BIOLOGY AND MANAGEMENT OF *FUSARIUM* SPECIES ON SUGAR BEET

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ABSTRACT

Minnesota and North Dakota together produce about 51% of the beet sugar in the United States of America. Fusarium diseases caused by *Fusarium oxysporum* f. sp. *betae* and *F. secorum* on sugar beet cause significant reduction in both root yield and sucrose concentration. This research was conducted to determine the best inoculation methods to induce Fusarium diseases on sugar beet seeds and plants and to evaluate fungicides for their efficacy at controlling Fusarium diseases in greenhouse conditions. The use of Fusarium colonized barley seeds in close proximity to sugar beet seeds and seedlings caused similar level of disease severity as the standard root-dipping method, and reduced the time for evaluation by directly inoculating seeds and 4-leaf stage plants rather than using older plants which have to be transplanted into new pots. Pydiflumetofen and metconazole fungicides used in-furrow have the potential to provide effective control of Fusarium diseases on sugar beet.

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CHAPTER ONE. LITERATURE REVIEW

Sugar beet history

Sugar can be classified into three groups: monosaccharides such as glucose, fructose, galactose, and ribose, disaccharides including sucrose, lactose, and maltose, and polysaccharides such as starch, glycogen, and cellulose. Sucrose is a disaccharide and is composed of two monosaccharides: glucose and fructose. Since sucrose is the common sugar that humans consume, sugar is commonly referred to sucrose.

Sucrose is obtained mainly from two crops: sugar cane (*Saccharum officinarum* L.) and sugar beet (*Beta vulgaris* L.). Sugar cane has been planted since 1000 BC in the tropical and sub-tropical regions of the world and accounts for 80% of sugar production worldwide (FAO, 2009). The top five sugar cane producers are Brazil, India, China, Thailand, and Pakistan (FAO, 2015). Compared to sugar cane, sugar beet is a relatively new source of sucrose in temperate regions and has been regularly cultivated since the 17th century (Sneep et al., 1979). In 2014, France was the largest sugar beet producer, followed by Russia, Germany, the United States, and Turkey (FAO, 2015).

The ancestor of sugar beet is considered to be the wild sea beet (*B. vulgaris* ssp. *Maritima*) which grows on the coasts of the UK, mainland Europe, and North Africa. In the beginning, people treated beets as vegetable and garden plants. In the 17th century, beets were cultivated as a field crop and used as fodder for cattle in France and Germany (Francis, 2006).

The foundation of the modern sugar beet industry was the discovery of crystallized sugar in sugar beet juice by Andreas Sigismund Marggraf in 1747. However, his discovery was not recognized by the public. Forty years later, Franz Carl Achard— Marggraf's student, who is recognized as the 'Father of the beet sugar industry', developed the industrial process of extracting

sugar from White Silesian beets and built the first beet sugar factory in 1801 at Cunern in Lower Silesia. The White Silesian beets, bred and cultivated by Achard himself, were described as white skin and flesh and having conical shape with the unique characteristic of high concentration of sucrose. The sugar beet industry flourished during the Napoleonic Wars. Since cane sugar that was produced in France's tropical colonies could not be shipped to France because all the imports to France were cut off by Great Britain, Napoleon provided financial support to promote local sugar beet industry in France from 1811 to 1813. Unfortunately, the sugar beet industry did not do well with the fall of Napoleon's empire. The second development of the beet sugar industry benefitted from the improvement of sugar extraction techniques and government's policy for sucrose (Winner, 1993; Francis, 2006).

In the early 18th century, the United States made several attempts to develop a beet sugar industry but failed due to the lack of techniques. In 1870, the first successful sugar factory was built in Alvarado, California by E. H. Dyer, which was soon followed by factories in Nebraska and Utah. Since the policy preferred to protect the domestic industry at that time, the beet industry developed rapidly in the United States (Winner, 1993).

In 2014, 266.8 million tons of sugar beet were produced worldwide. The United States produced 28.7 million tons which is 10.6% of the world sugar beet production (FAO, 2015). There are ten states which produce sugar beet in the United States, including Michigan, Minnesota, North Dakota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, and Oregon (USDA-ERS, 2016). The Red River Valley sugar beet-growing region, located in both Minnesota and North Dakota, is the largest compared to all other sugar beet producing regions in the United States. In the Red River Valley, the first beet sugar factory was built in East Grand Forks in 1926 after World War I. Later, six sugar beet processing factories were built. These seven factories currently belong

to three grower-owned cooperatives: American Crystal Sugar Company, Minn-Dak Farmers Cooperative, and Southern Minnesota Beet Sugar Cooperative. In 2015, the Red River Valley accounted for 56% of the sugar beet grow acreage and contributed 51% of the nation total sugar beet production (USDA-ERS, 2016).

Sugar beet diseases

Sugar beet biotic disease can be caused by bacteria, fungi, viruses, nematodes, rickettsias, oomycetes, parasitic plants, and arthropods (Hanson, 2009). In the Red River Valley, the common diseases on sugar beet are Rhizomania (Vector—*Polymyxa betae* Keskin, Virus—*Beet necrotic yellow vein virus* (BNYVV)), Aphanomyces root rot (*Aphanomyces cochlioides* Drechsler), Cercospora leaf spot (*Cercospora beticola* Saccardo), Rhizoctonia crown and root rot (*Rhizoctonia solani* Kühn), Fusarium yellows (*Fusarium oxysporum* f. sp. *betae* Snyder and Hansen), and Fusarium yellowing decline (*F. secorum*).

Rhizomania. Rhizomania is a viral disease caused by BNYVV that is transmitted by zoospores of the soil-borne *P. betae* (Mark et al., 2006). BNYVV was first reported on sugar beet in 1952 (Canova, 1959). In the United States, BNYVV was first reported in California in 1984 (Duffus et al., 1984; Rush, 2009) and later was confirmed in the Southern Minnesota Beet Sugar Cooperative growing area by Wisler et al. (1997) in 1996. Damages caused by this disease are reduced yield, sugar content and purity, and estimated economic loss can reach 50-60% (Asher, 1993). The disease symptoms are massive fine secondary roots, light brown discoloration in the central stele part of the root, and fluorescent yellow appearance of leaves (Rush, 2009). The most effective way to manage this disease is by planting Rhizomania resistant varieties (Asher, 1993; Mark et al., 2006; Rush, 2009). Most sugar beet seed companies have Rhizomania resistant varieties that are available for commercial use (Niehaus, 2016).

Aphanomyces root rot. Aphanomyces root rot caused by *A. cochlioides* was first found in the Red River Valley in the 1980s and became a major disease in the 1980s and 1990s. *A. cochlioide*s can infect anytime during the sugar beet growing season. It causes postemergence damping-off when plants are infected during the seedling stage and root rot when sugar beet plants get older. Root symptoms are present as water-soaked lesions with a tan-yellow color, and further develop as stunting and rotting of root tip. Under favorable plant growing conditions, infected plants may recover. The use of resistant varieties and seed treatment with hymexazol (Tachigaren, Mitsui Chemicals Agro, Inc.) may be used to control *A. cochlioider* (Windels and Harveson, 2009). Also, using precipitated calcium carbonate which is incorporated into the soil at least several months before planting can reduce Aphanomyces root rot (Windels et al., 2009).

Rhizoctonia crown and root rot. *Rhizoctonia solani* Kuhn (teleomorph, *Thanatephorus cucumeris* (A. B. Frank) Donk) is considered the biggest threat to sugar beet production in North Dakota and Minnesota (Carlson et al., 2012). Symptoms include damping-off that occurs on post-emergence seedlings, crown rot associated with hilling practice to remove weeds, and root rot that develops late in the season (Windels et al., 2009). This disease could be destructive with significant yield loss if not managed (Khan et al., 2010; Windels and Brantner, 2005). *R. solani* consists of different genetically isolated populations called anastomosis groups (AGs) (Ogoshi, 1987). In North Dakota and Minnesota areas, the frequently isolated strains are *R. solani* AG 2-2 IIIB and AG 2-2 IV (Brantner and Windels, 2007), while the former is more aggressive than the latter (Bolton et al., 2010). An integrated approach is used to manage *R. solani*. This include the use of tolerant varieties, fungicide seed treatment, and timely applicate of post-emergent fungicides (Khan, personal communication). No commercial cultivar immune to this disease is available, research has shown that resistant cultivars have significant lower disease severity than susceptible

ones (Liu and Khan, 2016; Noor and Khan, 2014). Seed treatments including Kabina ST (penthiopyrad, Mitsui Chemicals, Tokyo, Japan), Systiva XS (fluxapyroxad, BASF; Research Triangle Park, NC, USA), and Vibrance (sedaxane, Syngenta, Greensboro, NC, USA) are registered to manage *R. solani* (Khan, 2017). Applying azoxystrobin fungicides such as Quadris (Syngenta; Greensboro, NC, USA) while planting resistant cultivars can be an optimal practice in the sugar beet field with *R. solani* history (Khan, 2017).

Cercospora leaf spot. Cercospora leaf spot (CLS) is the most destructive foliar disease on sugar beet (Jacobsen and Franc, 2009; Skaracis et al., 2010). Under favorable conditions, losses caused by CLS can reach 40% (Jacobsen and Franc, 2009). In a 1998 epidemic, the estimated loss due to CLS was $40 million at American Crystal Sugar Company (Ellington et al., 2001). CLS was first reported by Saccardo, P. A. in 1876 (Chupp. 1953). In the United States, it was first reported in 1895 by Halsted (1895). Symptoms caused by *Cercospora beticola* Sacc. on sugar beet leaf are circular spots with a grey center and red-purple margins. As the disease progresses, spots coalesce and the entire leaf becomes necrotic and collapses, but remain attached to the plant (Weiland and Koch, 2004). Black pseudostromata are the overwintering structure that are located on the grey center, which germinate and produce conidia under favorable conditions (Ruppel, 1986). Resistant cultivars, crop rotation, and fungicide applications are the main methods used to manage CLS on sugar beet. Since CLS is a polycyclic disease, three to four fungicide applications are needed for one growing season (Secor et al., 2010). Fungicide-resistance management is also needed to reduce selection pressure (Windels, 2010). The methods used for fungicide-resistant management are mix, rotate, or base on prediction models to apply fungicides from different Fungicide Resistance Action Committee (FRAC) groups (Lamey et al., 1996). The classes of

fungicide registered on sugar beet to control CLS are quinone outside inhibitor (QoI), trazole (DMI), dithiocarbamate, triphenyltin hydroxide (TPTH), benzimidazole, mancozeb, and copper.

Fusarium diseases. *Fusarium* species can cause several different kinds of diseases on varieties sugar beet growing stages. *F. oxysporum* f. sp. *radicis-betae* can cause sugar beet root rot (Franc et al., 2001), while *F. oxysporum* f. sp. *betae* causes Fusarium yellows on sugar beet (Ruppel, 1991). *F. solani* was reported to cause both seedling damping-off and root rot on old plant (Abada, 1994). Also, Ruppel (1991) reported that *F. oxysporum*, *F. moniliforme*, *F. acuminatum*, and *F. avenaceum* can cause seedling yellows and seedling wilt; However, *F. oxysporum* f. sp. *betae* is the most devastating pathogen among *Fusarium* species mentioned above. Now, *Fusarium* species cause moderate to severe disease in the fields in the Moorhead and Crookston factory districts of American Crystal Sugar Company in the Red River Valley (Fig. 1.1).

Fusarium yellows

Disease history. *F. oxysporum* f. sp. *betae* (Steward) Snyd. and Hans. is the causal agent of Fusarium yellows. Stewart first described Fusarium yellows in 1931 (Stewart, 1931) and identified the causal agent as *F. conglutinans* var. *betae*, which was then reclassified as *F. oxysporum* f. sp. *betae* (Ruppel, 1991). Fusarium yellows has been reported at least in eight sugar beet-producing states of the United States (Harveson and Rush, 1997). It was also recently reported in Michigan (Hanson, 2006) and the Red River Valley of Minnesota and North Dakota (Windels et al., 2005). It was also reported in Germany, Iran, Belgium, India, Russia, and the Netherlands (Hanson and Jacobsen, 2009).

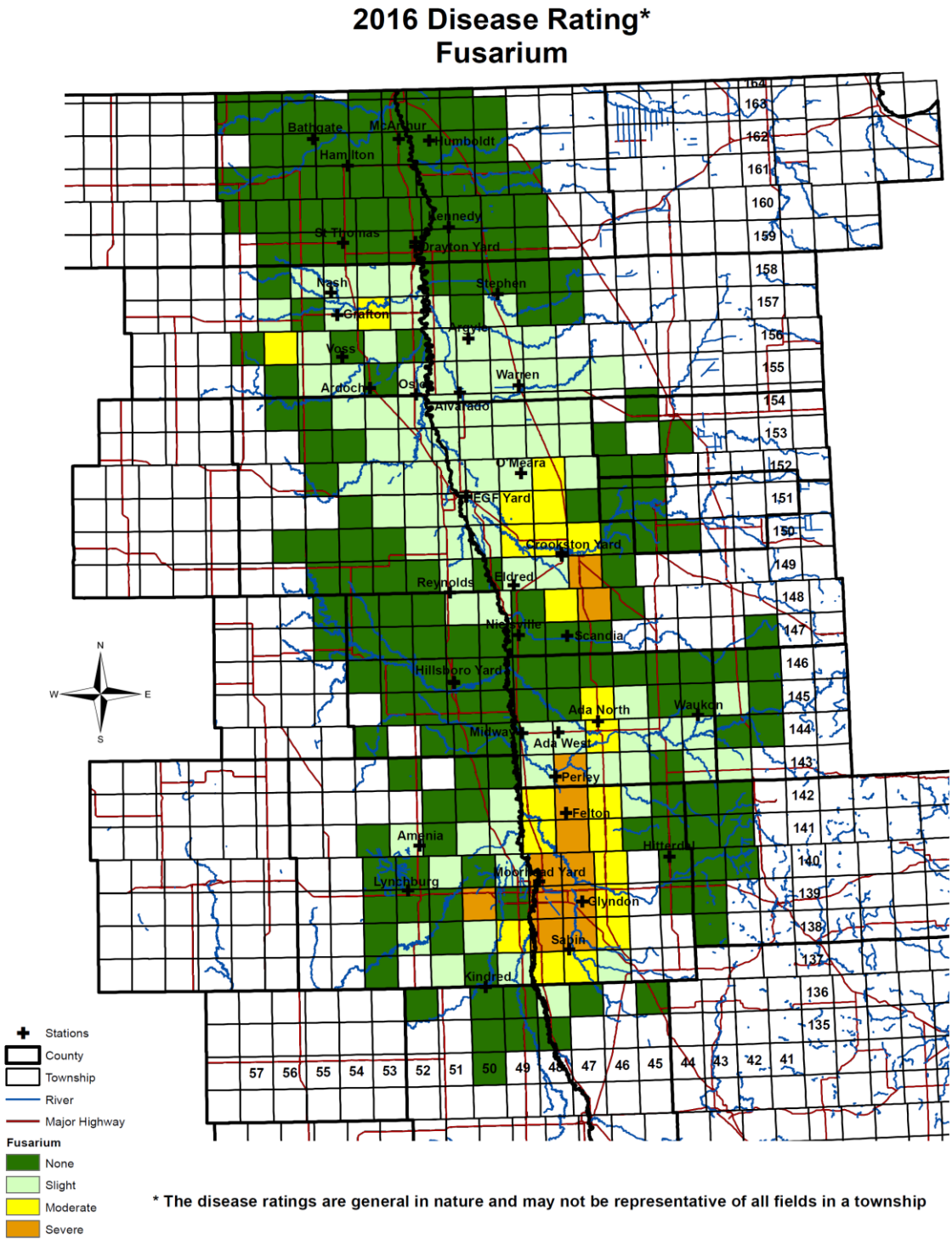

Figure 1.1: Map showing level of Fusarium in sugar beet field in the Red River Valley of Minnesota and North Dakota in 2016. https://www.crystalsugar.com/media/367934/fusarium-map-2016.pdf.

Morphology. *F. oxysporum* f. sp. *betae* is able to produce abundant macroconidia, microconidia, and chlamydospores on carnation leaf agar (CLA). However, the sexual stage is unknown. Macroconidia (3.5-5.5 × 21-35 µm) are formed from monophialides both on branched conidiophores and on hyphae. Macroconidia usually have 3-septate, slender, thin wall, and are straight to slightly curved. The basal cell is foot shaped to pointed, and the apical cell is tapered and curved, sometimes with a slight hook (Leslie and Summerell, 2006). Microconidia (2.5-4.5 × 6-15 µm) are formed in false heads on short monophialides. Microconidia usually has 0-septate, and are oval, kidney or elliptical shaped (Leslie and Summerell, 2006). Chlamydospores (7-11 µm) are formed singly or in pairs from either intercalary or terminal in aerial, surface, or submerged hyphae. Under unfavorable conditions, both hyphae and conidia can convert into chlamydospores (Alexopoulous et al., 1996; Leslie and Summerell, 2006). *F. oxysporum* f. sp. *betae* also produces pale to dark violet pigment in potato dextrose agar (PDA) (Leslie and Summerell, 2006). This pathogen can produce Fusaric acid that is toxic to host plants, causing symptoms of wilting and yellowing. *F. oxysporum* f. sp. *betae* isolated from sugar beet was found to produce mycotoxin trans-zearalenone (ZEA) (Bosch and Mirocha, 1992).

Disease cycle and symptoms. *F. oxysporum* f. sp. *betae* is a soil borne fungus that can survive as chlamydospores, macroconidia, and mycelium in plant debris. Under favorable conditions, *F. oxysporum* f. sp. *betae* starts to penetrate susceptible sugar beet root through wounds and moves toward the vascular system. Once this fungus successfully colonizes the plant, it moves upwards with the appearance of foliar symptoms. Initially, yellowing appears in the old leaves between the main veins. As the disease develops, the old yellow leaves become necrotic, while young leaves start to show yellowing. Yellowing may occur on the entire leaf or just half of the leaf. Some diseased plants may show wilting during the daytime and recover with watering or

overnight. The infected root vascular system shows a grayish brown discoloration. This discoloration may occur in the entire root vascular system or only on the area where the pathogen has penetrated. Finally, the whole leaves become dry and die, but remain attached to the plant. Seedlings may die if infection occurs early, while old plants can stay alive with reduced yield. This disease was reported to significantly reduce sugar beet yield, sugar purity, and recoverable sucrose percentage. At the end of growing season, the survival structure of this fungi returns to the soil and serves as initial inoculum in the next growing season (Draycott, 2006; Hanson and Jacobsen, 2009; Khan et al., 2009).

Another sugar beet pathogen, *F. oxysporum* f. sp. *radices-betae*, could be confused for *F. oxysporum* f. sp. *betae* because these two pathogens cause similar symptoms. Actually, the two pathogens are genetically distinct and the symptoms remain distinct when the environmental conditions change. The pathogen causing disease on sugar beet was redesignated as Fusarium root rot to distinguish it from Fusarium yellows. The major difference used to distinguish it from *F. oxysporum* f. sp. *betae* is that *F. oxysporum* f. sp. *radices-betae* can induce cortical root rot (Harveson and Rush,1997; Harveson and Rush, 1998).

Fusarium yellowing decline

Disease history. In 2005, a sugar beet pathogen was isolated from sugar beet plant in the Moorhead factory district field and in American Crystal Fusarium nursery in Sabin, MN (Burlakoti, 2007). Although this pathogen causes Fusarium yellows-like symptoms, it is also responsible for seedling infection, petiole vascular discoloration, and rapid death early in the growing season, which are distinct from Fusarium yellows (Burlakoti et al., 2012). Unlike other *Fusarium* species only isolated from taproots of sugar beet, this pathogen can be isolated from petioles. This disease is more aggressive than *F. oxysporum* f. sp. *betae* (Burlakoti et al., 2012). To differentiate this

disease from Fusarium yellows, it was named Fusarium yellowing decline (Rivera et al., 2008). Villamizar-Ruiz (2013) found that only canola and sugar beet are the hosts of *F. secorum* in the field, but infected canola did not show any symptoms.

***F. secorum*.** A new causal agent of Fusarium disease described above was found by Secor et al. (2014). Based on morphology and phylogenetic analysis, this new *Fusarium* strain was identified as *Fusarium secorum* and belongs to *Fusarium fujikuroi* species complex (FFSC). *F. acutatum* has the closest relationship with *F. secorum*. However, sugar beet is not a host of *F. acutatum* (Secor et al., 2014).

Morphology. *F. secorum* is able to produce microconidia, macroconidia, and chlamydospores. Microconidia (0-2 septates) are abundant even in young culture. Macroconidia (3-5 septates) are rarely produced on five weeks old cultures. Chlamydospores are subglobose and formed from intercalary or terminal mycelia. In addition, *F. secorum* produces frequently corkscrew-shaped circinate hyphae on CLA and 50% PDA. On PDA, *F. secorum* colonies are white color when they grow without light. Once the culture is exposed under fluorescent light, the color changes to light orange to pink with a white growing edge. No odor and sclerotia are detected from PDA cultures. The only mycotoxin produced by *F. secorum* is beauvericin (Secor et al., 2014).

Symptoms. *F. secorum* initiates infection on root, then develops upwards to petiole, and eventually moves to leaf. Fungal material was detected in sugar beet root, petiole, and leaves (Bian, 2015). It causes both root and petiole vascular discoloration and interveinal chlorosis on whole or half leaves. Disease symptoms first occur on old leaves and later moves on to young leaves. As disease develops, wilted and yellow leaves become scorched and eventually die. Dead leaves remain attached to plants. One characteristic of this disease also includes seedling infection. Since

this pathogen is aggressive, seedlings or young plants may die rapidly during early growing season (Burlakoti et al., 2012; Rivera et al., 2008; Secor et al., 2014).

Management

In the Red River Valley, both *F. oxysporum* f. sp. *betae* and *F. secorum* are present. There is no effective fungicide to control these two soilborne pathogens in the field. The most economic method to manage these two diseases is to use resistant varieties such as BTS 8500, BTS 8536, Crystal 574RR, Crystal D518, and Maribo MA502 (Khan et al., 2009; Niehaus, 2016).

Resistant varieties. The most efficient way to manage *Fusarium* species on sugar beet is to use resistant varieties (Burlakoti, 2007). Unfortunately, there is no immune variety currently available (Biancardi, 2005). Harveson and Rush (1994) found that Rhizomania resistant varieties were susceptible to Fusarium yellows. The American Crystal Company has included Fusarium resistance breeding since 2005 in North Dakota and Minnesota (Niehaus, 2005). Now, Fusarium resistant varieties are available in the Red River Valley. However, the resistance levels are vary from seed company to company.

Cultural practices. Cultural practices aim to reduce the pathogen population below the economic threshold. Crop rotation with non-host crops can be used to manage Fusarium diseases to reduce inoculum build-up in the field. However, the use of crop rotation is limited because *F. oxysporum* f. sp. *betae* has a wide host range and can survive as chlamydospores for many years (MacDonald and Leach, 1976).

Weeds such as Lambsquaters and pigweed are also hosts of *F. oxysporum* f. sp. *betae* in field. Villamizar-Ruiz (2013) found that Green foxtail may be the potential symptomless host of *F. secorum* in a greenhouse trial. Weeds can act a reservoir that helps pathogens survive and

increase its population. This makes removal of the weeds a necessary practice in the sugar beet field or field rotated with sugar beet.

Young sugar beet plants are more prone to infection than older plants. As such, early planting in cool soil can help to reduce the incidence of disease infection. Early harvesting and fast processing are also needed to prevent disease development. Avoiding movement of infested equipment and soil to non-infested areas may be used to prevent disease spread (Khan et al., 2009).

Biological control. Not all strains of *F. oxysporum* are plant pathogens. Some strains can compete for nutrients and growing space with pathogenic *Fusarium*, resulting in the biocontrol agent (Fravel and Alabouvette, 2003; Park et al., 1988). Several studies showed that *Bacillus megaterium*, *Burkbolderia cepacia*, *Trichoderma* spp., *Gliocladium virens*, and *Pseudomonas fluorescens* WCS365 are able to manage Fusarium wilt on tomato (Bolwerk et al., 2003; Dekkers et al., 2000; Larkin and Fravel, 1998). However, most biocontrol agents are limited to *in vitro* trial or greenhouse study.

Chemical control. There is no fungicide which is effective at controlling Fusarium diseases on sugar beet (Khan et al., 2009). However, it was reported that fumigants can control Fusarium diseases; in Texas, Harveson and Rush (1994) reported that Telone II effectively managed Fusarium root rot on sugar beet; Baker (1980) reported that methyl bromide also effectively suppressed *F. oxysporum* f. sp. *Dianthi*, the causal agent of Fusarium wilt on carnation. Unfortunately, methyl bromide was banned in 2005 because it damages the ozone layer and harm humans (Ristaino and Thomas, 1997; Schneider et al., 2003). Currently, metconazole, tebuconazole, and prothioconazole were able to control Fusarium head blight (FHB) caused by *F. graminearum* and reduce mycotoxin deoxynivalenol (DON) production on wheat (Edwards et al., 2001; Magan et al., 2002; Mesterhazy et al., 2003; Paul et al., 2008). Burlakoti (2010) found

thiabendazole, triticonazole, and metoconazole can suppress both *F. oxysporum* f. sp. *betae* and *F. secorum* growth in vitro. However, no fungicide has been shown to be effective in field conditions.

One objective of this research is to develop a more efficient inoculation method for both *F. secorum* and *F. oxysporum* f. sp. *betae*. Also, this research evaluates novel fungicides to determine their efficacy at controlling Fusarium diseases in greenhouse conditions.

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CHAPTER TWO. EVALUATE DIFFERENT INOCULATION METHODOLOGY FOR INFECTING SUGAR BEET WITH *FUSARIUM OXYSPORUM* F. SP. *BETAE* AND *F. SECORUM*

Introduction

Sugar beet (*Beta vulgaris* L.) is a major source of sucrose, especially in temperate region (FAO, 2009). The United States was the No. 4 sugar beet producer in the world in 2014 (FAO, 2015). Sugar beet was produced in ten states across the country. Minnesota and North Dakota accounted for 56% sugar beet grown and contributed 51% of the total sugar beet production in the USA (USDA-ERS, 2016). Diseases caused by *Fusarium* spp. on sugar beet may reduce yield and extractable sucrose (Hanson and Jacobsen, 2009).

In the Red River Valley, there were two *Fusarium* spp.: *F. oxysporum* f. sp. *betae* (D. Stewart) W.C. Snyder and H.N. Hansen and *F. secorum* are the pathogens most consistently associated with Fusarium diseases on sugar beet (Khan et al., 2009). Fusarium yellows caused by *F. oxysporum* f. sp. *betae* was first reported in the Red River Valley in 2002 (Windels et al., 2005). Symptoms characteristic of this disease are interveinal chlorosis, taproot vascular-discoloration without external symptoms, and foliar wilting. In 2005, a new disease Fusarium yellowing decline, caused by *F. secorum* was first reported by Rivera et al. (2008) in Minnesota (Secor et al., 2014). The difference between *F. oxysporum* f. sp. *betae* and *F. secorum* is that only the *F. secorum* causes seedling death, yellowing during early growing season, and petiole vascular discoloration (Burlakoti, 2012).

It is necessary to develop effective artificial inoculation methods for the identification of sources of resistance, host-parasite interactions, and studies on disease control (Das and Patil, 2015). The root-dipping inoculation method has been the standard method for evaluating reaction

of *F. oxysporum* which affect several plant species including chickpea, tomatoes, cotton, and cucumber (Dowd et al., 2004; Maitlo et al., 2016; Rowe, 1980; Williams, 1996). Root-dipping method has also been used to evaluate *F. oxysporum* f. sp. *betae* on sugar beet (Hanson, 2006). The same inoculation method has been used to study the effect of *F. secorum* on sugar beet (Burlakoti, 2007; Rivera et al., 2008). The root-dipping inoculation method causes damage to roots and allows the pathogen to invade plants through wounds and possibly avoids a natural barrier at the epidermis (Eynck et al., 2009). It will be useful to have alternative inoculation methods which do not result in wounding of root which will simulate natural conditions.

Materials and methods

Fungal isolates. Isolates used for this study were known pathogenic isolates *F. oxysporum* f. sp. *betae* F-19, which was isolated from Salem, Oregon in 2001, provided by the USDA-ARS Sugarbeet Research Unit, Fort Collins, CO., and *F. secorum* 784-12-4, which was isolated from Sabin, Minnesota in 2007, provided by Dr. G. A. Secor, North Dakota State University, Fargo, ND.

Inoculum preparation. Liquid and solid substrates were used. Liquid cultures were prepared using CMC (carboxymethylcellulose) medium. One liter of CMC medium contains 15 g of carboxymethylcellulose sodium salt (Sigma-Aldrich, USA), 1g of ammonium nitrate (ACS reagent, ≥98%; Sigma-Aldrich, USA), 1g of potassium phosphate monobasic (Sigma-Aldrich, USA), 0.5g of magnesium sulfate heptahydrate (ACS reagent, ≥98%; Sigma-Aldrich, USA), and 1g of yeast extract (Sigma-Aldrich, USA). All chemicals were dissolved in one liter distilled water and autoclaved at 170 kPa and 120 °C for 20 minutes. Fungal cultures were prepared by transferring hyphae from long term storage vial into 100 × 15 mm petri dishes (Falcon, USA) containing full strength PDA (potato dextrose agar; Sigma-Aldrich, USA), and incubating them

under fluorescent light at room temperature (24 °C) for one week. Erlenmeyer flasks containing 200 ml of CMC medium was inoculated with 20 pieces 5 mm long square plugs containing actively growing hyphae. The inoculated CMC medium was placed in a rotary shaker (Thermo Scientific MaxQ Shakers, USA), and incubated at 210 rpm under soft white fluorescent light at 25 °C. After 7 days, the CMC medium was passed through 2-layers of miracloth (Calbiochem, EMD Millipore Corporation, Billerica, USA) to collect spores. A hemacytometer (Propper Manufacturing Co., Inc., USA) was used to estimate the concentration. The spore suspension was adjusted to 5×104 spores/ml with distilled water and used immediately.

Barley seeds (non-treated) were used as a solid substrate. Fusarium infested barley inoculum were produced following the same method used for producing Rhizoctonia solani-infested barley grains (Kirk et al., 2008; Noor and Khan, 2014). Mixtures of 4.8 g potato dextrose broth (PDB; Sigma-Aldrich, USA), 200 ml barley, and 120 ml distilled water (the volume ratio is barley: distilled water=5:3) were placed into 500-ml flasks (Pyrex, USA) and autoclaved at 170 kPa and 120 °C for 30 minutes, and left to cool to room temperature overnight. The initial inoculum was grown on PDA as described above, cut into 3 mm square plugs and transferred into autoclaved flasks containing barley. One flask of barley was inoculated with plugs from one petri dish. Inoculated flasks were sealed, mixed every two days by hand-shaking, and incubated at room temperature for two weeks and then air dried under a laminar flow hood for 2-days. The air dried barley grains were stored at 4 °C until used. Colony forming units (CFU) were calculated for each isolate by grinding 50 grains in 100 ml autoclaved distilled water for 5 minutes using a lab blender. Ten-fold serial dilutions, $1\text{-}10^{-3}$ were prepared and three 100 µl samples for each concentration were plated into 100 × 15 mm PDA plates. The number of CFU was estimated after 24 h incubation at room temperature.

Sugar beet plants. This study was conducted in a greenhouse (Argus Control Systems, Ltd.; British Columbia, Canada) of the Agricultural Experiment Station of North Dakota State University in Fargo, ND, USA. Three seeds of *Fusarium*-susceptible variety Maribo 409 (Niehaus, 2015) were planted in 10 × 10 × 12 cm plastic pot (T. O. Plastic Inc.; Clearwater, MN, USA) filled with Sunshine mix 1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada). One teaspoon fertilizer of Osmocote 15-9-12 (3-4 months' formula) (Everris NA Inc., Dublin, OH, USA) was added and mixed to each pot before seeding. One-week after planting, seedlings were thinned to one plant per pot. The greenhouse conditions were set to an average temperature of 24 °C and 16-hour photoperiod. Plants were watered as needed. Three-weeks old sugar beet plants (4-leaf stage) were used for inoculation.

To identify the most effective inoculation methods, root-dipping, drench without injury, drench with injury, Fusarium colonized barley seeds placed next to sugar beet plants, and Fusarium colonized barley seeds placed next to sugar beet seeds at planting were evaluated. After inoculation, all the plants were kept in the greenhouse set at a temperature of 24 °C and 16-hour photoperiod, and watered as needed. There were six replicates for each isolate. This experiment was repeated once using a completely randomized design (CRD).

Root-dipping (root-dipping). Three-weeks old plants were carefully removed from the pots. Roots were washed with distilled water, dried with tissue paper, and soaked in *Fusarium* spore suspension (5×10^4 spores/ml) for eight minutes (Hanson and Hill, 2004). A 10^{-2} dilution of CMC medium in distilled water was used as control. After inoculation, plants were transplanted into wet plastic pots as described above. Inoculated plants were not watered until next day. Old-yellow leaves were removed three days after inoculation (Hanson and Hill, 2004). One cup of soluble fertilizer 20-20-20 (JR Peters, Inc., Allentown, PA, USA) was dissolved in five-gallon of

water and added at two and four weeks after inoculation, one cup of dissolved liquid fertilizer per pot.

Drenching without injury (drenching). Inoculation was conducted by directly and evenly pouring 20 ml *Fusarium* spore suspension (5×10^4 spores/ml) on to the soil surface of pots containing one three-weeks old plant each (Maitlo et al., 2016). Control pots had distilled water poured instead of spore suspension. Inoculated plants were not watered until next day.

Drenching with injury (cutting). To injure three-weeks old sugar beet root, two longitudinal cuts with 10 cm deep were made about 1.3 cm away from opposite side of the root using a knife. These two cuts were parallel to each other. Inoculation was performed the same way as drench inoculation without injury. The control was inoculated with distilled water with longitudinal two cuts on the opposite side of the root. Inoculated plants were not watered until next day.

Fusarium colonized barley seeds placed next to sugar beet plants (barley to root). Inoculation was conducted by placing one *Fusarium* colonized barley seed 1 cm away from root and 2 cm deep from soil surface and then covered with Sunshine mix 1 peat for each sugar beet plant (Liu and Khan, 2016). Control used sterilized barley seed without *Fusarium* infection. Inoculated plants were not watered until next day.

Fusarium colonized barley seeds placed next to sugar beet seeds at planting (barley to seed). For this inoculation method, 28 ×12 × 12 cm plastic trays were used and fertilizer was added when potting. Ten sugar beet seeds were planted in 2 cm deep furrows and one *Fusarium* colonized barley seed was placed 1 cm to the side of each sugar beet seed and the inoculum and seeds were then covered with Sunshine mix 1 peat (Liu and Khan, 2016). Control used sterilized barley seed that was not inoculated with the pathogen. Trays were watered after inoculation.

Disease evaluation and data analyses. Disease evaluation was based on Fusarium yellows and Fusarium yellowing decline symptoms. The severity scale used in the study was as follows (Fig. 2.1) 0 = no disease; 1 = leaves wilted, small chlorotic areas on lower leaves, most of leaf green; 2 = leaves showing interveinal yellowing; 3 = leaves with small areas of necrosis or becoming necrotic and dying, less than half of the leaves affected; 4 = more than half of leaves dead, plant stunted, most living leaves showing symptoms; 5 = plant death (Hanson et al., 2009). Disease incidence and severity were taken every week for five weeks after inoculation.

Five weeks after inoculation, plants were carefully removed from pots, washed under tap water, and roots were longitudinally cut to check for discoloration on the vascular system. The severity scale used for root rating was as follows (Fig. 2.2) 0 = no internal browning; 1 = slight internal browning, usually at the tip of the tap root; 2= moderate to severe internal browning of the entire tap root; and 3 = severe internal browning extending from the tap root into the lower stem above the soil line (Rowe, 1980).

Levene's test was used to determine whether the data sets for incidence had homogeneous variances and could be combined for analyses. The data were analyzed by non-parametric method, using SAS (Version 9.4, SAS Institute Inc.; Cary, NC, USA) commands Proc Rank and Proc Mixed with module F2_LD_F1, LD_CI, and mult macro to separate treatments and obtain their confidence intervals (Shah and Madden, 2004).

Figure 2.1: Severity scale used for foliar rating of *Fusarium*-infected sugar beet plants.

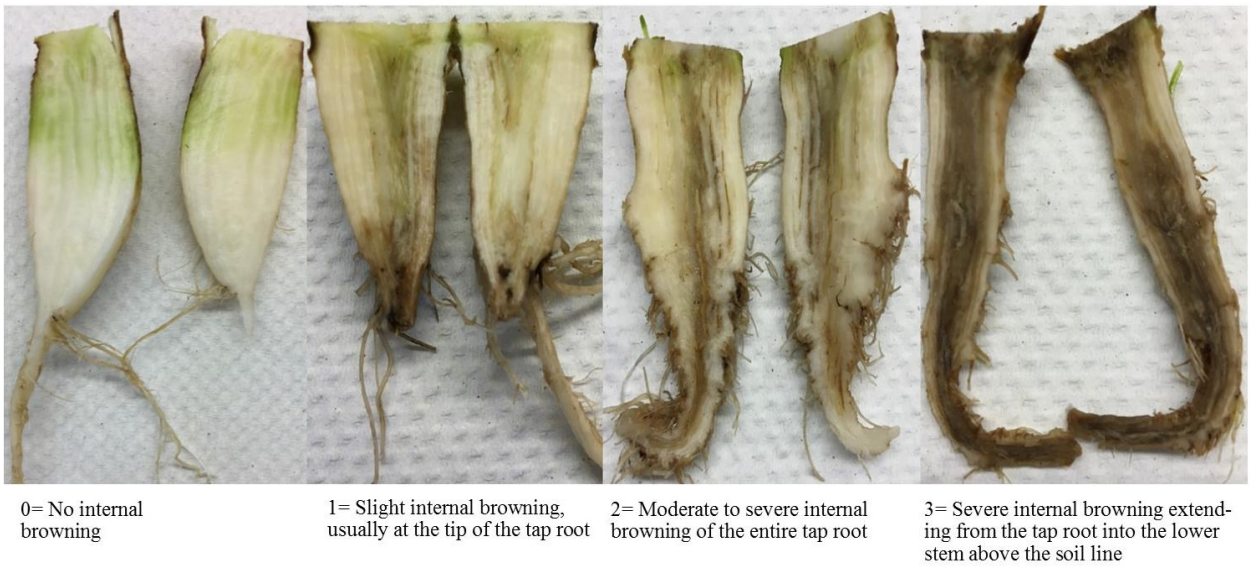

Figure 2.2: Severity scale used for root rating of *Fusarium*-infected sugar beet plants.

Results

The two runs of data for this experiment were combined because their homogeneity for variance were not significantly different (*P*-value = 0.6727). The negative controls for each inoculation method were without foliar or root symptoms of Fusarium yellows or Fusarium yellowing decline and were not included in data analyze.

In Table 2.1, sugar beet disease severity based on foliar symptom observation was significantly different across the two species and five inoculation methods. *F. oxysporum* f. sp. *betae* caused significantly higher disease severity on sugar beet than *F. secorum* (Fig. 2.3). Of the five inoculation methods, the most effective methods were barley to seed and root-dipping causing the highest disease severity, which were not significantly different from one another (Fig. 2.4). Cutting was between drenching and barley to root, but not statistically differ from these two methods. Figure 2.5 and table 2.3 showed the disease severity for all the treatments of inoculated sugar beet at 7, 14, 21, and 35 DAI (days after inoculation). For most of the treatments, leaf symptoms caused by two fusarium species were first observed at 14 DAI, except for the treatments with *F. secorum* using barley to root where symptoms were first observed at 21 DAI. Root-dipping, barley to root, and barley to seed had the similar high rate of the increase in foliar symptoms. For the barley to root inoculation method, the use of *F. secorum* resulted in significantly lower disease development than *F. oxysporum* f. sp. *betae*.

Root disease severity was significantly different between the two *Fusarium* species and among the five inoculation methods (Table 2.2). Among all the treatments, root-dipping and barley to seed with both species, and barley to root with *F. oxysporum* f. sp. *betae* resulted in the highest disease severities (Fig. 2.6, Table 2.4). Cutting with *F. oxysporum* f. sp. *betae* was not significantly

different from root-dipping and barley to seed methods with a lower infection on sugar beet roots. Drenching induced root symptoms, but was inconsistent (Fig. 2.6).

Discussion

The standard root-dipping method was the most effective inoculation method for both *Fusarium* species inoculation on sugar beet (Fig. 2.5; Fig. 2.6). Root-dipping method included soaking seedlings in spore suspension followed by transplanting. During this process, spores could directly get in contact with the damaged root system and lead to pathogens entering the vascular system through wounds. Therefore, root-dipping method allowed pathogen escape resistance mechanisms at the root epidermal level (Eynck et al., 2009; Michielse and Rep 2009). Studies showed *F. oxysporum* f. sp. *betae* could directly penetrate root epidermis by forming net-like hyphae and accumulating on the surface of root tip to reach a certain density, and then colonizing tissue intracellularly and intercellularly (Bishop and Cooper, 1983; Czymmek et al., 2007; Mendgen et al., 1996; Van Peer and Schippers, 1992). This also explains why drench inoculation without injury (drenching) and with injury (cutting) caused the same level of disease severity. However, these two inoculation methods caused significantly lower disease severity than the standard root-dipping method. Spore distribution in soil was limited by spore morphology and electrical charge, and soil physical features (Hepple, 1960; Wallace, 1978). Gracia-Garza and Favel's (1998) study showed spores were unevenly distributed in soil, and CFU at 0-2 cm depth was 10-times higher than at 8-10 cm depth. It is possible that, most of the spores applied in the drench remained on the surface and the top 2 cm of the soil and thus reduced the chance of spores getting in contact with sugar beet roots and thus resulted in low disease severity.

Barley inoculum was used for study of *Rhizoctonia solani* on sugar beet (Gaskill, 1968; Kirk et al., 2008; Noor and Khan, 2014). By using barley inoculum with *Fusarium*, barley to seed

Table 2.1: Test statistics for the effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Effect	ANOVA A-type statistic (ATS)			
	df_N[b]	df_D[c]	ATS	*P*-value
Isolate	1	1	28.78	<0.0001***
Inoculation Method	3.35	1	27.98	<0.0001***
Isolate × Inoculation Method	3.35	4.09	5.54	0.0005***
Time	2.85	1	400.14	<0.0001***
Isolate × Time	2.85	1	11.03	<0.0001***
Inoculation Method × Time	7.52	1	9.15	<0.0001***
Isolate × Inoculation Method × Time	7.52	1	3.02	0.0028**

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.
** Significantly different at P ≤0.01
***Significantly different at P ≤0.001

Table 2.2: Test statistics for the effect of five different inoculation methods and two *Fusarium* species on disease severity of sugar beet root at 35 DAI[a].

Effect	ANOVA A-type statistic (ATS)			
	df_N[b]	df_D[c]	ATS	*P*-value
Isolate	1	47.9	49.54	<0.0001***
Inoculation Method	2.24	47.9	24.6	<0.0001***
Isolate × Inoculation Method	2.24	47.9	10.85	<0.0001***

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.

Table 2.3: Effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Inoculation method	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Dipping	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Dipping	14	4.00	3.00	373.33	332.66	0.621	0.554	0.562-0.677	0.510-0.596
Dipping	21	5.00	4.50	487.08	452.99	0.811	0.754	0.752-0.858	0.693-0.806
Dipping	28	5.00	5.00	501.75	482.24	0.835	0.803	0.801-0.865	0.750-0.846
Dipping	35	5.00	5.00	501.75	491.98	0.835	0.819	0.801-0.865	0.774-0.857
Drenching	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Drenching	14	0.00	0.00	146.63	134.60	0.244	0.223	0.196-0.300	0.186-0.266
Drenching	21	1.00	0.00	240.40	210.57	0.400	0.350	0.295-0.516	0.257-0.456
Drenching	28	2.00	1.50	328.01	272.01	0.546	0.452	0.403-0.681	0.366-0.542
Drenching	35	3.50	3.00	402.30	308.44	0.670	0.513	0.575-0.753	0.424-0.601
Cutting	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Cutting	14	0.00	0.00	134.59	149.15	0.223	0.248	0.186-0.267	0.195-0.309
Cutting	21	3.50	1.00	323.36	257.28	0.538	0.428	0.396-0.674	0.336-0.526
Cutting	28	5.00	3.00	367.30	280.68	0.611	0.467	0.434-0.762	0.378-0.558
Cutting	35	5.00	3.00	438.55	307.42	0.730	0.512	0.590-0.834	0.414-0.608

Table 2.3: Effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a] (continued).

Inoculation method	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Barley to root	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Barley to root	14	2.50	0.00	304.56	122.50	0.507	0.203	0.394-0.619	0.187-0.220
Barley to root	21	5.00	0.00	435.87	176.48	0.726	0.293	0.613-0.814	0.223-0.376
Barley to root	28	5.00	1.00	467.65	236.03	0.778	0.393	0.715-0.830	0.313-0.479
Barley to root	35	5.00	3.00	496.93	334.72	0.827	0.557	0.775-0.869	0.518-0.596
Barley to Seed	7	0.00	0.00	122.50	122.50	0.203	0.203	0.187-0.220	0.187-0.220
Barley to Seed	14	0.50	2.00	241.33	280.38	0.401	0.466	0.275-0.543	0.333-0.604
Barley to Seed	21	5.00	4.50	454.67	357.61	0.757	0.595	0.629-0.850	0.425-0.744
Barley to Seed	28	5.00	5.00	511.50	450.06	0.852	0.749	0.834-0.867	0.632-0.837
Barley to Seed	35	5.00	5.00	511.50	493.79	0.852	0.822	0.834-0.867	0.758-0.871

[a]DAI=days after inoculation

[b]MDS=median disease rating. Disease severity was evaluated every week for five weeks based a 0 to 5 scale: 0 (no disease), 1 (leaves wilted, small chlorotic areas on lower leaves, most of leaf green), 2 (leaves showing interveinal yellowing), 3 (leaves with small areas of necrosis or becoming necrotic and dying, less than half of the leaves affected), 4 (more than half of leaves dead, plant stunted, most living leaves showing symptoms), 5 (plant death).

[c]MR=mean rank

[d]REDS=relative effect of disease severity

[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

Table 2.4: Effect of five different inoculation methods and two *Fusarium* species on root disease severity of sugar beet at 35 DAI[a].

Inoculation method	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
	F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Dipping	3	3	80.50	80.50	0.667	0.667	0.641-0.691	0.641-0.691
Drenching	3	2	56.04	32.50	0.463	0.267	0.337-0.595	0.177-0.391
Cutting	3	2	63.63	27.04	0.526	0.221	0.392-0.656	0.149-0.327
Barley to root	3	1	80.50	23.29	0.667	0.190	0.641-0.691	0.122-0.302
Barley to Seed	3	3	80.50	80.50	0.667	0.667	0.641-0.691	0.641-0.691

[a]DAI=days after inoculation

[b]MDS=median disease rating. Sugar beet plants were hand harvested at 35 DAI and root disease severity was rated with a 0 to 3 scale: 0 (no internal browning), 1 (slight internal browning, usually at the tip of the tap root), 2 (moderate to severe internal browning of the entire tap root), and 3 (severe internal browning extending from the tap root into the lower stem above the soil line).

[c]MR=mean rank

[d]REDS=relative effect of disease severity

[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

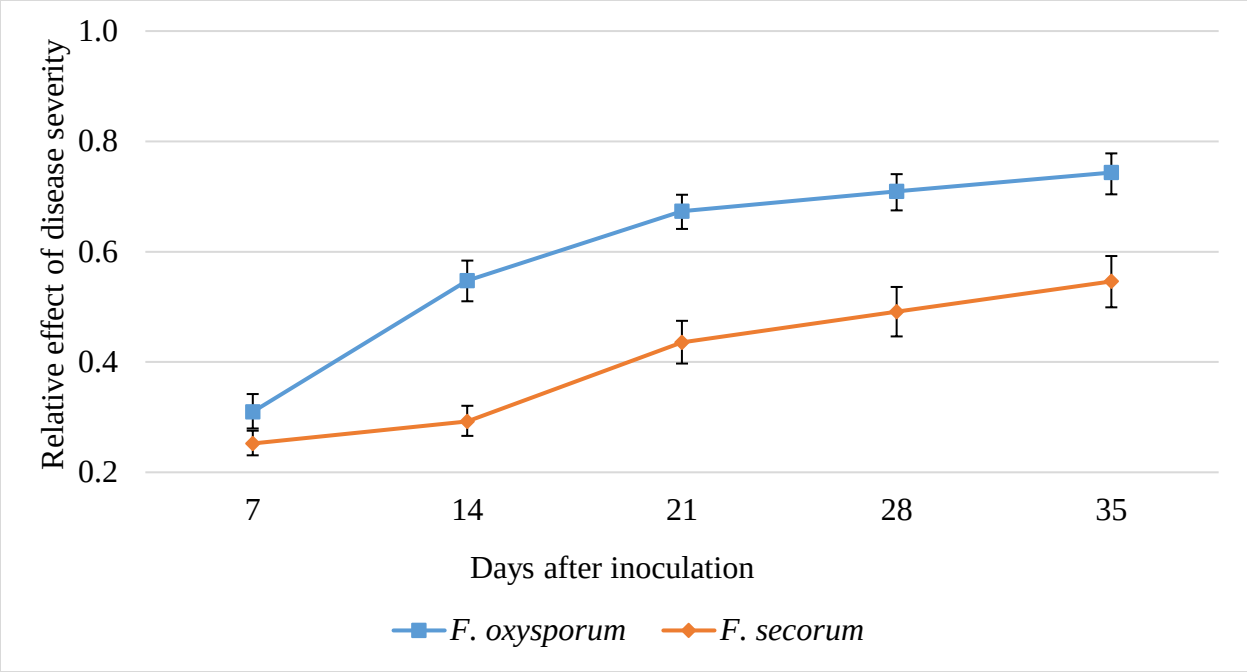

Figure 2.3: Effect of *F. oxysporum* f. sp. *betae* and *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI.

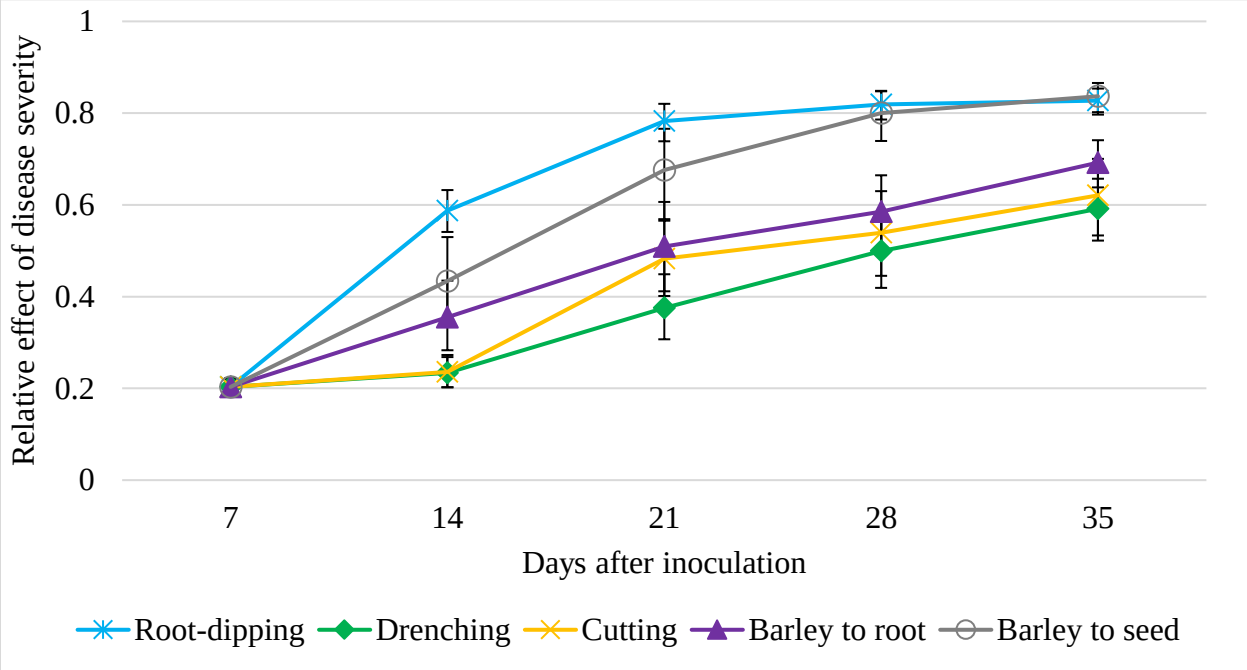

Figure 2.4: Effect of five different inoculation methods of *F. oxysporum* f. sp. *betae* and *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI.

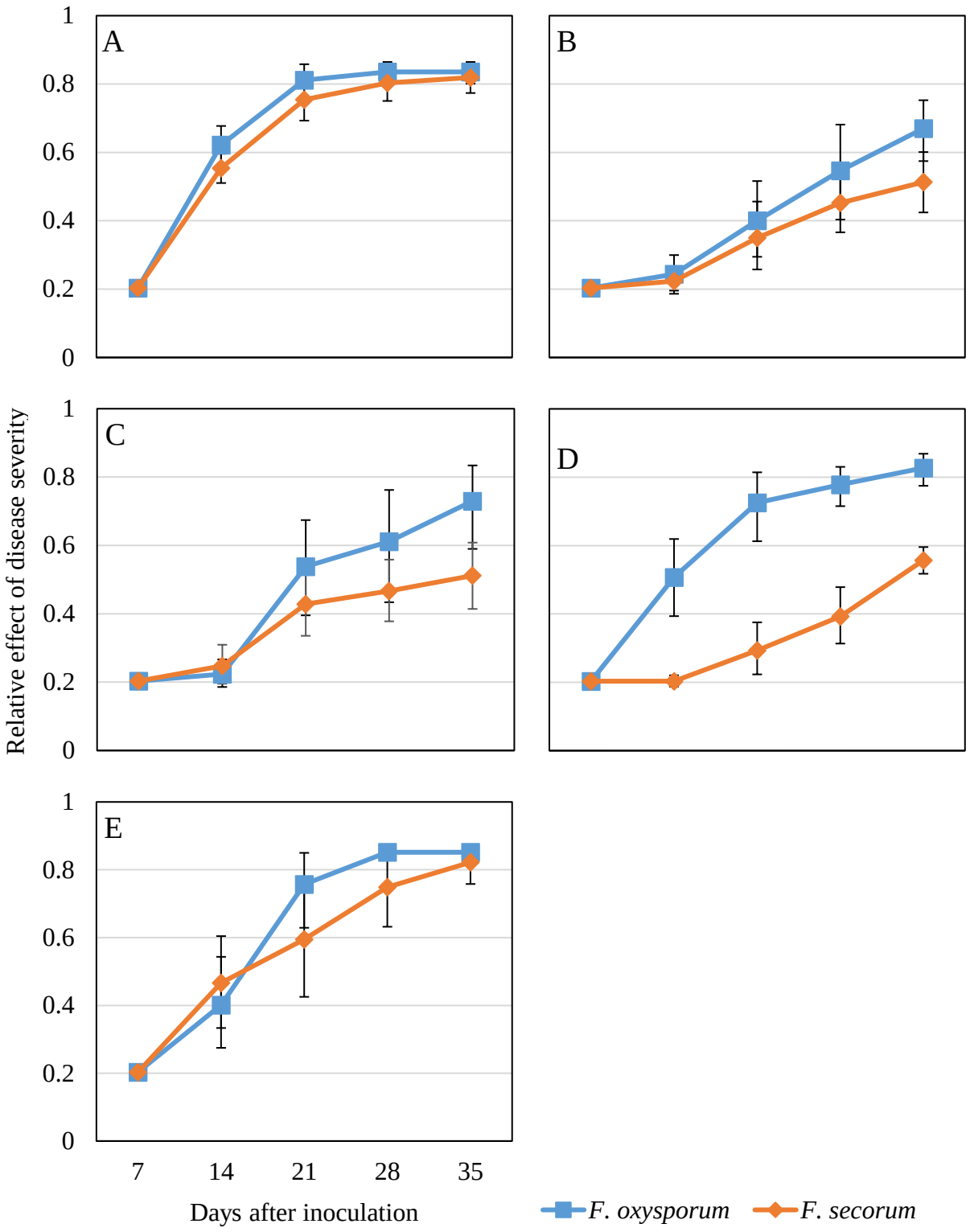

Figure 2.5: Effect of five different inoculation methods and two *Fusarium* species on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI. A Root-dipping, B Drenching, C Cutting, D Barley to root, and E Barley to seed.

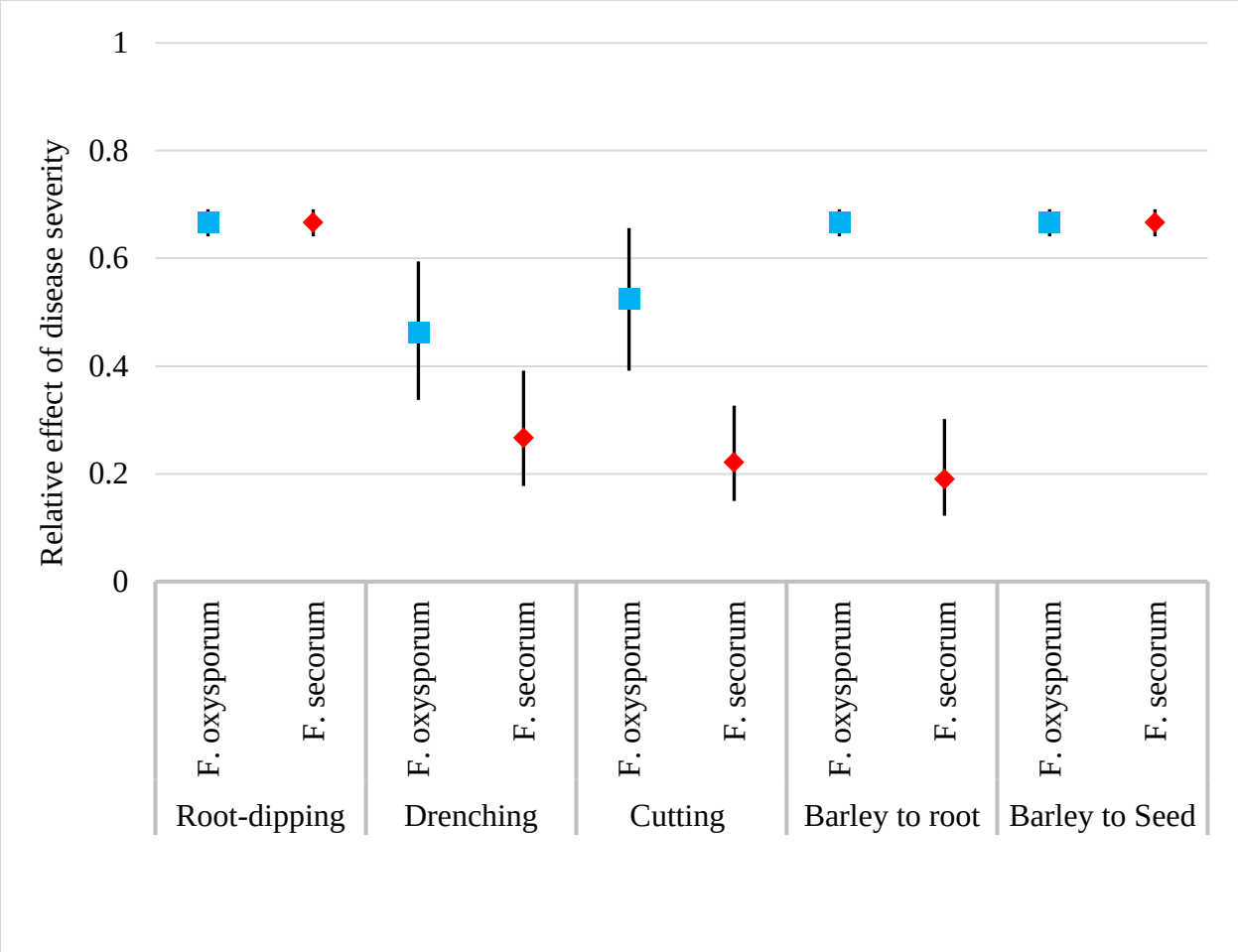

Figure 2.6: Relative effect of five different inoculation methods and two *Fusarium* species on disease severity of root of sugar beet at 35 DAI.

inoculation method caused the highest disease severity with early onset of symptoms (7 DAI) compared with the standard root-dipping method (14 DAI). We first observed 1-2 seedlings per replicate have disease symptoms at 7 DAI for the treatments with both Fusarium species using barley to seed, but there were ten samplings for each replicate and the median disease ratings were taken for the data analyses. As such, the results did not show the symptoms caused by the two Fusarium species were first observed at 7 DAI in Table 2.3 and Figure 2.5. However, for the barley to root inoculation method, F. secorum caused significantly lower disease severity with delayed

onset of symptoms (21 DAI) compared with F. oxysporum f. sp. betae (14 DAI). During this experiment, F. oxysporum f. sp. betae (F-19) grew faster than F. secorum (784-12-4). CFU for F. oxysporum f. sp. betae (F-19) was 4.8 × 105 CFU/barley, which was 2.6-times higher than the CFU for F. secorum (784-12-4) (Data not shown). Plant stage also had an effect on sugar beet disease severity and younger plants were more susceptible than older plants.

Burlakoti et al. (2012) reported that different *Fusarium* species could have similar foliar symptoms at 60 DAI, but when evaluating the diseased roots, the more-virulent isolates resulted in more vascular discoloration than the less-virulent ones. In this study, foliar symptoms were evaluated by recording the yellowing scales at 0, 7, 14, 21, and 35 DAI contributing to disease severities for each *Fusarium* isolate. This evaluation method for foliar symptoms caused by *Fusarium* species could be reliable, because both foliar (Fig. 2.5) and root (Fig. 2.6) evaluations indicated that *F. oxysporum* f. sp. *betae* (F-19) induced significantly higher disease severity than *F. secorum* isolate (784-12-4). Burlakoti et al. (2012) reported that *F. secorum* was more aggressive than *F. oxysporum* f. sp. *betae* on sugar beet. However, the specific isolate number of *F. secorum* was unknown. Since *F. secorum* was a relatively new species (Rivera et al., 2008), the differentiation in pathogenicity and virulence among its isolates was still unclear. Given the fact by Hill et al. (2011) that *F. oxysporum* f. sp. *betae* (F-19) was evaluated as highly pathogenic to sugar beet, *F. oxysporum* f. sp. *betae* (F-19) could be more aggressive than the specific *F. secorum* isolate 784-12-4.

In conclusion, this study evaluated artificial inoculation methods to induce *Fusarium* diseases on sugar beet in greenhouse conditions. The results showed both root-dipping and barley to seed were effective inoculation methods that could be used for *Fusarium* study on sugar beet. However, for large scale sugar beet germplasm resistant selection, root-dipping method is time

consuming, labor intensive, impractical for field study since this method requires transplanting after inoculation. Therefore, the barley to seed can be an alternative inoculation method that could be used for *Fusarium* study on sugar beet.

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CHAPTER THREE. DETERMINE WHICH FUNGICIDES HAVE THE POTENTIAL TO CONTROL *FUSARIUM OXYSPORUM* F. SP. *BETAE* AND *F. SECORUM* ON SUGAR BEET

Introduction

The Red River Valley of Minnesota and North Dakota is the largest sugar beet production region in the United States and contributed 51% of total U.S. sugar beet production in 2016 (USDA-ERS, 2016). Total economic activities in this region's sugar beet industry was valued at $4.9 billion dollars (Bangsund et al., 2012). Factors which limit sugar beet production include diseases such as Aphanomyces root rot, Rhizomania, Cercospora leaf spot, and Rhizoctonia crown and root rot. Fusarium yellows caused by *F. oxysporum* f. sp. *betae* was reported in 2002 and caused serious problems in the Moorhead factory district (Khan et al., 2009; Windels et al., 2005). The novel pathogen, *F. secorum* was first reported to cause Fusarium yellowing decline in Sabin, MN, in 2005 (Rivera et al., 2008). The distinct characteristics of Fusarium yellowing decline from Fusarium yellows are *F. secorum*, unlike *F. oxysporum* f. sp. *betae*, not only caused Fusarium yellows-like symptoms, but also caused seedling death, petiole vascular discoloration, and early season yellowing. *F. secorum* can be isolated from petioles while other *Fusarium* species have only been isolated from infected taproot of sugar beet.

Crop rotation, early planting, and use of resistant cultivars can be used to manage Fusarium yellows and Fusarium yellowing decline in sugar beet (Khan et al., 2009). However, crop rotation is unreliable because *Fusarium* species can survive as chlamydospores for many years and *F. oxysporum* f. sp. *betae* have a wide host range such as field weeds (MacDonald and Leach, 1976). Planting resistant varieties is the most efficient method to manage *Fusarium* species on sugar beet (Biancardi, 2005; Burlakoti, 2007). The American Crystal Company has included *F. oxysporum* f.

sp. *betae* and *F. secorum* in resistance breeding in the Red River Valley since 2005 (Niehaus, 2005). Currently, there is no commercial variety available which is immune to Fusarium diseases (Biancardi, 2005). Thus, fungicides may be needed to control these diseases.

Currently, there is no fungicide that can effectively control Fusarium diseases on sugar beet (Khan et al., 2009). Triazole fungicides were the most effective fungicides to control Fusarium head blight (FHB) caused by *F. graminearum* (Homdork et al., 2000; Mesterhazy et al., 2003; Hershman and Draper, 2004). Fungicides such as metconazole, tebuconazole, and prothioconazole can control Fusarium head blight (FHB) and reduce mycotoxin deoxynivalenol (DON) production on wheat (Edwards et al., 2001; Magan et al., 2002; Mesterhazy et al., 2003; Paul et al., 2008). Paul et al. (2010) reported that prothioconazole, metconazole, and prothioconazole + tebuconazole resulted in 13.8~15.0% increase in wheat mean yield and metconazole was the best one to control FHB in wheat. Burlakoti et al. (2010) reported that the *Fusarium* species isolated from sugar beet were sensitive to metconazole, triticonazole, and thiabendazole fungicides. However, this study was limited on *in vitro* assay to test the effectiveness of fungicides on controlling Fusarium diseases on sugar beet.

Benzovindiflupyr (Solatenol) and pydiflumetofen (Adepidyn) are novel QoI (Succinate dehydrogenase inhibitor) fungicides in FRAC (Fungicide Resistance Action Committee) group 7 with a broad spectrum. Which were recently registered by Syngenta (FRAC, 2017). These two fungicides have not been registered and used for Fusarium disease control on sugar beet in the United States. Benzovindiflupyr with seven other fungicides belong to a chemical group of pyrazole-4- carboxamides (FRAC, 2017). This fungicide was designed to control both foliar diseases and soil pathogens including rusts, many different leaf spots, apple scab, powdery mildew and *Rhizoctonia* on a wide range of crops (http://www.syngentacropprotection.com/news_releases

/news.aspx?id=183015). Pydiflumetofen belongs to a new chemical group of N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide (FRAC, 2017). This fungicide was designed to control leaf spots, blights, powdery mildew such as *Cercospora*, *Alternaria*, *Venturia*, *Botrytis*, *Sclerotinia*, *Corynespora*, and Fusarium Head Blight (http://www4.syngenta.com/what-we-do/crops-and-products/products-and-innovation/adepidyn).

This study was conducted to determine the efficacy of Topsin (thiophanate-methyl, United Phosphorus, Inc., King of Prussia, PA, USA), Quadris (azoxystrobin, Syngenta, Greensboro, NC, USA), Headline (pyraclostrobin, BASF Corporation, Florham Park, NJ, USA), Solatenol (benzovindiflupyr, Syngenta, Greensboro, NC, USA), Adepidyn (pydiflumetofen, Syngenta, Greensboro, NC, USA), and Caramba (metconazole, BASF Corporation, Florham Park, NJ, USA) at controlling *F. oxysporum* f. sp. *betae* and *F. secorum* on sugar beet in greenhouse conditions.

Materials and methods

Fungal isolates. In this study, two known pathogenic isolates were used: *F. oxysporum* f. sp. *betae* F-19 which was isolated from sugar beet in Salem, Oregon in 2001, provided by the USDA-ARS Sugarbeet Research Unit, Fort Collins, CO., and *F. secorum* 784-12-4 which was isolated from sugar beet in Sabin, Minnesota in 2007, provided by Dr. G. A. Secor, North Dakota State University, Fargo, ND.

Preparation of *Fusarium* inoculum. *Fusarium*-infested barley seeds (non-teated) were used in this study. Following the method of *Rhizoctonia solani*-infested barley grains were produced (Noor and Khan, 2014; Kirk et al., 2008), *Fusarium*-infested barley grains were produced by mixing 4.8 grams of potato dextrose broth (PDB; Sigma-Aldrich, USA), 200 ml of barley, and 120 ml of distilled water (the volume ratio is barley: distilled water=10:6) in a 500 ml flask (Pyrex, USA) and autoclaved at 170 kPa and 120 °C for 30 minutes. After the autoclaved

barley cooled to room temperature, it was inoculated with 3 mm square plugs from one week old culture, and plugs from one 100 × 15 mm Petri dish for one flask. Inoculated flasks were sealed, and hand-shaken to mix the plugs and barley. After two weeks, non-contaminated barley grains were air dried and stored at 4 °C until used. 50 inoculated barley grains with 100 ml autoclaved distilled water were grounded for 5 minutes in a lab blender. To detect if both fungal mycelium and conidia were present, 2 ml subsample was examed under a compound microscope. To calculate the colony forming units (CFU), 1, 10, 100, and 1000 times diluted solutions were obtained, followed by placing three 100 µl samples for each concentration into 100 × 15 mm PDA plates, and incubated at room temperature for 24 hours. The CFU were counted under compound microscope.

Fungicide spray and inoculation. Fungicides included in this study were thiophanate-methyl (Topsin, 45% a.i., United Phosphorus, Inc.), azoxystrobin (Quadris, 22.9% a.i., Syngenta), pyraclostrobin (Headline, 23.6% a.i., BASF Corporation), benzovindiflupyr (Solatenol, 10.3% a.i., Syngenta), pydiflumetofen (Adepidyn, Syngenta) and metconazole (Caramba, 8.6% a.i., BASF Corporation). The rate applied in this study was Topsin at 20 fl oz/acre, Quadris at 9.2 fl oz/acre, Headline at 12 fl oz/acre, Solatenol at 4.1 fl oz/acre, Adepidyn at 13.7 fl oz/acre, and Caramba at 14, 10, 5, and 2.5 fl oz/acre.

This study was conducted in the Agriculture Experiment Station (AES) at North Dakota State University, Fargo, ND, USA. A 2-cm deep furrow was made in the middle of a 28 ×12 × 12 cm plastic tray and filled with Sunshine mix 1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada). Ten seeds were placed evenly in the furrow, followed by fungicide application directly over the seeds. The spraying booth (Devries Manufacturing, Hollandaise, MN, USA) was set to deliver 47 L ha^{-1} solution at 138 kPal through a single flat fan nozzle (4001E). After fungicides were applied,

Fusarium-infested barley grains were placed 1 cm away from seeds, one grain for each seed. Sunshine mix 1 peat was used to cover the inoculated seeds and then compacted. The positive control was inoculated with *Fusarium*-infested barley grains and no fungicides application, and the negative control was inoculated with sterilized barley grains without fungicides application. The greenhouse (Argus Control Systems, Ltd.; British Columbia, Canada) condition was maintained at 75 °F with a 16-hour photoperiod. Plants were watered as needed to maintain sufficient soil moisture. This experiment was conducted twice with four replicates for each treatment. The completely randomized design (CRD) was used for this study.

Disease evaluation and data analysis. For the foliar disease symptoms, disease evaluation was conducted every week for five weeks after inoculation. The disease scale used for this study was 0 = no disease; 1 = leaves wilted, small chlorotic areas on lower leaves, most of leaf green; 2 = leaves showing interveinal yellowing; 3 = leaves with small areas of necrosis or becoming necrotic and dying, less than half of the leaves affected; 4 = more than half of leaves dead, plant stunted, most living leaves showing symptoms; 5 = plant death (Hanson et al., 2009).

Five weeks after inoculation, the plants were harvested from the tray, and cleaned under tap water. The symptom of discoloration on the vascular part was detected by longitudinally cutting the sugar beet root. Disease scale used for rate root disease was 0 = no internal browning; 1 = slight internal browning, usually at the tip of the tap root; 2= moderate to severe internal browning of the entire tap root; and 3 = severe internal browning extending from the tap root into the lower stem above the soil line (Rowe, 1980).

To test if these two runs' data can be combined, the homogeneity of variances were tested by Levene's test using SAS version 9.4 (SAS Institute Inc.; Cary, NC, USA). Non-parametric method was used for analysis of the disease scales, and commands Proc Rank and Proc Mixed

with module F2_LD_F1, LD_CI, and mult macro were used to separate treatments and obtain their confidence intervals (Shah and Madden, 2004).

Results

The homogeneity test based on disease severity of the two experiments confirmed that there was no difference (*P*-value = 0.5840) between them. Therefore, the data were combined. Plants from the negative control remained symptomless and plants from the positive control had the highest disease severity among all treatments.

The effect of the fungicides applied in-furrow followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* were significantly different (Table 3.1). The disease severity at 7, 14, 21, 28, and 35 days after inoculation (DAI) for all the treatments are shown in Fig. 3.5. In the positive controls, seedling wilt was first observed at 7 DAI when sugar beet seeds were inoculated with *F. oxysporum* f. sp. *betae* and at 14 DAI with *F. secorum*. Wilted seedlings were observed to show yellowing and necrosis, and eventually died quickly.

For those treatments where fungicides were applied followed by inoculation with *F. oxysporum* f. sp. *betae*, compared with the positive control where disease symptoms was first observed at 7 DAI, the fungicides delayed the disease symptoms onset which was first observed at 14 DAI except for the treatments applied with Quadris, Headline, and Caramba at 14 fl oz/Acre. Fungicides significantly reduced disease severity caused by *F. oxysporum* f. sp. *betae* compared to the positive control, but there were no differences among all the fungicide treatments except for Adepidyn which had lowest disease severity compareed to the other fungicide trreatments.

For those treatments with fungicides followed by inoculation with *F. secorum*, most of the fungicides delayed the disease symptoms onset compared with the positive control except for the treatments applied with Topsin, Headline, and Solatenol where disease symptoms were observed

at 14 DAI. The treatments applied with Caramba at 10 fl oz/Acre, 5 fl oz/Acre, and 2.5 fl oz/Acre, and Quadris where disease symptoms were first observed at 21 DAI, Caramba at 14 fl oz/Acre where disease symptoms were observed at 28 DAI, and Adepidyn where no symptoms observed. Fungicides significantly reduced disease severity caused by *F. secorum* compared to the positive control. Adepidyn performed a complete disease control when seeds were infested with *F. secorum* in greenhouse conditions. Caramba worked better at a low concentration of 5 fl oz/Acre to control *F. secorum* than to control *F. oxysporum* f. sp. *betae*.

When comparing the two species, *F. oxysporum* f. sp. *betae* caused significantly more disease on sugar beet plants than *F. secorum* (Fig. 3.2). When comparing the fungicides, the fungicide treatments were significantly different from the positive control and negative control (Fig. 3.3). The most effective fungicide to control Fusarium diseases was Adepidyn, where the disease severity was significantly lower than all the other fungicides treatments and the positive control. There were no significant differences among Caramba at 14, 10, 5, and 2.5 fl oz/Acre, among Topsin, Quadris, Headline, and Solatenol, and among Caramba (at 2.5 fl oz/Acre), Quadris, and Solatenol. The second effective fungicides to control Fusarium diseases was Caramba applied at 14, 10, and 5 fl oz/Acre. Lollipop shaped leaves resulted from phytotoxicity were observed in Caramba treatments (Fig. 3.1). In our result, decreased rates of Caramba resulted in reduced phytotoxicity with increased disease severity. No phytotoxicity was observed when Caramba was applied at 2.5 fl oz/Acre.

In Table 3.2, root disease severity was evaluated and calculated as the relative effects were also significantly different across the two species' fungicide treatments. Among all the treatments, similar results of the effect of fungicides described in Fig. 3.4 and Fig. 3.5. Adepidyn was the most effective fungicide at controlling both *F. oxysporum* f. sp. *betae* and *F. secorum*. Caramba was

effective at controlling *F. secorum* at the rates of 14, 10, and 5 fl oz/Acre. Topsin, Quadris, Headline, and Solatenol reduced disease severity, but were not as effective as Adepidyn and Caramba.

Discussion

For *Fusarium* species, conidia can be produced in 10-day old culture on half-strength PDA (Burlakoti et al., 2012). In this study, barley inoculum was incubated for 14 days after inoculation and both fungal mycelium and conidia were detected in barley inoculum which was inoculated with either *F. oxysporum* f. sp. *betae* or *F. secorum*. These two species can survive as chlamydospores, conidia, and mycelium in soil and plant material, and cause infection under favorable conditions (Khan et al., 2009). Thus, the inoculation method used in this study was very similar to the field situation. To prevent pathogens from causing infection on sugar beet, fungicides are needed to be sprayed on sugar beet before Fusarium inoculation to inhibit spore germination and mycelia growth.

Adepidyn excellently controlled Fusarium diseases on both sugar beet foliar and root in greenhouse conditions. Succinate dehydrogenase inhibitor (SDHI) fungicides interrupt fungal respiration by targeting and binding the succinate dehydrogenase (SDH) complex II to block mitochondrial electron transport chain (Beckerman, 2013). Fungicides in this group have been reported to inhibit conidia germination, germ tube elongation, and mycelia growth (Amiri et al., 2014; Thomas et al., 2012; Villani et al., 2016). Hou et al., (2017) reported Adepidyn can suppress mycelial growth against *F. asiaticum* at the concentration of 0.0745 µg/ml and conidial germination at the concentration of 0.1813 µg/ml in an EC_{50} test, and provide more than 80% of disease control in field conditions, indicating that Adepidyn has the potential to be used to control

Figure 3.1: Lollipop shaped leaves resulted from phytotoxicity when Caramba was applied in-furrow on sugar beet seeds.

Table 3.1: Test statistics for the relative effect of fungicides applied in-furrow on sugar beet seed, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Effect	ANOVA A-type statistic (ATS)			
	df_N[b]	df_D[c]	ATS	*P*-value
Isolate	1	9.01	162.52	<0.0001***
Fungicides	8.51	1	30.82	<0.0001***
Isolate × Fungicides	8.51	10.4	4.54	<0.0001***
Time	2.47	1	368.73	<0.0001***
Isolate × Time	2.47	267	25.36	<0.0001***
Fungicides × Time	17.4	1	9.63	<0.0001***
Isolate × Fungicides × Time	17.4	268	5.68	<0.0001***

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.
***Significantly different at P ≤0.001

Table 3.2: Test statistics for the relative effect of fungicides applied in-furrow on sugar beet seed, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on the root disease severity of sugar beet at 35 DAI[a].

Effect	ANOVA-type statistic (ATS)			
	df_N[a]	df_D[b]	ATS	*P*-value
Isolate	1	80.2	69.83	<0.0001***
Fungicides	6.33	80.2	22.42	<0.0001***
Isolate × Fungicides	6.33	80.2	3.93	0.0005***

[a]DAI=days after inoculation
[b]df_N=numerator degrees of freedom.
[c]df_D=denominator degrees of freedom.

Table 3.3: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a].

Treatment	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Negative Control	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Negative Control	14	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Negative Control	21	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Negative Control	28	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Negative Control	35	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Positive Control	7	1.00	0.00	653.00	310.50	0.618	0.294	0.421-0.701	0.238-0.267
Positive Control	14	2.50	0.50	934.94	543.90	0.885	0.515	0.794-0.909	0.307-0.635
Positive Control	21	4.00	3.00	1002.19	961.83	0.949	0.910	0.904-0.960	0.849-0.924
Positive Control	28	5.00	4.00	1028.12	1023.38	0.973	0.969	0.936-0.983	0.949-0.972
Positive Control	35	5.00	4.50	1039.12	\1031.26	0.984	0.976	0.968-0.987	0.956-0.981
Topsin	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Topsin	14	1.00	0.00	615.00	361.22	0.582	0.342	0.409-0.635	0.219-0.391
Topsin	21	1.00	0.00	784.82	411.96	0.743	0.390	0.610-0.762	0.240-0.463
Topsin	28	2.00	0.00	822.83	481.69	0.779	0.456	0.656-0.800	0.270-0.565
Topsin	35	3.50	2.00	956.97	790.93	0.906	0.749	0.780-0.944	0.556-0.828
Quadris	7	0.00	0.00	462.73	310.50	0.438	0.294	0.270-0.521	0.238-0.267
Quadris	14	1.00	0.00	735.50	310.50	0.696	0.294	0.589-0.679	0.238-0.267

Table 3.3: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a] (continued).

Treatment	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Quadris	21	1.50	0.50	792.49	532.56	0.750	0.504	0.634-0.759	0.311-0.606
Quadris	28	2.00	1.00	834.11	690.46	0.789	0.653	0.660-0.818	0.433-0.760
Quadris	35	2.00	1.50	890.48	771.53	0.843	0.730	0.731-0.871	0.525-0.815
Headline	7	0.00	0.00	462.75	310.50	0.438	0.294	0.271-0.520	0.238-0.267
Headline	14	1.00	0.00	634.00	412.01	0.600	0.390	0.417-0.667	0.238-0.465
Headline	21	1.50	1.00	753.06	754.49	0.713	0.714	0.525-0.785	0.597-0.712
Headline	28	2.00	2.00	834.13	879.80	0.789	0.833	0.662-0.817	0.765-0.829
Headline	35	2.00	2.00	852.50	879.80	0.807	0.833	0.663-0.847	0.765-0.829
Solatenol	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Solatenol	14	1.00	0.00	583.06	411.98	0.552	0.390	0.358-0.642	0.240-0.463
Solatenol	21	1.00	0.50	754.39	532.72	0.714	0.504	0.599-0.710	0.312-0.605
Solatenol	28	1.00	1.00	784.60	621.62	0.743	0.588	0.612-0.761	0.374-0.702
Solatenol	35	1.50	1.50	840.23	803.98	0.796	0.761	0.632-0.846	0.631-0.782
Adepidyn	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Adepidyn	14	0.50	0.00	513.83	310.50	0.486	0.294	0.311-0.564	0.238-0.267
Adepidyn	21	1.00	0.00	564.61	310.50	0.534	0.294	0.357-0.601	0.238-0.267
Adepidyn	28	1.00	0.00	564.61	310.50	0.534	0.294	0.357-0.601	0.238-0.267

Table 3.3: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a] (continued).

Treatment	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Adepidyn	35	1.00	0.00	583.71	310.50	0.552	0.294	0.360-0.639	0.238-0.267
Caramba 14 fl oz/Acre	7	0.00	0.00	361.46	310.50	0.342	0.294	0.218-0.392	0.238-0.267
Caramba 14 fl oz/Acre	14	1.00	0.00	615.49	310.50	0.582	0.294	0.411-0.634	0.238-0.267
Caramba 14 fl oz/Acre	21	1.50	0.00	776.71	310.50	0.734	0.294	0.531-0.818	0.238-0.267
Caramba 14 fl oz/Acre	28	2.00	0.00	802.82	360.88	0.759	0.342	0.548-0.848	0.218-0.392
Caramba 14 fl oz/Acre	35	2.50	0.00	840.27	411.27	0.794	0.390	0.572-0.887	0.239-0.465
Caramba 10 fl oz/Acre	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Caramba 10 fl oz/Acre	14	1.00	0.00	715.04	310.50	0.677	0.294	0.493-0.740	0.238-0.267
Caramba 10 fl oz/Acre	21	2.00	0.00	834.05	462.46	0.789	0.438	0.661-0.817	0.272-0.518
Caramba 10 fl oz/Acre	28	2.50	0.00	875.66	462.46	0.829	0.438	0.702-0.863	0.272-0.518
Caramba 10 fl oz/Acre	35	2.50	0.00	875.66	462.46	0.829	0.438	0.702-0.863	0.272-0.518
Caramba 5 fl oz/Acre	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267
Caramba 5 fl oz/Acre	14	0.50	0.00	539.80	310.50	0.504	0.294	0.309-0.609	0.238-0.267
Caramba 5 fl oz/Acre	21	2.00	0.00	824.82	441.65	0.789	0.390	0.660-0.818	0.240-0.462
Caramba 5 fl oz/Acre	28	2.00	0.00	866.64	441.65	0.832	0.390	0.694-0.872	0.240-0.462
Caramba 5 fl oz/Acre	35	2.00	0.00	866.64	441.65	0.832	0.390	0.694-0.872	0.240-0.462
Caramba 2.5 fl oz/Acre	7	0.00	0.00	310.50	310.50	0.294	0.294	0.238-0.267	0.238-0.267

Table 3.3: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI[a] (continued).

Treatment	DAI	MDS[b]		MR[c]		REDS[d]		95%CI[e]	
		F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Caramba 2.5 fl oz/Acre	14	1.50	0.00	751.34	310.50	0.713	0.294	0.520-0.788	0.238-0.267
Caramba 2.5 fl oz/Acre	21	2.00	0.50	913.79	476.58	0.861	0.543	0.781-0.874	0.317-0.686
Caramba 2.5 fl oz/Acre	28	3.00	1.00	945.18	520.18	0.889	0.591	0.812-0.907	0.367-0.715
Caramba 2.5 fl oz/Acre	35	3.00	1.00	945.18	520.18	0.889	0.591	0.812-0.907	0.367-0.715

[a]DAI=days after inoculation

[b]MDS=median disease rating. Disease severity was evaluated every week for five weeks based a 0 to 5 scale: 0 (no disease), 1 (leaves wilted, small chlorotic areas on lower leaves, most of leaf green), 2 (leaves showing interveinal yellowing), 3 (leaves with small areas of necrosis or becoming necrotic and dying, less than half of the leaves affected), 4 (more than half of leaves dead, plant stunted, most living leaves showing symptoms), 5 (plant death).

[c]MR=mean rank

[d]REDS=relative effect of disease severity

[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

Table 3.4: Effect of fungicides applied in-furrow on sugar beet seed, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on root disease severity of sugar beet at 35 DAI[a].

Treatment	MDS[b]		MR[c]		RRDS[d]		95%CI[e]	
	F. oxysporum	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*	*F. oxysporum*	*F. secorum*
Negative Control	0.00	0.00	33.00	33.00	0.185	0.185	0.163-0.209	0.163-0.209
Positive Control	3.00	3.00	158.88	158.88	0.900	0.900	0.844-0.934	0.844-0.934
Topsin	3.00	0.50	146.75	71.00	0.831	0.401	0.569-0.937	0.253-0.571
Quadris	2.00	1.00	123.62	84.75	0.700	0.479	0.597-0.784	0.345-0.616
Headline	1.50	1.00	102.13	98.50	0.577	0.557	0.442-0.701	0.477-0.633
Solatenol	1.50	1.00	121.38	85.63	0.687	0.484	0.532-0.806	0.283-0.690
Adepidyn	0.00	0.00	46.75	33.00	0.263	0.185	0.180-0.371	0.163-0.209
Caramba 14 fl oz/Acre	1.50	0.00	106.25	33.00	0.601	0.185	0.444-0.738	0.163-0.209
Caramba 10 fl oz/Acre	2.00	0.00	114.25	46.75	0.646	0.263	0.557-0.725	0.180-0.371
Caramba 5 fl oz/Acre	1.00	0.00	103.75	53.63	0.587	0.302	0.499-0.668	0.207-0.420
Caramba 2.5 fl oz/Acre	2.00	1.00	119.50	72.63	0.676	0.410	0.594-0.748	0.285-0.549

[a]DAI=days after inoculation

[b]MDS=median disease rating. Sugar beet plants were hand harvested at 35 DAI and root disease severity was rated with a 0 to 3 scale: 0 (no internal browning), 1 (slight internal browning, usually at the tip of the tap root), 2 (moderate to severe internal browning of the entire tap root), and 3 (severe internal browning extending from the tap root into the lower stem above the soil line).

[c]MR=mean rank

[d]REDS=relative effect of disease severity

[e]95% CI=upper-lower values of 95% confidence interval (CI) of relative effect.

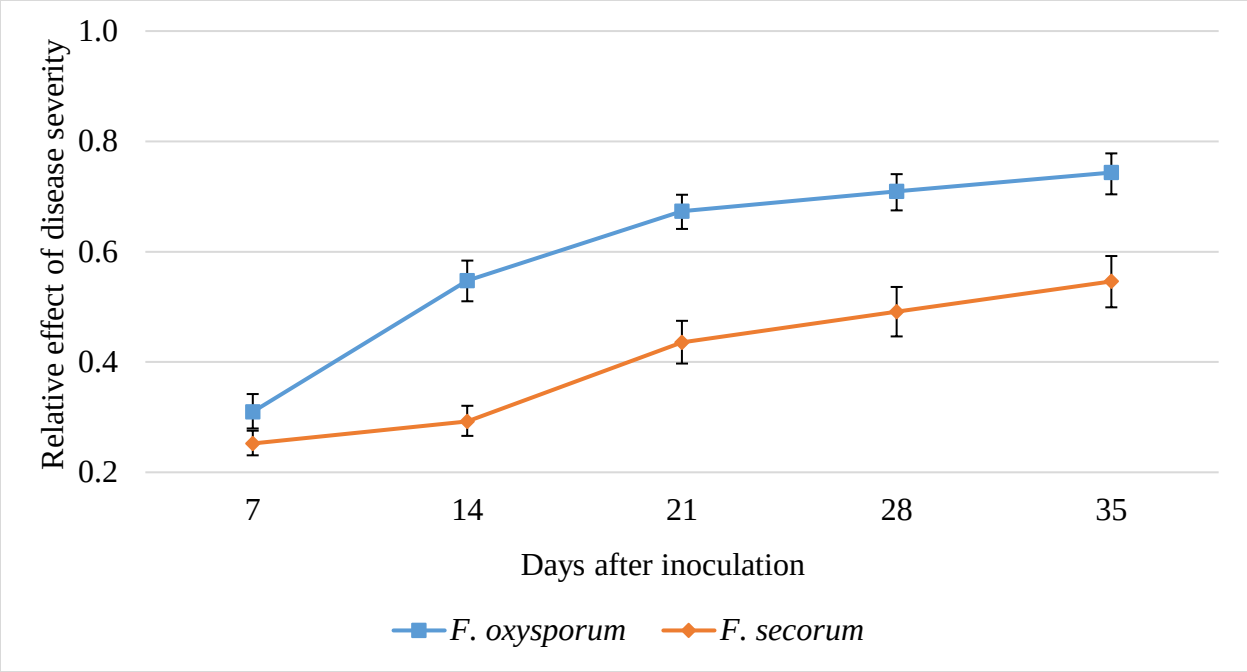

Figure 3.2: Effect of *F. oxysporum* f. sp. *betae* and *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI.

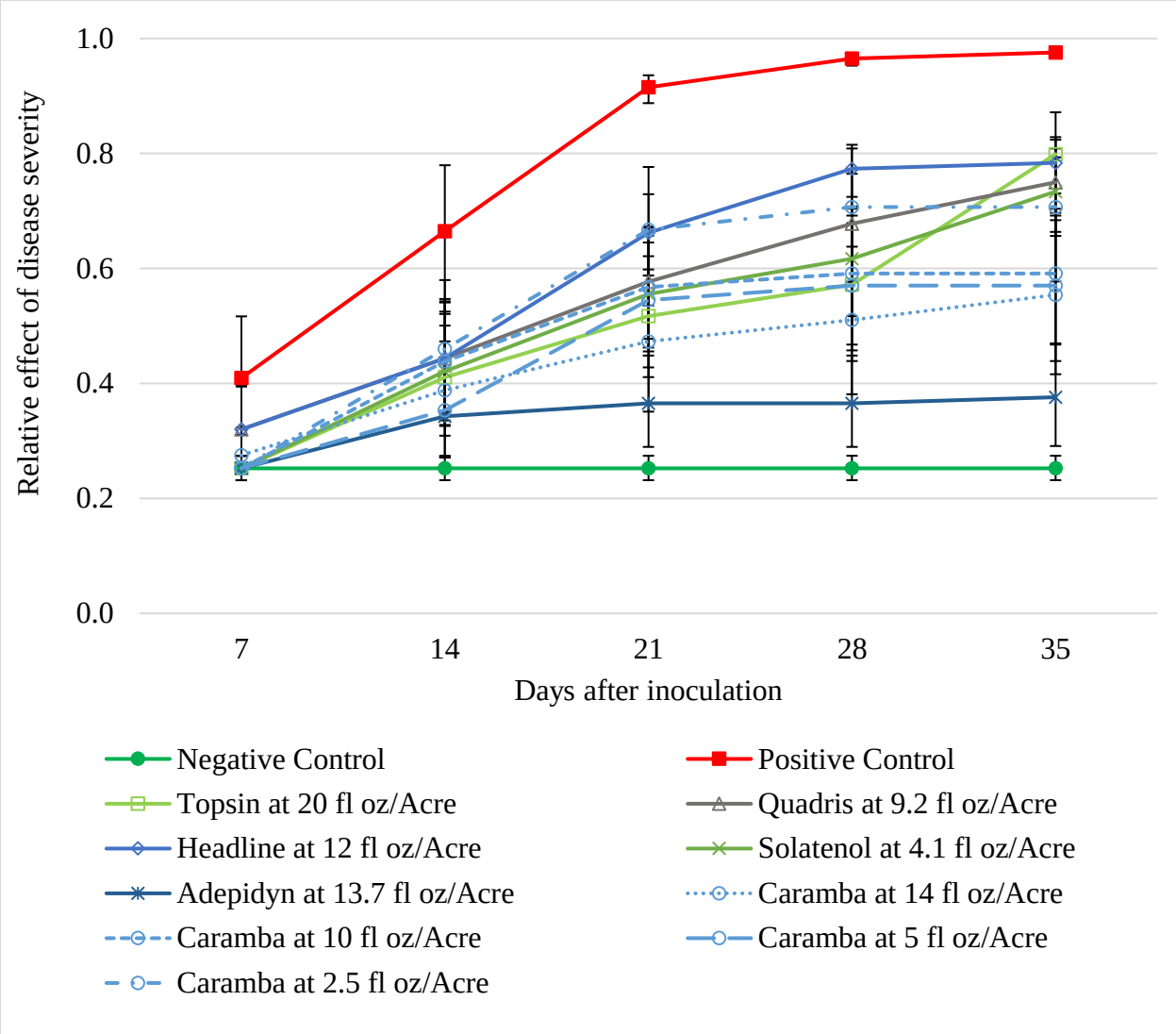

Figure 3.3: Effect of fungicides applied in-furrow to control *F. oxysporum* f. sp. *betae* and *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI.

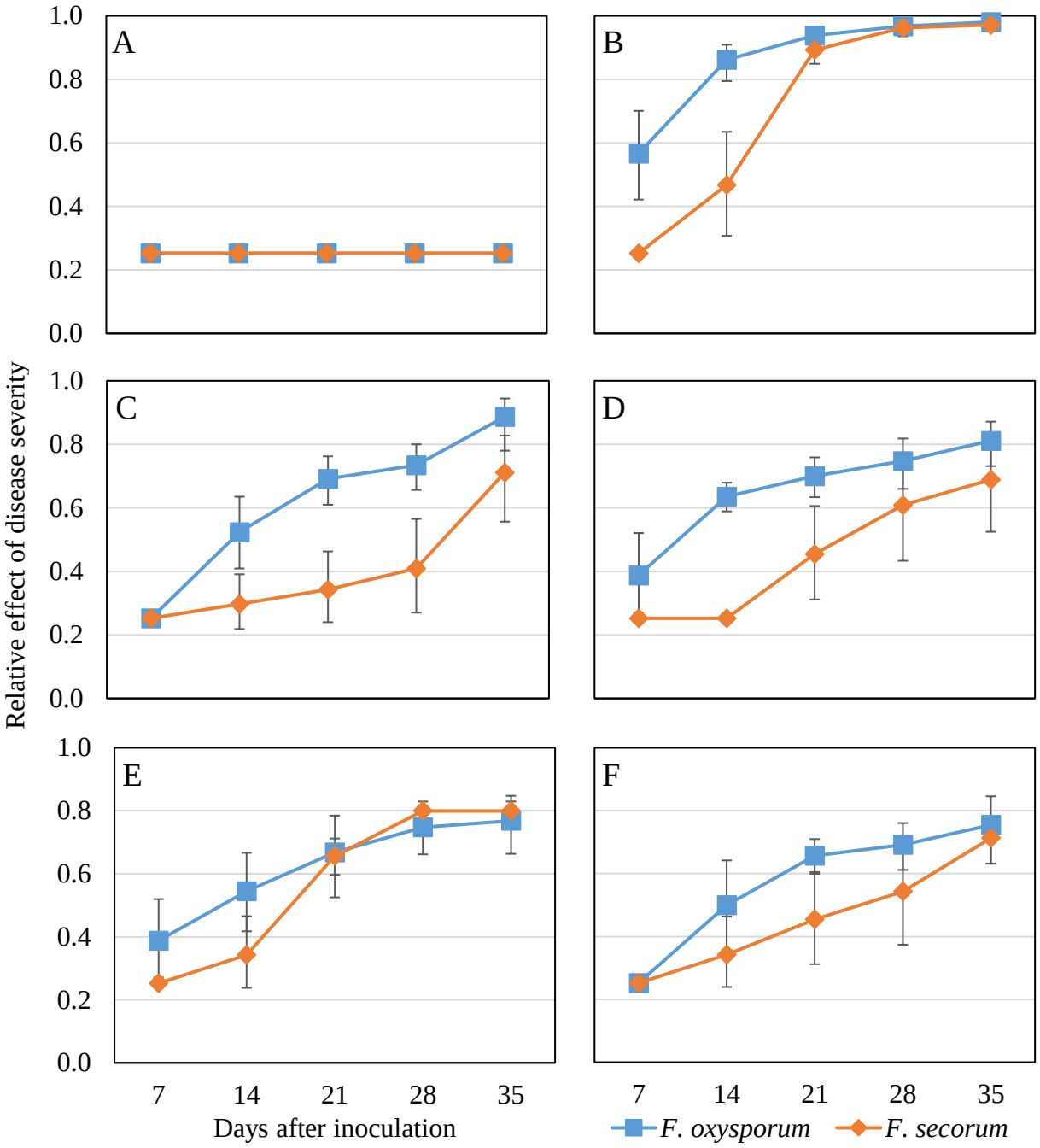

Figure 3.4: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI. A Negative control, B Positive control, C Topsin at 20 fl oz/acre, D Quadris at 9.2 fl oz/acre, E Headline at 12 fl oz/acre, F Solatenol at 4.1 fl oz/acre, G Adepidyn at 13.7 fl oz/acre, H Caramba at 14 fl oz/Acre, I Caramba at 10 fl oz/Acre, J Caramba at 5 fl oz/Acre, and K Caramba at 2.5 fl oz/Acre.

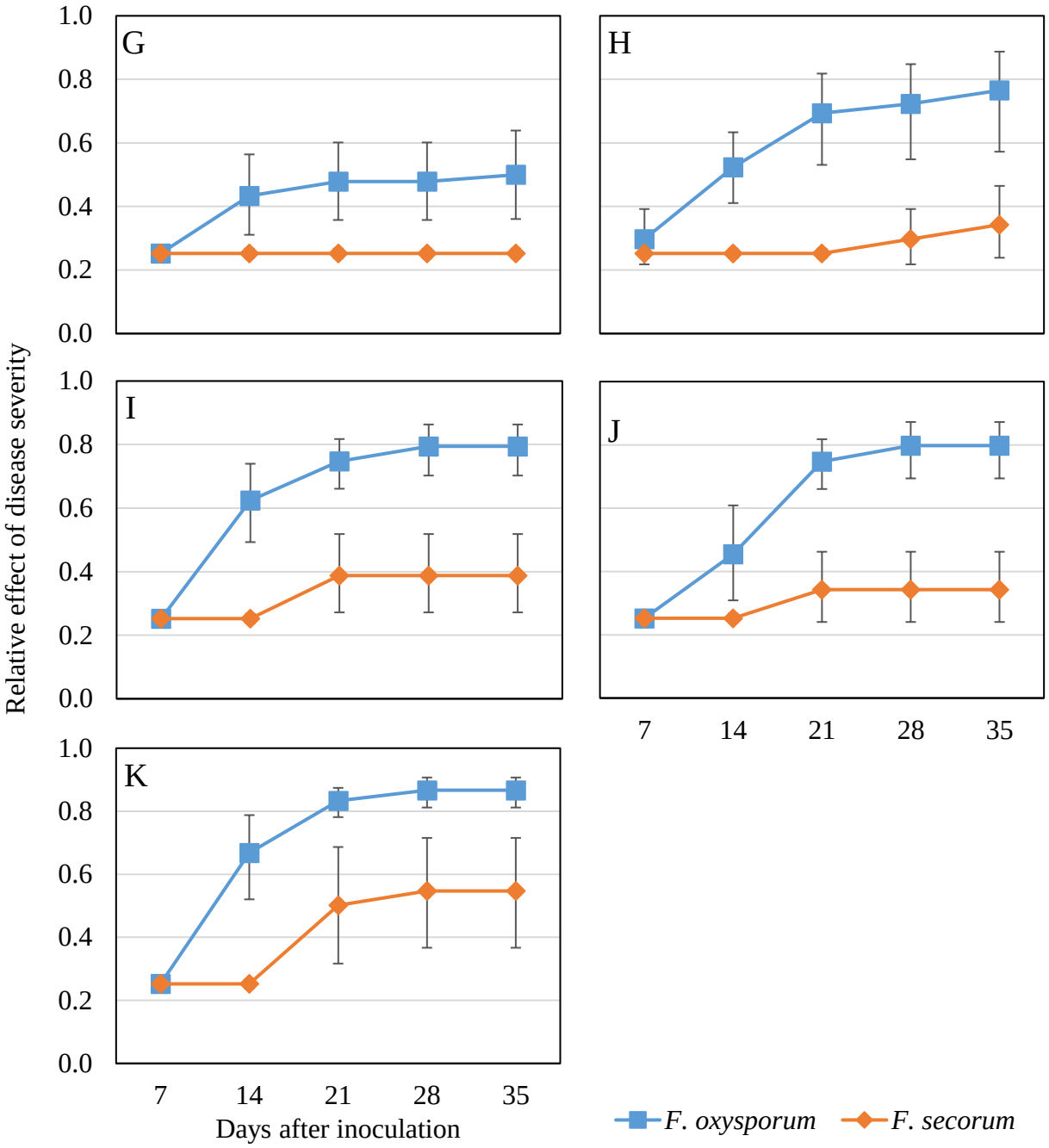

Figure 3.4: Effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on foliar disease severity of sugar beet at 7, 14, 21, 28, and 35 DAI (continued). A Negative control, B Positive control, C Topsin at 20 fl oz/acre, D Quadris at 9.2 fl oz/acre, E Headline at 12 fl oz/acre, F Solatenol at 4.1 fl oz/acre, G Adepidyn at 13.7 fl oz/acre, H Caramba at 14 fl oz/Acre, I Caramba at 10 fl oz/Acre, J Caramba at 5 fl oz/Acre, and K Caramba at 2.5 fl oz/Acre.

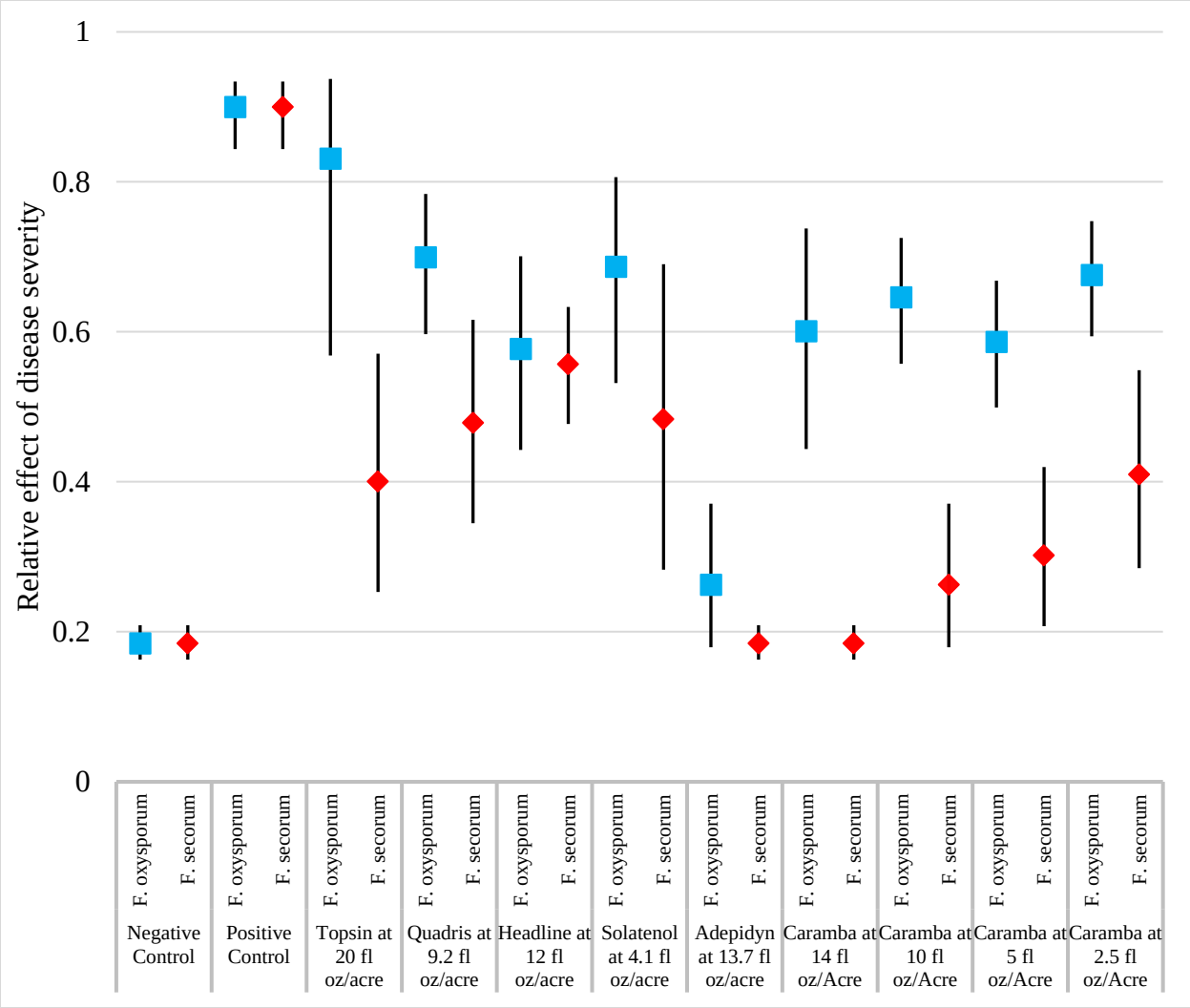

Figure 3.5: Relative effect of fungicides applied in-furrow, followed by inoculation with either *F. oxysporum* f. sp. *betae* or *F. secorum* on root disease severity of sugar beet at 35 DAI.

Fusarium diseases on sugar beet. Based on the data, the efficacy of Adepidyn is recommended to be tested for controlling *Fusarium* species in field studies.

Caramba (metconazole, 8.6% a.i., BASF Corporation) is labeled to control Powdery mildew (*Erysiphe betae*) on sugar beet using recommended rate of 14 fl oz/Acre (BASF Corporation, 2013). *In vitro* assay showed *F. oxysporum* f. sp. *betae* and *F. secorum* collected from sugar beet fields had low EC_{50} values (0.02-0.04 µg ml^{-1}) against metconazole (Burlakoti et al., 2010). In this study, Caramba applied at 14, 10, and 5 fl oz/Acre provided effective control of Fusarium diseases on sugar beet. Pirgozliev et al. (2002) had similar results finding metconazole could significantly reduce disease severity of FHB and the DON concentration when it was applied at double, full, half, and quarter of the recommended rate. However, in this study, significantly higher disease severity was observed when Caramba was applied at 2.5 fl oz/Acre, which is about 0.18-times of the recommended rate. The colony forming units (CFU) of barley inoculum used in this study was 1.8-4.8×10^5 CFU/barley which produced a very high disease pressure so that Caramba at 2.5 fl oz/Acre could not suppress all the colonies to cause infection. In this study, phytotoxicity was observed in Caramba treatments at 14, 10, and 5 fl oz/Acre. Strausbaugh et al. (2012) also observed phytotoxicity on sugar beet when metconazole was applied with the Nipslt INSIDE seed treatment and plants recovered to normal during later growth stages. Everts et al. (2014) reported that metconazole could control Fusarium wilt of watermelon, but also caused phytotoxicity with leaf thickening, darkened leaf color, and reduced vine length. More studies are needed to determine if the phytotoxicity caused by metconazole affects sugar beet yield.

Topsin (thiophanate-methyl, 45% a.i., United Phosphorus, Inc.), Headline (pyraclostrobin, 23.6% a.i., BASF Corporation), and Quadris (azoxystrobin, 22.9% a.i., Syngenta) are used to effectively manage Rhizoctonia crown and root rot and Cercospora leaf spot on sugar beet (Khan,

2017). Headline, Quadris and their generics are applied in-furrow to control *Rhizoctonia solani* during the early growing season (Khan, et al. 2017, Khan, 2017). In this study, those three fungicides did not provide effective control of Fusarium diseases compared with other fungicides. The disease severity was significantly reduced by those three fungicides compared with the positive control, but the difference was marginally significant and the disease severity was significantly higher than the treatments with Adepidyn and Caramba at 14, 10, and 5 fl oz/Acre. Other reports also showed azoxystrobin could significantly reduce FHB incidence and severity, but it was not as effective as metconazole (Jones, 2000; Pirgozliev et al., 2002). Madden et al., (2014) reported that pyraclostrobin reduced FHB incidence, but increased DON concentration. Elmer and McGovern (2004) reported thiophanate-methyl alone failed to reduce disease severity of Fusarium wilt on cyclamen.

This study demonstrated that Adepidyn applied at 13.7 fl oz/Acre and Caramba applied at 14, 10, and 5 fl oz/Acre provided effective control of *Fusarium* species on sugar beet when the fungicides were applied in-furrow. The greenhouse study shows the first disease symptoms observed at 7-14 DAI when the temperature was constantly at 75 °F. In the field, planting is conducted from mid-April to early June with an average soil temperature of 46 to 63 °F, in Sabin, MN (NDAWN 2007-2017; http://ndawn.ndsu.nodak.edu/). Fusarium diseases symptoms was observed in May, 2017 at the Moorhead research site which indicated that infection took place at a low temperature and at an early plant stage (2-leaf stage) (Khan, personal communication). It is possible that in-furrow application of fungicides at planting would be a useful tool for controlling Fusarium diseases on sugar beet.

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