-3	*Alternaria solani*	Minden, NE	0.10	1.54	0.10
1998	22-1	*Alternaria solani*	Staples, MN	1.60	1.46	1.18
1998	30-1	*Alternaria solani*	Buxton, ND	1.69	1.35	0.10
1998	31-1	*Alternaria solani*	O'Neil, NE	2.45	1.48	0.10
1998	31-4	*Alternaria solani*	O'Neil, NE	1.24	1.33	0.10

Year	Isolate	Species	Location	AP and PP EC_{50} (µg/ml)		
				Pyrimethanil	Cyprodinil	Fludioxonil
1998	32-1	*Alternaria solani*	Park Rapids, MN	1.20	0.10	1.11
1998	33-1	*Alternaria solani*	Park Rapids, MN	1.18	0.10	0.10
1998	37-1	*Alternaria solani*	O'Neil, NE	1.17	0.10	1.04
1998	37-4	*Alternaria solani*	O'Neil, NE	1.21	0.10	1.07
1998	38-1	*Alternaria solani*	Minden, NE	1.25	0.10	1.06
1998	38-4	*Alternaria solani*	Minden, NE	1.31	0.10	1.24
1998	40-1	*Alternaria solani*	Watertown, SD	1.21	0.10	1.21
1998	68-1	*Alternaria solani*	Shelley, ID	1.26	0.14	1.10
1998	83-1	*Alternaria solani*	Hamer, ID	1.19	0.10	0.10
1998	88-1	*Alternaria solani*	Hancock, WI	1.19	1.70	1.13
2001	521-1	*Alternaria solani*	O'Neil, NE	1.38	1.09	1.18
2001	528-2	*Alternaria solani*	O'Neil, NE	1.21	0.10	1.12
2001	528-3	*Alternaria solani*	O'Neil, NE	1.12	0.09	0.10
2001	532-2	*Alternaria solani*	O'Neil, NE	1.25	0.10	1.12
2001	535-2	*Alternaria solani*	O'Neil, NE	1.62	0.11	0.10
2001	537-2	*Alternaria solani*	O'Neil, NE	1.15	0.10	0.10
2001	545-1	*Alternaria solani*	O'Neil, NE	1.12	1.08	0.10
2001	547-1	*Alternaria solani*	Dawson, ND	1.20	0.10	0.10
2001	547-2	*Alternaria solani*	Dawson, ND	1.23	1.03	0.10
2001	547-4	*Alternaria solani*	Dawson, ND	1.55	1.24	0.10
2001	549-1	*Alternaria solani*	Dawson, ND	1.72	1.34	0.10
2001	549-2	*Alternaria solani*	Dawson, ND	1.19	0.10	0.10
2001	549-3	*Alternaria solani*	Dawson, ND	1.36	0.20	0.10
2001	572-1	*Alternaria solani*	Minden, NE	1.21	0.10	0.10
2001	574-1	*Alternaria solani*	Columbus, NE	1.34	0.10	0.10
2001	574-3	*Alternaria solani*	Columbus, NE	1.16	0.10	0.10
2001	577-1	*Alternaria solani*	Minden, NE	1.10	0.10	1.18
2001	578-1	*Alternaria solani*	Minden, NE	1.15	0.10	1.09
2001	583-2	*Alternaria solani*	Dawson, ND	1.13	0.10	0.10
2001	583-3	*Alternaria solani*	Dawson, ND	1.10	1.19	0.10
2001	584-6	*Alternaria solani*	Dawson, ND	1.17	0.10	1.14
2001	585-1	*Alternaria solani*	Browerville, MN	1.10	0.14	1.09
2001	586-1	*Alternaria solani*	Browerville, MN	1.13	0.10	0.10
2001	586-2	*Alternaria solani*	Browerville, MN	1.10	0.10	1.06
2001	587-3	*Alternaria solani*	Browerville, MN	1.10	0.10	1.17
2001	587-4	*Alternaria solani*	Browerville, MN	1.21	0.10	0.10
2001	587-5	*Alternaria solani*	Dawson, ND	1.09	0.10	0.10
2001	588-1	*Alternaria solani*	Kearney, NE	1.14	0.10	0.10
2001	588-2	*Alternaria solani*	Kearney, NE	1.19	0.11	0.10
2001	589-1	*Alternaria solani*	Kearney, NE	1.15	0.10	1.06
2001	590-1	*Alternaria solani*	Pettibone, ND	1.15	0.34	0.10
2010	1179-3	*Alternaria solani*	Pettibone, ND	1.1467	0.1	0.1

Year	Isolate	Species	Location	AP and PP EC_{50} (µg/ml)		
				Pyrimethanil	Cyprodinil	Fludioxonil
2011	1184-14	*Alternaria solani*	Acequia, ID	1.12235	0.1	0.1
2013	1332-6	*Alternaria solani*	Dalhart, TX	1.2235	0.1	0.1

APPENDIX L. SUMMARY OF STATISTICAL ANALYSIS FOR *ALTERNARIA* SPP. IN VITRO SENSITIVITY ASSAYS

Table L1. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria alternata* baseline isolate EC_{50} values for pyrimethanil, cyprodinil, and fludioxonil

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.004	0.00	0.9583
Fungicide	2	23.142	15.7	<0.0001
Trial x fungicide	2	0.838	0.57	0.5673

Table L2. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria arborescens* baseline isolate EC_{50} values for pyrimethanil, cyprodinil, and fludioxonil

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.032	0.16	0.6915
Fungicide	2	0.808	4.02	0.0288
Trial x fungicide	2	0.113	0.56	0.7338

Table L3. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria tenuissima* baseline isolate EC_{50} values for pyrimethanil, cyprodinil, and fludioxonil

Source of Variation	Degrees of Freedom	Mean Square Error	F-value	Pr > F
Trial	1	0.342	0.12	0.7334
Fungicide	2	25.078	8.57	0.0004
Trial x fungicide	2	0.0524	0.02	0.2939

Table L4. Combined analysis of variance for in vitro fungicide sensitivity of the *Alternaria solani* baseline isolate EC_{50} values for pyrimethanil, cyprodinil, and fludioxonil

Source of Variation	**Degrees of Freedom**	**Mean Square Error**	**F-value**	**Pr > F**
Trial	1	0.044	0.17	0.6813
Fungicide	2	42.618	162.85	<0.0001
Trial x fungicide	2	0.040	0.15	0.8584

APPENDIX M. SUMMARY OF STATISTICAL ANALYSIS FOR *ALTERNARIA SOLANI* IN VIVO SENSITIVITY ASSAYS: AP AND PP FUNGICIDES

Table M1. Combined analysis of variance of in vivo percentage disease control of *Alternaria solani* isolates provided by pyrimethanil

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	130.243	0.44	0.5070
Rep	2	76.861	0.26	0.7707
Fungicide concentration	3	28737.900	97.56	<0.0001
Rep x fungicide concentration	6	58.799	0.20	0.9765
Trial x fungicide concentration	3	69.330	0.24	0.8716
Trial x rep	2	49.527	0.17	0.8454
Trial x rep x fungicide concentration	6	68.989	0.23	0.9649

Table M2. Combined analysis of variance of in vivo percentage disease control of *Alternaria solani* isolates provided by cyprodinil

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	249.645	0.34	0.5619
Rep	2	49.575	0.07	0.9351
Fungicide concentration	3	34404.040	46.56	<0.0001
Rep x fungicide concentration	6	167.058	0.23	0.9678
Trial x fungicide concentration	3	109.093	0.15	0.9311
Trial x rep	2	93.772	0.13	0.8809
Trial x rep x fungicide concentration	6	50.762	0.07	0.9987

Table M3. Combined analysis of variance of in vivo percentage disease control of *Alternaria solani* isolates provided by fludioxonil

Source of variation	Degrees of Freedom	Mean Square Error	F value	Pr > F
Trial	1	11.274	0.06	0.8082
Rep	2	1.710	0.01	0.9911
Fungicide concentration	3	10470.310	54.88	<0.0001
Rep x fungicide concentration	6	18.482	0.10	0.9966
Trial x fungicide concentration	3	7.908	0.04	0.9887
Trial x rep	2	19.083	0.10	0.9049
Trial x rep x fungicide concentration	6	7.897	0.04	0.9997