DETERMINATION OF DISEASE IMPACTS ON SUNFLOWER YIELD

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Determination of Disease Impacts on Sunflower Yield

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ABSTRACT

Diseases that infect sunflower frequently occur in North Dakota, but the impact they have on yield is unclear. The objectives of this research are to 1) evaluate fungicide efficacy, application timing and yield impact of Phoma black stem of sunflower and 2) determine the impact of diseases on sunflower yield in North Dakota and Minnesota. Results of 14 fungicide trials conducted between 2017 and 2019 showed that yield losses to Phoma black stem were infrequent, but the disease could be managed by application of several available and efficacious fungicides applied at growth stage R1. Analysis of survey data collected over 11 years from 1,003 sunflower fields revealed that when diseases were determined to be a production-limiting factor, mean yield was 427 kg/ha less than in fields where no was production-limiting factor was reported. Results of these studies may help sunflower growers make decisions that optimize yield on their farms.

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Sunflower

Origin and History

Domestication of sunflower (*Helianthus annuus* L.) began in eastern North America thousands of years ago (Blackman et al. 2011; Heiser 1955). Seeds of sunflower have been recovered from fossilized human feces that was estimated to be up to 6,000 years old, and remnants of roasted seeds have been recovered from archeological sites dating back to 1000 BC (Harveson 2016a). Indigenous cultures used sunflower as a food source since the plant's edible seeds are a good source of fat (Putt 1997). Additionally, they used sunflower as medicines, such as using the juice of a freshly cut stem to dress a wound. Sunflower was also used as dyes and as parts of religious ceremonies (Heiser 1955).

Sunflower was believed to first be introduced into Europe by the Spanish sometime around 1500 AC, but other separate introductions likely occurred from French and British explorers and traders (Heiser 1955). In Europe, popularity of sunflower initially grew because of its aesthetic value, with the first sunflower plants in Europe described as having purple ray flowers and diversely colored seeds. Human consumption of sunflower seeds did not become common in Europe until the mid-1700's. The potential of sunflower as an oil crop wasn't recognized until the early-1700's. In 1716, an English patent was registered to Arthur Bunyan for the creation and protection of an oil-extraction technique involving sunflower seed. It wasn't until sunflower became popular in Russia that mass production occurred for oil extraction. Sunflower production grew rapidly because of Russia's wide adoption of the crop. The development of Russian breeding programs in the early 1900's successfully improved seed size, head size, oil content, and disease resistance (Harveson 2016a).

Breeding efforts have resulted in two different types of sunflower, which are both commercially grown. Oilseed type hybrids are grown primarily for high-value cooking oil, while non-oil type (confectionary) hybrids are grown for direct human consumption (Berglund 2007a). In 2018/2019, approximately 26 million hectares of sunflower were harvested globally, with a yield of 1.95 metric tons per hectare (USDA-FAS 2020). Ukraine, Russia and the European Union led world production of sunflower with 15 million metric tons, 12.7 million metric tons and 9.5 million metric tons, respectively. The next leading producers of sunflower were Argentina, China, Turkey, the US and South Africa, although the combined production of all these countries is only approximately equal to the European Union.

Production in the US

Even though sunflower is native to North America, commercial production in Canada and the US didn't begin until the late 1800s when Russian immigrants reintroduced more productive sunflower genotypes (Putt 1997). After reintroduction to North America, sunflower was mainly grown as a silage crop since it had better frost and drought tolerance than corn (*Zea mays* L.) and sunflower was believed to have the same nutritional value as corn silage. Little seed production occurred and of the limited amount, most was used as animal feed (Putt 1997). In the second half of the 20th century, sunflower production in the US grew rapidly. Expanding markets in Canada and increases in oil concentrations and yields from Russian cultivars expanded interest in growing sunflower in the US. Additionally, the discovery of cytoplasmic male sterility (Leclercq 1968) and fertility-restoring genes (Enns et al. 1970) allowed development of hybrid sunflower. According to USDA-NASS data, the harvested area of oilseed and non-oilseed sunflower in North Dakota was around 4,000 hectares in 1949. This increased to approximately 45,000 hectares in 1969 and reached a high of 1,367,000 hectares in

1979. High oil prices due to increased demand in Europe drove US sunflower production to its peak in the late-1970's/early-1980's (Putt 1997). Production has trended downward since the 1980's. In 2019, 83% of the total 504,000 hectares of sunflower were harvested in the Northern Great Plains (NGP) of the US, an area that includes North Dakota, South Dakota and the western edge of Minnesota (USDA-NASS 2019).

Sunflower Diseases

Sunflower is a known host for over 30 pathogens, many of which cause yield limiting diseases (Markel et al 2015). However, the number of diseases that occur, and the severity of those diseases are thought to be higher in North America than in other sunflower producing regions globally. Likely, this is in part due to the center of origin of *Helianthus* being in North America, where co-evolution of sunflower pathogens occurred for thousands of years. Additionally, commercial domestication of *Helianthus* occurred in Asia (Russia), and early cultivars introduced into North America would have been selected for in an environment with different pathogens. Many of these sunflower diseases occur frequently and/or are yield limiting.

Sunflower Rust

Sunflower rust, caused by the fungus *Puccinia helianthi* Schw. is an economically important disease in all sunflower growing regions of the US and Canada (Bradley et al. 2007). *P. helianthi* is an autoecious and macrocyclic rust, and can overwinter as thick-walled teliospores in the cold climate of the NGP. In the spring, teliospore germination and development through the next three spore stages (basidia, pycnia, and aecia) result in the production of asexual cinnamon-colored urediniospores. The urediniospores are a repeating stage and can replicate rapidly throughout the growing season. Uredinia will cycle in as little as 10-14 days under

conditions with 24 hours of free moisture, temperatures of 10-25˚C, and low light intensity (Friskop and Markell 2016). Early season development of urediniospores and favorable environments for reproduction can lead to severe epidemics (Markell et al. 2009). Cool temperatures and host maturity initiate the change from urediniospores to teliospores (Friskop and Markell 2016). A high density of rust on the upper leaves of a sunflower plant can reduce their photosynthetic processes resulting in lower yield, oil content, seed size and test weight (Bradley et al. 2007; Friskop and Markell 2016; Gulya et al. 1997). Documented yield losses due to rust in the NGP have exceeded 80%; although, that level of yield loss is considered rare (Markell et al. 2009, Friskop et al. 2011).

Multiple management tools are available for rust, but the most effective strategy is to use multiple tools (Friskop et al. 2011). Resistance genes are commonly incorporated into commercial hybrids for rust management; however, many races of *P. helianthi* occur in the NGP which challenge their effectiveness (Friskop et al 2015b). Crop rotation and tillage can reduce the level of inoculum from a previous year's crop, as telia survive on infected residue. However, urediniospores are capable of traveling long distances, so rotation is of moderate effectiveness. Destruction of wild sunflowers, which harbor disease and provide a site for overwintering, can help reduce early-season inoculum and a potential site of sexual recombination leading to new pathogen races. Friskop et. al. (2015a) and Berghuis et al. (2018) demonstrated that multiple effective fungicides could reduce rust severity and protect yield if applied at optimal timing.

Sclerotinia Diseases

Several species of *Sclerotinia* can infect sunflower. *Sclerotinia minor* Jag. is known to cause sclerotinia wilt on sunflower in Australia, Argentina, Uruguay, Chile, China, Spain and California (Farr and Rossman 2020, Li et al. 2016), but is not found in the NGP (Gulya et al.

1997). Similarly, *Sclerotium rolfsi* Sacc. will cause infections resulting in wilt in warmer climates, but is not known to occur in the NGP. In the NGP, sclerotinia diseases of sunflower are caused by the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary.

The fungus, *S. sclerotiorum*, occurs globally and can cause three distinct diseases on sunflower: sclerotinia head rot, sclerotinia mid-stalk rot or sclerotinia wilt (Gulya et al. 1997). *Sclerotinia* diseases are distinguished by what part of the plant is infected and how infection occurs. The germination of sclerotia bodies, which appear within plant tissue as large (1-5 cm), irregularly-shaped brown to black fungal masses, determine the mode of infection. Sclerotia bodies in the presence of root exudates may undergo myceliogenic germination and produce mycelia that infect the sunflower directly through the roots, causing sclerotinia wilt to develop (Huang 1985; Woodward 2016). Sclerotinia wilt affects the ability of roots to absorb water, so the first symptom is often a sudden wilting of the plant accompanied by a small, water-soaked lesion completely encircling the base of the stem near the soil line (Markell et al. 2014g). White mycelial growth and black sclerotia may be visible on or inside the lesion. Sclerotinia wilt often appears in a field on single plants, small patches of plants or plants in a row.

Carpogenic germination of sclerotia results in development of apothecia, small reproductive structures resembling a mushroom. Apothecia release sexual ascospores that are aerially disseminated and dispersed. Sunflower infection frequently begins when ascospores colonize flower tissue (Bradley et al. 2007). Mycelial spread of the pathogen from the flower tissue can cause infections of the sunflower head, resulting in sclerotinia head rot, or the stem, resulting in sclerotinia mid-stalk rot (Rashid et al. 2016). Head rot typically first appears on the back of the head and bracts as light-brown, water-soaked tissue or on the face of the head as white mycelium (Markell et al. 2014c). The head starts to appear bleached and disintegrate as

infection starts to move into the neck of the sunflower, giving it a skeletonized appearance (Bradley et al. 2007). Mid-stalk rot infections appear as small, water-soaked lesions, often near the petiole insertion on the stem (Markell et al. 2014a). Lesions will quickly expand up to 20-30 centimeters in length, and have a tan-manila color with concentric rings (Rashid et al. 2016). White mycelial growth may develop on the lesion under humid conditions. As lesions mature, plant material will disintegrate taking on a 'shredded' appearance. The presence of sclerotia on or inside the lesion is common near the end of the growing season (Bradley et al. 2007). Infected plants are more susceptible to lodging due to deterioration of the pith. The incidence and severity of each type of infection varies year to year, and is partially based on soil conditions affecting sclerotia germination and environmental conditions promoting infection and disease development (Gulya et al. 1997; Rashid et al. 2016). Saturated soils and warm temperatures promote carpogenic germination (Hao et al. 2003), thus increasing the impact of sclerotinia head rot and sclerotinia mid-stalk rot in years with high precipitation (Gulya 1996). For example, in 1986, heavy rainfalls occurred during the summer and sclerotinia head rot was observed in 98% of surveyed fields in North Dakota (N = 80), affecting an estimated 10.2% of the sunflower crop, which was a 200-fold increase from the fields surveyed in 1984 when below-average to average rainfall occurred (Gulya et al. 1989). The incidence of sclerotinia wilt is generally consistent between years in the US (Gulya 1996).

Management of diseases caused by *Sclerotinia sclerotiorum* is difficult in the US. Crop rotation is of limited value due to the long-lived nature of sclerotia, and the broad host range of the pathogen. Genetic resistance has improved in recent years, but is still insufficient for effective management. Management and/or mitigation of infection by foliar fungicides is generally considered to be ineffective.

Rhizopus Head Rot

Rhizopus head rot is caused by the fungus *Rhizopus oryzae* Fisch., *R. stolonifera* Ehrenb.:Fr. and *R. mircrosporus* Tiegh. Infections in the NGP are only known to occur from *R. oryzae* and *R. stolonifera*, with *R. oryzae* more prevalent and virulent (Gulya et al. 1997). Sporangiospores of *Rhizopus* spp. cause infection, and are considered ubiquitous and likely present in most field environments (Harveson 2016b). Rhizopus enters the head through a wound caused by hail, birds and/or insects and has been associated with head moth (*Homoeosoma electellum* Hulst.) and midge (*Contarinia schulzi* Gagné) damage (Bradley et al. 2007; Rogers et al. 1978). Frequent summer hailstorms are thought to contribute to initiation of infection (Harveson 2016b). Symptoms generally appear similar to those of other head rots caused by bacteria, *Sclerotinia* spp., and *Botrytis* spp. Initially, brown, water-soaked lesions appear on the back of the head originating from a wound (Harveson et al. 2014). Lesions will enlarge to cover the entire head, turning a dark brown as rotten tissue dries. Inside the head, fluffy white mycelia with black sporangia can be observed. In the US, Rhizopus head rot is considered a sporadic disease, but it is more common in seed production fields of California and in commercial production fields in states such as Nebraska, Colorado, Kansas and Texas where up to 100% yield loss can occur in severely affected fields under the right environmental conditions (Harveson 2016b). No adequate management tools exist for Rhizopus head rot. Rotation and tillage are generally considered ineffective as the pathogens are considered ubiquitous. Limited information on genetic resistance in commercial hybrids exists.

Downy Mildew

Downy mildew of sunflower, caused by the oomycete *Plasmopara halstedii* (Farl.) Berl. and de Toni, is an economically important seedling disease observed throughout sunflower

growing regions of the US. Prevalence and incidence vary year to year but are higher when soils are cool and wet in the spring (Markell et al. 2014f). Additionally, it is more serious in low lying areas of the field and in soils with a high water-holding capacity (Bradley et al. 2007). Two types of infection occur that cause different symptoms and have different impacts on yield: systemic infections and secondary foliar infections. The pathogen overwinters as thick-walled sexual oospores, and in spring will germinate and form motile zoospores (Humann et al. 2016). Systemic infection occurs when *P. halstedii* zoospores infect the roots of sunflower seedlings, often causing pre-emergent damping off. Seedlings that emerge will become moderately to severely stunted and rarely produce a viable head (Markell et al. 2014f). Leaves will have a puckered appearance, with chlorosis developing along the veins and the presence of white asexual spores on the undersides of infected leaves. When systemic infections occur in large patches, yield loss can be 100%; however, if infection is sporadic, adjacent sunflower plants may compensate for the stand loss (Humann et al. 2016). Secondary foliar infections occur when asexual spores from infected plants are windblown or rain-splashed onto leaves of adjacent sunflowers resulting in small, chlorotic, angular lesions to develop on leaves (Humann et al. 2016). This type of infection rarely results in any additional systemic infections and therefore is not considered economically important (Bradley et al. 2007). Management of downy mildew can be accomplished with genetic resistance and seed-applied fungicides; however, the pathogen is genetically variable and has overcome resistance genes and fungicide chemistries (Gilley and Markell 2019; Humann et al. 2016). Seed-applied fungicides provide an excellent management tool for systemic infection occurring shortly after planting. Fungicides in FRAC (Fungicide Resistance Action Committee) group 4 (such as metalaxyl-M and mefenoxam) were very effective until the late 1990's, when the pathogen evolved resistance to the chemistry.

Oxathiapiprolin (FRAC group 49) can very effectively reduce infections when applied as a seed treatment (Human et al. 2019). The FRAC group 11 fungicide azoxystrobin can suppress infection, but it is less effective than oxathiapiprolin. Crop rotation is ineffective at managing sunflower downy mildew because the oospores can survive in soils for up to 10 years (Humann et al. 2016).

Charcoal Rot

Charcoal rot, caused by the fungus *Macrophomina phaseolina* (Tassi) Goid., can be an economically important disease problem on sunflower. Severe infections can result in premature senescence, leading to reductions in head size, test weight, oil content and ultimately yield (Ryley 2016). Although charcoal rot is found in all sunflower growing regions of the US, disease development is more severe in areas with hot and dry growing conditions (Bradley et al. 2007; Gulya et al. 1997; Gulya et al. 2010). Infection can occur early in the growing season, but symptoms generally will not develop until flowering (Ryley 2016). Microsclerotia, present in the soil or on previously infected residue, will germinate in the presence of sunflower root exudates resulting in production of mycelia that infect the root system and subsequently the vascular tissue (Gulya et al. 1997). As infection spreads into the stem, the pith will compress horizontally and start to deteriorate (Bradley et al. 2007). A lesion will develop at the base of the plant, appearing brown-black near the soil line turning a gray-silver color as it progresses up the stem. Microsclerotia will develop on the infected stem internally and externally, with highest densities found closest to the soil line (Markell et al. 2014b). Additional stresses on the plant, such as high temperatures and low soil moisture, will result in more pronounced symptoms (Ryley 2016). Seed-borne infections have been documented (Raut 1983) and resulted in pre-emergent and post-emergent damping off; however, when soil temperatures were less than 35˚C,

seedling death did not occur nor did systemic infection. Minimizing water stress through irrigation, especially during flowering, will help minimize the impact of charcoal rot. Reducing inoculum with crop rotations would be beneficial, but may prove difficult in practice since many major crops, such as corn, soybean (*Glycine max* (L.) Merr.), and sorghum (*Sorghum bicolor* (L.) Moench), are also hosts to *M. phaseolina* (Ryley 2016).

Verticillium Wilt

Verticillium dahliae Kleb., the causal agent of Verticillium wilt of sunflower, is a fungus that has a worldwide distribution and a host range of more than 350 dicotyledonous species (Gulya et al. 1997). Verticillium wilt of sunflower, also called leaf mottle, can be an important disease in many sunflower growing regions globally (Gulya et al. 1997). Incidence and severity are largely determined by the amount of *V. dahliae* microsclerotia in the soil and susceptibility of the hybrid (Erreguerena et al. 2019; Hoes et al. 1973). Sunflower root exudates will stimulate the germination of microsclerotia and produce hyphae that infect sunflower roots and colonize xylem tissue of the plant. Symptoms are generally first noticed in foliar tissue, and become apparent between late vegetative to early flower growth stages (Harveson and Markell 2016). Leaf symptoms appear to progress from lower leaves to upper leaves (Markell et al. 2014d). Initially, small yellow spots will appear interveinally on a sunflower leaf, and as symptoms develop, spots will coalesce and become necrotic in the center. The "Verticillium leaf mottle" name comes from this striking contrast between the brown necrotic tissue and the healthy green tissue, divided only by a small chlorotic area. This symptom is characteristic of Verticillium wilt on sunflower (Bradley et al. 2007). The production of these chlorotic/necrotic leaf areas are from a toxin produced by the pathogen and not the pathogen itself (Gulya et al. 1997). Stem symptoms are initially only apparent internally. Cross-sections of infected stems and petioles

will have a reddish-brown ring near the outer pith, but as plants mature, the pith turns black and becomes shrunken (Markell et al. 2014d). In severely infected plants, the outside of the lower stem can turn a dark silver/gray to black color, as microsclerotia begin to colonize the outer stem tissue. Microsclerotia will also develop on the seed, and act as a primary source of infection (Harveson and Markell 2016). Management practices include using sanitized seed and avoiding fields with a history of disease. Genetic resistance has been effective historically, but a strain of V. dahliae has been identified that overcomes the V1 resistance gene found in most sunflower hybrids (Gulya 2007).

Phomopsis Stem Canker

Phomopsis stem canker of sunflower is caused by a fungal complex of *Diaporthe*/*Phomopsis* spp. and is found in all sunflower growing regions of the world (Masirevic et al. 2016; Thompson et al. 2011). In the US, *D. helianthi* Munt.-Cvetk., Mihaljč and Petrov and *D. gulyae* Shivas., Thomps. and Young are the primary causal agents of Phomopsis stem canker; however, in 2016, *D. stewartii* was also isolated from a sunflower field in Minnesota (Mathew et al. 2015; Olson et al. 2016). In the 2000s, incidence of the disease began increasing in the US and in 2010, when an outbreak of Phomopsis stem canker occurred in the NGP, 40% yield reductions were observed in some grower fields (Mathew et al. 2015). The fungus will overwinter on infected sunflower debris as mycelium and pycnidia, and in the spring, perithecia will develop (Mathew et al. 2018a). Infection begins when *Diaporthe/Phomopsis* ascospores, either wind-blown or rain-splashed, germinate in guttation drops on margins of leaves (Masirevic et al. 2016). Symptoms start to develop in the late-vegetative or early reproductive stage. Early leaf lesions appear on the margins of leaves as triangular necrotic areas surrounded by a yellow border. The pathogen will progress through the petiole and into the stem resulting in a small

brown lesion, centered on the leaf axil (Markell et al. 2014e). Stem lesions can rapidly expand up to 15-20 cm long. Stem lesions destroy internal tissue, creating a 'hollow' stem symptom and lead to an increased susceptibility of lodging for the plant (Harveson et al. 2018). Applying light finger pressure on a lesion to reveal a soft stem can be diagnostic (Markell et al. 2014e). Disease outbreaks are more severe when temperatures are 20-25˚C and frequent or abundant rainfalls occur between budding and flowering stages (Masirevic et al. 2016). Management practices are recommended that reduce the buildup of inoculum; for example, long rotations not including other host crops such as soybean (Masirevic et al. 2016; Mathew et al. 2017), burying residue to promote microbial degradation (Gulya et al. 1997) and managing weed hosts (Thompson et al. 2015). Incorporation of resistance genes and selection of resistant hybrids in greenhouse and field nurseries has led to improved hybrids in the US, but the use of resistant hybrids is complicated since differences in host susceptibility were observed between *D. helianthi* and *D. gulyae* (Mathew et al. 2018b). Several foliar fungicide modes of action can reduce disease, but often provide insufficient control.

Phoma Black Stem

Phoma macdonaldii Boerma is a fungal pathogen of *Helianthus annuus* and other wild *Helianthus* spp. (Gulya et al. 1997; Farr and Rossman 2020). In culture, pycnidia of *P. macdonaldii* are subglobose, light brown, and range in size from 150-300 microns (Gulya et al. 1997). In the US, the sexual stage, *Leptosphaeria lindquistii* Frezzi., was first reported by Donald and Venette (1985). Perithecia of *L. lindquistii* are globose and measure to 150-315 microns by 130-230 microns, and are often interspersed with pycnidia on host tissue (Gulya et al. 1997).

Phoma black stem is thought to occur wherever cultivated sunflowers are produced (Farr and Rossman 2020), but the disease is not considered economically important in most areas (Gulya et al. 1997). However, research trials in France have indicated yield losses of 8-20% (Penaud 1996; Quiroz et al. 2014). Additionally, a research trial in Argentina reported reductions in stem diameter, head diameter, yield per head, seed weight and oil content in a field trial that had high levels of Phoma black stem (Velazquez and Formento 2003). Pathogen inoculated research trials in South Dakota recorded reductions in seed weight and oil content, but yield was not significantly impacted (Carson 1991).

Phoma black stem appears on the stem as a small (5-6 centimeters) jet-black lesion with well-defined margins centered on the leaf axil (Markell et al. 2014h). These lesions occur when leaf infections develop and progress through the petiole and into the stem (Harveson et al. 2018). Infected petioles and leaves become necrotic and turn black. Additionally, lesions may develop directly on the stem when stem weevils carrying *P. macdonaldii* spores oviposit on the leaf axil, also resulting in colonization of pith tissue and increased damage (Donald 1988; Gaudet and Schulz 1981). It is common that multiple lesions form on a single stem (Markell et al. 2014h). Lesions on older lower leaves develop quicker than lesions on newer leaves (Larfeil et al. 2010), which might explain why lesions begin to appear on the lower stem first. Symptoms of Phoma black stem can be confused with those of Phomopsis stem canker. Both diseases begin as a leaf lesion that spreads through the petiole and into the stem. The dark colored lesions that form on the lower stem can be hard to differentiate early in their development. This similarity can lead to a misdiagnosis between the two diseases. As lesions develop, differentiation becomes easier. The expanding brown Phomopsis stem canker lesions will have less of a defined margin and be much larger (up to 20 centimeters in length) than the small (5-6 centimeters) black lesions of

Phoma black stem (Harveson et al. 2018). Lesions of Phoma black stem are often superficial, rarely developing into the pith, while Phomopsis stem canker lesions cause severe degradation of the pith and vascular tissue, resulting in weak stems that can be easily crushed with little pressure.

P. macdonaldii overwinters on infected crop residues as mycelia and pycnidia, and perithecia develop in the spring (Gulya et al. 1997). Throughout the growing season, perithecia will release ascospores that can infect leaf tissue of surrounding sunflower plants. Ascospores are aerially dispersed and can travel to adjacent fields (Charlène et al. 2012; Schwanck 2016b). Infection can occur at any plant stage, but lesions do not generally develop until flowering (Schwanck et al. 2016a). *Phoma* infected leaf tissue is difficult to distinguish from normal leaf senescence and other diseases; therefore, the disease is not accurately visually diagnosable until lesions develop on the stem. Pycnidia may develop on seed, resulting in introduction of the pathogen into new areas as was suggested what happened in the Xinjiang province in China (Wu et al. 2012).

Long rotations and tillage practices that bury residues will reduce buildup of *Phoma*-infected residues in soils (Schwartz and Markell 2016). Debaeke and Pérès (2003) showed that dense canopies facilitate higher disease intensity, and adjustments to plant populations will have an effect on incidence and severity of Phoma black stem lesions. Insect control may be beneficial since seed weevils have the ability to transmit *Phoma* spores (Gaudet and Schulz 1981). Sunflower lines with partial resistance to Phoma black stem have been identified (Bordat et al. 2017), but commercial hybrids in the US are not given resistance ratings. In the US, fungicide management strategies have not been researched; however, Yugoslavian trials have

indicated that thiophanate methyl, chlorothalonil and mancozeb were effective at reducing Phoma black stem in seed production fields (Gulya et al. 1997).

National Sunflower Association (NSA) Survey

Throughout the 1980s-1990s, disease surveys within the NGP (Gulya 1996; Lamey et al. 2001) and production questionnaires (Lamey et al. 1992, 1993, 1999) were evidence for the NSA that the sunflower industry could greatly benefit from a nationwide comprehensive survey. For example, Gulya (1996) was able to see that prevalence of some diseases, such as rust and Sclerotinia head rot, fluctuated greatly year to year, while other diseases, such as Sclerotinia wilt, were consistent. Additionally, it was recognized that Phomopsis stem canker was becoming a growing problem for producers and needed combative action. In 1984, shortly after the discovery of the pathogen in the US, the disease was found in 0.6% of fields in the NGP, and by 1995 it was found in 82% of fields (Gulya 1996).

In 2002, a national effort to evaluate sunflower production practices and identify production issues in the US was supported by the NSA. The NSA is a nonprofit commodity group based in Mandan, ND that works to solve the problems arising within the sunflower industry and create opportunities for growth and development (https://www.sunflowersnsa.com). The NSA survey was primarily designed to identify yield-limiting factors in sunflower production (Gulya et al. 2019). Data on weeds, diseases, insects, bird damage, field management and yield components were collected from all sunflower growing regions in the US (Gulya and Mathew 2018). Between 2002 and 2013, the national survey was conducted annually (with the exception of 2004). Between 2015 and 2019, the survey was conducted only on odd number years. The survey is conducted in all major sunflower growing states, including North Dakota, Minnesota, South Dakota, Nebraska, Colorado, Kansas and Texas and in the Canadian province

of Manitoba. Periodically, other states were included in the survey; however, those states often had few fields sampled. The number of fields selected within each state (and county) is based on a proportion of sunflower acres planted. Initially, one field was surveyed for every 2,023 hectares of sunflower planted (Gulya 2003), but this high geo-spatial density of surveying proved to be excessively intensive and was changed to one field for every 4,046 hectares in 2003 (Gulya 2004). In recent years, North Dakota State University Extension has led and coordinated the NSA survey effort.

The NSA survey is an in-field survey conducted by volunteer experts from multiple disciplines. However, the wide geographic distribution of sunflower created a logistical problem because it meant that a large number of surveyors would be needed and that they would need to be spread out across all growing regions (Gulya et al 2019). Additionally, the survey was designed to be comprehensive: the goal was to identify pests across a wide range of disciplines (weeds, diseases, and insects). However, that meant surveyors needed to have a wide range of diagnostic skills to be able to accurately identify many types of pests. It is uncommon for individuals to have such a diverse skill-set spanning across multiple disciplines. Therefore, within each region, surveys were conducted in teams of two or more individuals from varying disciplines.

To ensure consistent and accurate data collection across teams, one-day courses were developed where researchers would teach surveyors diagnostic skills. Initially, courses were held in multiple states, but as surveyors became more experienced and remote technology became more efficient, online-training presentations replaced most in-field courses (Gulya et al. 2019). Once a field was selected, researchers would collect data from two separate sites that were visually representative of the field as a whole. Observations at each site were done on two

adjacent sunflower rows 7.62 meters long. Data collected in each field included hybrid type (oilseed/confection), previous crop, row spacing, irrigation/rainfed, tillage, plant population, head size, head fill (the area of the head in which seed actually develops), seed size, percent of seeds with kernel development, and bird damage. Insect, weed and disease data were also collected at each site. From 2002-2005, diseases evaluated in the NSA survey included Sclerotinia head rot, Sclerotinia wilt, Sclerotinia mid-stalk rot, Phomopsis stem canker, Rhizopus head rot, downy mildew, rust, charcoal rot, and Verticillium wilt (Gulya 2003). In 2006, Phoma black stem was added to the list of diseases surveyed (Berglund 2007b). Results of the survey are presented at the National Sunflower Association Annual Research Forum in Fargo, ND (Gulya et al. 2019).

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CHAPTER 1: EFFECT OF FUNGICIDE AND TIMING OF APPLICATION ON MANAGEMENT OF PHOMA BLACK STEM

Introduction

Phoma black stem, caused by *Phoma macdonaldii* Boerema (teleomorph *Leptosphaeria lindquistii* Frezzi), is one of the most prevalent diseases of sunflower (*Helianthus annuus* L.) in the US Northern Great Plains (NGP) region. Phoma black stem was present in 66.8% of North Dakota fields surveyed between 2006-2017 (N = 1003) (Hansen nonpublished [Chapter 2]). The fungus overwinters on infected residues as perithecia, pycnidia or mycelium (Gulya et al. 1997). Phoma black stem lesions result when ascospores infect through the margins of leaves and progress into the stem through the petiole, leaving necrotic tissue behind as it develops (Harveson et al. 2018). A black circular lesion with distinct margins, often no bigger than 5-6 centimeters, forms on the stem centered over the petiole (Markell et al. 2018). Multiple infections may occur on a single plant. Lesions may coalesce to completely encircle the stem. Infection can occur at any growth stage, but epidemics generally occur after flowering (Schwartz and Markell 2016). Typically, lesions will begin developing on the lower stem first, and gradually progress higher up the stem. When infections are located on the base of the stem, premature ripening may occur, resulting in loss of leaf tissue and stem greenness (Donald 1988).

Phoma black stem has been yield-limiting in some sunflower growing regions internationally. In France during the 1990s, short rotations away from sunflower and reduced tillage practices were implicated as major reasons the number of Phoma black stem outbreaks increased at that time (Pérès and Poisson 2000) and losses as high as 20% were recorded through the early-2000s. (Penaud 1996; Quiroz et al. 2014). In Argentina, Velazquez and Formento (2003) reported reductions in stem diameter, head diameter, seed weight, oil yield and grain

yield in a field trial with high levels of Phoma black stem. However, the disease has not been thought to be yield-limiting in the US (Carson 1991). In the US, producers often do not target disease management practices directly at Phoma black stem, but management strategies currently practiced may provide a level of management for Phoma black stem. Crop rotations are used to reduce the buildup of pathogen inoculum that cause soil-borne diseases like downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni), sclerotinia diseases (*Sclerotinia sclerotiorum* Lib.), and Phomopsis stem canker (*Phomopsis* spp.) Longer rotations also help reduce Phoma black stem since the primary inoculum survives on infected sunflower residues. Tillage practices that bury debris infected with *P. macdonaldii* promote its breakdown, therefore reducing the inoculum source. There is a lack of research investigating the effect of fungicides on Phoma black stem. However, in Yugoslavia, research in seed production fields have shown that thiophanate methyl (FRAC 1: methyl benzimidazole carbamate), chlorothalonil (FRAC M: multi-site activity), and mancozeb (FRAC M: multi-site activity) can be effective at reducing Phoma black stem (Gulya et al. 1997), but these chemicals are not labeled for sunflower in the US.

In 2017, a natural epidemic of Phoma black stem occurred in a research trial designed to investigate the effect of fungicide timing on Phomopsis stem canker management in North Dakota. In that study, higher yields and reduced Phoma incidence were observed with multiple fungicide treatments. Thus, it is prudent to investigate the impact of Phoma black stem to determine if unappreciated losses are being incurred by producers in the NGP. The objectives of this study were to determine fungicide efficacy and timing of application on management of Phoma black stem and determine the effect on sunflower yield.

Materials and Methods

Locations and Experimental Design

Field trials were conducted at two sites in North Dakota: Davenport (DAV), and the NDSU Carrington Research Extension Center (CAR) (Table 1.1). Locations had a history of Phoma black stem and trials relied on natural inoculum to incite epidemics. Two fungicide timing trials, differing only by hybrid, were conducted each year at DAV from 2017 to 2019, and at CAR in 2018 and 2019. Hybrids were selected based on their level of resistance to Phomopsis stem canker. Phomopsis stem canker resistance was used as a proxy since sunflower hybrids are not given Phoma black stem resistance scores and infection of both diseases develop in a similar manner. However, it is unclear if resistance to Phomopsis stem canker has any impact on (or is correlated to) resistance to Phoma black stem. Each year, one of the trials was planted with a moderately susceptible (PscMS) hybrid and the other was planted with a moderately resistant hybrid (PscMR). The PscMS hybrids used in this study were MY8N421CLDM (Mycogen Seeds, San Diego, CA) and N4HM354 (Nuseed, Breckenridge, MN), and PscMR hybrids used included MY8N449CLDM (Mycogen Seeds, San Diego, CA) and Camaro II (Nuseed, Breckenridge, MN). Fungicide efficacy trials were conducted from 2018 to 2019 at DAV and CAR, and were planted with N4HM354. All trials were non-irrigated and designed in a randomized complete block with four replications. Each plot consisted of four rows with a 76.2 cm row width. Previous crop, planting date, plot length and harvest date varied by location, and sunflower hybrid varied by year (Table 1.1).

Table 1.1. Location, trial type, agronomic information, and growth stages of fungicide application in Phoma black stem fungicide trials conducted in North Dakota from 2017 to 2019.

Loc[z]	Trial Type	Prev Crop[y]	Planting date	Hybrid[x]	Growth stage of fungicide application[v]	Plot length (m)	Row width (cm)	Harvest date
DAV	Timing	S. Beet	18 May 17	MY8N421 (PscMS)	V8-V10, R1, R5	10.4	76.2	12 Oct 17
DAV	Timing	S. Beet	18 May 17	MY8N449 (PscMR)	V8-V10, R1, R5	10.4	76.2	12 Oct 17
DAV	Timing	S. Beet	21 May 18	N4HM354 (PscMS)	V8-V10, R1, R5	10.4	76.2	18 Oct 18
DAV	Timing	S. Beet	21 May 18	Camaro II (PscMR)	V8-V10, R1, R5	10.4	76.2	18 Oct 18
DAV	Efficacy	S. Beet	21 May 18	N4HM354 (PscMS)	R1	10.4	76.2	18 Oct 18
CAR	Timing	Flax	07 Jun 18	N4HM354 (PscMS)	V8-V10, R1, R5	4.6	76.2	01 Nov 18
CAR	Timing	Flax	07 Jun 18	Camaro II (PscMR)	V8-V10, R1, R5	4.6	76.2	01 Nov 18
CAR	Efficacy	Flax	07 Jun 18	N4HM354 (PscMS)	R1	4.6	76.2	01 Nov 18
DAV	Timing	Corn	21 May 19	N4HM354 (PscMS)	V8-V10, R1, R5	10.4	76.2	18 Oct 19
DAV	Timing	Corn	21 May 19	Camaro II (PscMR)	V8-V10, R1, R5	10.4	76.2	18 Oct 19
DAV	Efficacy	Corn	21 May 19	N4HM354 (PscMS)	R1	10.4	76.2	18 Oct 19
CAR	Timing	Canola	04 Jun 19	N4HM354 (PscMS)	V8-V10, R1, R5	4.6	76.2	No data
CAR	Timing	Canola	04 Jun 19	Camaro II (PscMR)	V8-V10, R1, R5	4.6	76.2	No data
CAR	Efficacy	Canola	04 Jun 19	N4HM354 (PscMS)	R1	4.6	76.2	No data

[z] Loc = Location – DAV = Davenport, ND; CAR = Carrington Research Extension Center, Carrington, ND.
[y] Prev Crop = Previous Crop – S. Beet = Sugarbeet (*Beta vulgaris* L.); Flax (*Linum usitatissimum* L.); Corn (*Zea mays L.*); Canola (*Brassica napus* L.).
[x] Hybrid used across the entire trial. Phomopsis stem canker resistance ratings (in parenthesis, [PscMS = moderately susceptible; PscMR = moderately resistant]) was used as a proxy for resistance to Phoma black stem.
[v] Growth stages are based on Schneiter and Miller (1981).

Fungicide Timing

Ten trials were conducted between 2017 and 2019 to investigate timing of fungicide application on management of Phoma black stem. Two trials, one with a PscMS hybrid and one with a PscMR hybrid, were established at each site per year. Fungicide applications were made singly and/or in combination at three growth stages as described by Schneiter and Miller (1981): V8-V10 (late-vegetative), R1 (budding), and R5 (flowering). Timings were selected, in part, because these growth stages coincide with other established sunflower production practices. Herbicide applications can take place during late-vegetative growth stages before the canopy fully closes, fungicide applications for management of Phomopsis (Olson 2017) and non-disease plant health effects have been recommended at the budding stage, and a pesticide application for management of sunflower rust (*Puccinia helianthi* Schw.) and seed insects have been

recommended at the flowering stage (Friskop et al. 2015; Knodel et al. 2015). All fungicide applications were made with pyraclostrobin (Headline, BASF, Research Triangle Park, NC) at 109.2 grams of active ingredient per hectare, as efficacy had been established in previous studies (Gilley et al. 2018). A non-treated control (NTC), in which no applications were made, was also included in each trial. Trials had fungicide applied in a 187 liter per hectare suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer. The boom had three 8002 Teejet flat fan nozzles with a 50.8 cm nozzle spacing. Spray was targeted to all four rows of a plot.

Fungicide Efficacy

Four trials were conducted between 2018 and 2019 to investigate the efficacy of nine different fungicides on Phoma black stem (Table 1.2). Fungicides used included chemicals from at least one of three FRAC groups: FRAC 3 (demethylation inhibitors – DMIs), FRAC 7 (succinate dehydrogenase inhibitors – SDHIs) and FRAC 11 (quinone outside inhibitors – QoIs). Fungicides included in the trials were DMIs; tebuconazole (Folicur, Bayer CropScience, Research Triangle Park, NC) and metconazole (Quash, Valent USA, Walnut Creek, CA), SDHIs; pydiflumetofen (Miravis, Syngenta Crop Protection, Greensboro, NC) and boscalid (Endura, BASF, Research Triangle Park, NC) and QoIs; azoxystrobin (Quadris, Syngenta Crop Protection), picoxystrobin (Aproach, DuPont Agricultural Products, Wilmington, DE) and pyraclostrobin (Headline, BASF). Other fungicides included were a combination of a SDHI and DMI; fluopyram and tebuconazole (Luna Experience, Bayer CropScience), a combination of a QoI and SDHI; pyraclostrobin and fluxapyroxad (Priaxor, BASF) and a combination of a QoI, SDHI and DMI; pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF). A treatment without any type of application was included in every trial as the nontreated control

(NTC). All fungicides were applied at the R1 growth stage. Fungicide application was carried out in the same manner as described above.

Table 1.2. Trade name, mode of action, FRAC group, active ingredient and rate of fungicides used in Phoma black stem efficacy trials in North Dakota from 2018 and 2019.

Tradename	Mode of Action[z]	FRAC[y] group	Active ingredient	Rate[x]
Headline	QoI	11	Pyraclostrobin	109.8 g ai/ha
Aproach	QoI	11	Azoxystrobin	109.2 g ai/ha
Quadris	QoI	11	Picoxystrobin	109.2 g ai/ha
Priaxor	QoI	11	Pyraclostrobin	97.4 g ai/ha
	SDHI	7	Fluxapyroxad	48.7 g ai/ha
Miravis	SDHI	7	Pydiflumetofen	150.6 g ai/ha
Endura	SDHI	7	Boscalid	392.3 g ai/ha
Luna Experience	SDHI	7	Fluopyram	131.6 g ai/ha
	DMI	3	Tebuconazole	131.6 g ai/ha
Luna Experience	SDHI	7	Fluopyram	187.2 g ai/ha
	DMI	3	Tebuconazole	187.2 g ai/ha
Folicur	DMI	3	Tebuconazole	126.1 g ai/ha
Quash	DMI	3	Metconazole	140.1 g ai/ha
Revytek	QoI	11	Pyraclostrobin	103.7 g ai/ha
	SDHI	7	Fluxapyroxad	51.8 g ai/ha
	DMI	3	Mefentrifluconazole	77.8 g ai/ha

[z] Fungicide mode of actions. QoI – Quinone outside inhibitors; SDHI – Succinate dehydrogenase inhibitors; DMI – demethylation inhibitors.
[y] Fungicide Resistance Action Committee classification of fungicides (FRAC 2020).
[x] Rate of fungicide applied in grams of active ingredient per hectare.

Data Collection

Plots were rated for Phoma black stem at the R7 growth stage in 2017 and additionally at the R8 growth stage (Schneiter and Miller 1981) in 2018 and 2019. Disease ratings were done on ten randomly selected sunflower plants from the center two rows of each plot. Incidence of a plot was calculated as the percent of plants with at least one Phoma stem lesion, and severity was calculated as the average number of stem lesions per diseased plant. A disease index (DSI) was

calculated for each plot by multiplying incidence and severity. The middle two rows of the plot were harvested for yield and adjusted to be based on a ten percent moisture content.

Statistical Analysis

A generalized linear model (PROC GLM) was used to analyze trials with disease pressure individually using SAS (version 9.4; SAS Institute, Cary, NC, U.S.A.). Fisher's protected least significant difference at $\alpha = 0.05$ was used to determine significant differences between treatments. Following the results of individual trial analyses, combined analyses was conducted using a generalized linear mixed model (PROC GLIMMIX) for timing trials and efficacy trials. Fungicide timing and fungicide represented the fixed effects in the combined analyses of the timing trial and efficacy trial, respectively. Random effects included trial, replications within trial, and trial by treatment. If treatment effects were detected, differences between individual treatment pairs were determined using pairwise t-tests of least-square means at $\alpha = 0.05$. Yields across experiments varied and a PROC GLIMMIX analysis was used to categorize trials into environments: high-yielding, moderate-yielding, or low-yielding. Yield loss relationships were assessed using regression analyses (PROC REG) on each environment separately by comparing the treatment means of disease index values at R7 and the treatment means of yield.

Results

Timing Trials

In 2017, a natural epidemic of Phoma black stem occurred in the trials at DAV. The DSI of the NTC was 6.6 and 4.8 for the PscMS and PscMR hybrids, respectively, which was among the highest in the three years study (Table 1.3). Equal DSI values were observed in the NTC and the R5 timing in the PscMS hybrid in 2017. The lowest DSI values were observed in treatments

that included an R1 timing application. Yield was not different among treatments. Differences in DSI and yield were observed among different timing treatments in the PscMR hybrid in 2017. A lower DSI was observed for all timings, except V8-V10. Higher yields were observed for a single application at R1 and for treatments with combination timings (V8-V10 + R1, V8-V10 + R5, R1 + R5 and V8-V10 + R1 + R5).

Table 1.3. Disease severity index and yield of Phoma black stem fungicide timing trials conducted in Davenport, ND 2017.

Growth stage of fungicide application[z]	MY8N421CLDM		MY8N449CLDM	
	R7 DSI[y]	Yield (kg/ha)	R7 DSI	Yield (kg/ha)
NTC	6.6 a[x]	3472	4.8 a	3679 b
V8-V10	3.8 b	3395	5.3 a	3719 b
R1	1.8 cd	3453	1.0 c	4001 a
R5	5.8 a	3277	3.5 b	3658 b
V8-V10+R1	1.1 d	3707	0.5 c	4050 a
V8-V10+R5	2.9 bc	3552	2.8 b	3948 a
R1+R5	2.3 bcd	3389	1.0 c	4004 a
V8-V10+R1+R5	1.3 d	3570	0.7 c	3967 a
p-value	<0.0001	0.5639	<0.0001	<0.0001

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.
[y] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.
[x] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

No disease established at CAR in 2018; therefore, no data is available to present. High levels of Phoma black stem infections occurred at DAV in 2018, with R7 DSI values of the NTC at 4.8 and 4.2 in the PscMS and PscMR hybrids, respectively (Table 1.4). Differences in disease index were observed among treatments in the PscMS hybrid in 2018. The DSI for the R1 timing was equal to the combination timings, and all fungicide treatments were lower than the NTC at

the R8 rating. Yields were not different. In the PscMR hybrid in 2018, higher DSI was observed in the NTC than all other timings at both the R7 and R8 ratings (Table 1.4). Lower DSI was observed in the V8-V10 and R1 application timings than the R5 timing at both ratings. Yield was not different for any of the application timings.

Table 1.4. Disease severity index and yield of Phoma black stem fungicide timing trials conducted in Davenport, North Dakota, 2018.

Growth stage of fungicide application[z]	N4HM354			Camaro II		
	R7 DSI[y]	R8 DSI	Yield (kg/ha)	R7 DSI	R8 DSI	Yield (kg/ha)
NTC	4.8 a[x]	7.9 a	3564	4.2 a	6.0 a	3558
V8-V10	2.5 b	4.2 b	3734	1.4 c	2.9 c	3934
R1	2.2 b	2.2 cd	3613	1.4 cd	2.5 c	3494
R5	3.7 a	4.7 b	3917	3.6 b	4.3 b	3188
V8-V10+R1	0.8 c	1.1 cd	4104	0.6 ef	1.1 de	3821
V8-V10+R5	1.5 bc	2.4 c	3639	1.0 cd	1.9 cd	3655
R1+R5	1.8 bc	2.2 cd	3852	1.0 de	2.0 cd	3698
V8-V10+R1+R5	1.8 bc	2.5 c	3795	0.2 f	0.8 e	3787
p-value	<0.0001	<0.0001	0.1588	<0.0001	<0.0001	0.0755

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.
[y] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.
[x] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Very low levels of disease established in 2019 at the CAR. Consequently, disease ratings only occurred at the R8 growth stage. The DSI of the NTC was 1.0 and 0.8 for the PscMS and PscMR hybrids, respectively. (Table 1.5). In both trials, equal DSI values were observed in the R5 timing and the NTC. Yield data was not collected in CAR because a snow storm at the end of the growing season prevented harvest.

Table 1.5. Disease severity, incidence and disease severity index of Phoma black stem fungicide timing trials conducted in Carrington, North Dakota, 2019.

	N4HM354			Camaro II		
Growth stage of fungicide application[z]	R8 Severity[y]	R8 Incidence[x]	R8 DSI[w]	R8 Severity	R8 Incidence	R8 DSI
NTC	1.5 a[v]	65.0 a	1.0 a	1.3 a	55.0 a	0.8 a
V8-V10	0 c	0 c	0 b	0 b	0 b	0 b
R1	0.8 b	17.5 b	0.2 b	0.5 b	5.0 b	0.1 b
R5	1.4 a	57.5 a	0.9 a	1.4 a	50.0 a	0.7 a
V8-V10+R1	0 c	0 c	0 b	0 b	0 b	0 b
V8-V10+R5	0.3 c	2.5 c	0 b	0 b	0 b	0 b
R1+R5	0.5 bc	7.5 bc	0.1 b	0.5 b	15.0 b	0.2 b
V8-V10+R1+R5	0 c	0 c	0 b	0 b	0 b	0 b
p-value	>0.0001	>0.0001	>0.0001	>0.0001	>0.0001	>0.0001

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.
[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.
[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.
[w] DSI = Disease severity index is calculated from a plot as incidence times severity.
[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Results of the DAV PscMS hybrid in 2019 demonstrated a lower DSI for the R1 timing than the V8-V10 and R5 timings for both ratings (Table 1.6). Yields between treatments were not different. Results of the PscMR hybrid in 2019 showed the DSI ratings for the R1 timing and all combination applications were equal, and were lower than the V8-V10 and R5 timing (Table 1.6). Differences between yields were not observed.

Table 1.6. Disease severity index and yield of Phoma black stem fungicide timing trials conducted in Davenport, North Dakota, 2019.

Growth stage of fungicide application[z]	N4HM354			Camaro II		
	R7 DSI[y]	R8 DSI	Yield (kg/ha)	R7 DSI	R8 DSI	Yield (kg/ha)
NTC	3.2 a[x]	5.0 a	2357	3.1 a	6.0 a	2232
V8-V10	2.2 b	4.4 a	2246	2.1 b	4.6 b	2207
R1	1.1 c	1.5 c	2491	0.5 cd	1.2 cd	2124
R5	2.9 a	4.1 a	2500	2.7 ab	4.1 b	2201
V8-V10+R1	0.2 d	1.2 c	2398	0.2 d	0.7 d	2199
V8-V10+R5	1.8 b	2.6 b	2334	0.9 c	2.2 c	2423
R1+R5	0.6 cd	1.5 c	2456	0.7 cd	1.4 cd	2303
V8-V10+R1+R5	0.3 d	0.6 c	2338	0.2 d	0.6 d	2266
p-value	<0.0001	<0.0001	0.8863	<.0001	<0.0001	0.9316

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Timing trials with disease development by R7 were included in a combined analysis; namely, the 2017 DAV PscMS trial, the 2017 DAV PscMR trial, the 2018 DAV PscMS trial, the 2018 DAV PscMR trial, the 2019 DAV PscMS trial and the 2019 DAV PscMR trial. The combined analysis indicated the DSI of fungicide applications at R5 were not different than at the NTC (Table 1.7). Of the single fungicide applications, the lowest DSI was observed with R1 fungicide applications. No difference in DSI was observed between the R1 application and any of the combination applications. However, incidence of all combination treatments that included an R1 application were lower than all combination treatments that did not include an R1 application. Similarly, severity was lower in combination treatments when an R1 timing was included, except for the R1+R5 treatment. Yield was not significantly different when analyzed at $\alpha = 0.05$; however, differences were observed at $\alpha = 0.1$. Yields higher than the NTC were

observed in treatments with multiple fungicide applications that included R1; namely, V8-V10+R1, R1+R5 and V8-V10+R1+R5.

Table 1.7. Disease severity, incidence, disease severity index and yield of six Phoma black stem fungicide timing trials conducted between 2017 and 2019 in North Dakota.

Growth stage of fungicide application[y]	Timing Trials Combined[z]							
	R7 Severity[x]		R7 Incidence[w]		R7 DSI[v]		Yield (kg/ha)	
NTC	4.4	a[u]	100.0	a	4.4	a	2801	c[t]
V8-V10	3.0	b	92.1	a	2.9	b	2861	bc
R1	1.8	cde	67.9	bc	1.3	cd	2851	bc
R5	3.9	a	93.8	a	3.7	a	2786	c
V8-V10+R1	1.2	e	38.8	d	0.6	d	3015	a
V8-V10+R5	2.3	bc	75.8	b	1.8	c	2899	abc
R1+R5	1.9	cd	59.2	c	1.2	cd	2929	ab
V8-V10+R1+R5	1.4	de	40.8	d	0.7	d	2936	ab
p-value	<0.0001		<0.0001		<0.0001		0.0726	

[z] Timing trials included in the analysis were the 2017 Davenport PscMS trial, the 2017 Davenport PscMR trial, the 2018 Davenport PscMS trial, the 2018 Davenport PscMR trial, the 2019 Davenport PscMS trial and the 2019 Davenport PscMR trial.

[y] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[x] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[w] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[v] DSI = DSI = Disease severity index is calculated from a plot as incidence times severity.

[u] Comparisons of severity, incidence and disease severity index were made using pairwise comparisons between all treatments at an $\alpha = 0.05$. Means with the same letter are not significantly different.

[t] Comparisons of yield were made using pairwise comparisons between all treatments at an $\alpha = 0.10$. Means with the same letter are not significantly different.

Efficacy Trials

In 2018, disease pressure was high in DAV, with R7 DSI of the NTC being 6.8 (Table 1.8). The DSI was higher for the NTC than all fungicide treatments, with the exception of tebuconazole, at the R8 rating. Higher DSI was observed in the DMIs as compared to any fungicide that included an SDHI. The lowest DSI of the three QoIs was pyraclostrobin, which was statistically equal to all premix fungicide combinations. The DSI values were lowest for

pydiflumetofen and boscalid at the R8 rating. No disease established in the efficacy trial at CAR in 2018; therefore, no data is available to present.

Table 1.8. Disease severity index and yield values for the 2018 and 2019 fungicide efficacy trials conducted in Davenport, ND

	2018 N4HM354					2019 N4HM354				
Fungicide[z]	R7 DSI[w]		R8 DSI		Yield (kg/ha)	R7 DSI		R8 DSI		Yield (kg/ha)
NTC	6.8	a[v]	8.7	a	2724	3.2	a	5.7	a	1935
PYR	1.8	fgh	3.4	ef	3200	1.5	bcd	2.3	de	2214
AZO	3.1	de	4.2	de	3221	1.2	cde	3.8	bc	1872
PIC	3.7	cd	5.3	cd	3045	1.8	bc	2.7	cde	2296
TEB	5.3	b	7.3	ab	2948	3.0	a	4.9	ab	2092
PYD	0.2	i	0.6	h	3115	0.2	f	0.3	g	1981
BOS	0.8	hi	1.4	gh	3012	0.4	ef	0.3	g	1779
FLU+TEB[y]	2.5	def	4.3	de	3045	0.8	def	2.2	def	2190
FLU+TEB[x]	2.2	efg	3.3	ef	2759	0.6	ef	1.6	ef	1976
PYR+FLX	1.5	fgh	3.1	ef	2452	1.0	de	1.7	ef	2106
PYR+FLX+MEF	1.3	ghi	2.4	fg	2997	0.8	def	0.9	fg	2257
MET	4.5	bc	6.3	bc	2902	2.1	b	3.4	cd	1802
p-value	<0.0001		<0.0001		0.1467	<0.0001		<0.0001		0.0704

[z] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.

[x] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

In the 2019 DAV efficacy trial, differences in DSI were observed among treatments (Table 1.8). For both DSI ratings, tebuconazole was the only fungicide not different than the NTC. The DSI values for pydiflumetofen and boscalid were lower than all other fungicides, except pyraclostrobin + fluxapyroxad + mefentrifluconazole, at the R8 rating. The efficacy trial at CAR in 2019 had low disease pressure; therefore, ratings were only conducted at the R8

growth stage. The highest severity was observed in the NTC, with a value of 1.4 (Table 1.9). Equal levels of incidence and DSI were observed between the NTC and tebuconazole. Yield data is not available because an early snowstorm prevented harvest before winter.

Table 1.9. Severity, incidence and disease severity index of Phoma black stem fungicide efficacy trials in Carrington, ND 2019.

Fungicide[z]	2019 Carrington					
	R8 Severity[w]		R8 Incidence[v]		R8 DSI[u]	
NTC	1.4	a[t]	20.0	a	0.3	a
PYR	0.3	bc	2.5	c	0	c
AZO	0	c	0	c	0	c
PIC	0.3	bc	2.5	c	0	c
TEB	0.7	b	12.5	ab	0.2	ab
PYD	0	c	0	c	0	c
BOS	0.5	bc	5.0	bc	0.1	bc
FLU+TEB[y]	0.3	bc	2.5	c	0	c
FLU+TEB[x]	0	c	0	c	0	c
PYR+FLX	0	c	0	c	0	c
PYR+FLX+MEF	0	c	0	c	0	c
MET	0.3	bc	2.5	c	0	c
p-value	0.0007		0.0017		0.0023	

[z] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.

[x] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.

[w] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[v] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[u] DSI = DSI = Disease severity index is calculated from a plot as incidence times severity.

[t] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

A combined analysis of efficacy trials with disease development by R7, namely the 2018 DAV trial and the 2019 DAV trial, indicated that tebuconazole was the only fungicide with a DSI value the same as the NTC (Table 1.10). The R7 DSI levels were the same among

pyraclostrobin, pydiflumetofen, boscalid, and all the multiple site of action fungicides. Pydiflumetofen, boscalid and pyraclostrobin + fluxapyroxad + mefentrifluconazole had the lowest DSI levels at R8.

Table 1.10. Disease severity index and yield of two Phoma black stem fungicide efficacy trials conducted in North Dakota in 2018 and 2019.

	Efficacy Trials Combined[z]				
Fungicide[y]	R7 DSI[v]		R8 DSI		Yield (kg/ha)
NTC	5.0	a[u]	7.2	a	2332
PYR	1.6	cdef	2.9	def	2710
AZO	2.2	cde	4.0	cd	2549
PIC	2.7	bcd	4.0	cd	2673
TEB	4.2	ab	6.1	ab	2522
PYD	0.2	f	0.5	g	2550
BOS	0.6	ef	0.8	g	2397
FLU+TEB[x]	1.7	cdef	3.2	de	2620
FLU+TEB[w]	1.4	def	2.4	ef	2370
PYR+FLX	1.2	def	2.4	ef	2281
PYR+FLX+MEF	1.1	def	1.6	fg	2630
MET	3.3	bc	4.8	bc	2354
p-value	0.0015		<0.0001		0.3970

[z] Trials included in this combined analysis were the 2018 Davenport efficacy trial and 2019 Davenport efficacy trial.
[y] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.
[x] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.
[w] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.
[v] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.
[u] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Yield Loss Assessment

Yield loss assessments only included trials with disease development by R7; namely, the 2017 DAV PscMS timing trial, 2017 DAV PscMR timing trial, 2018 DAV PscMS timing trial, 2018 DAV PscMR timing trial, 2018 DAV efficacy trial, 2019 DAV PscMS timing trial, 2019

DAV PscMR timing trial and the 2019 DAV efficacy trial. Trials were categorized into low-yielding, moderate-yielding and high-yielding environments based on the mean yield of each trial (Table 1.11). Each of the three environments were regressed to determine if a relationship between DSI and yield existed. The R7 DSI significantly affected yield in the high-yielding environment ($p = 0.0018$; $R^2 = 0.2811$), but the relationship wasn't significant in the moderate-yielding ($p = 0.5306$; $R^2 = 0.0405$) or low-yielding ($p = 0.9426$; $R^2 = 0.0002$) environments (Figure 1.1). Since a significant relationship was indicated in the high-yielding environment, an additional linear regression analysis was performed, in which the R7 DSI was regressed against the treatment means of yield as a percent of the NTC (Figure 1.2). A 1.8% loss in yield was observed for every one unit increase in DSI at R7 ($p = 0.0004$; $R^2 = 0.3425$).

Table 1.11. Yield of the timing and efficacy trials.

Year	Trial Type	Hybrid	No of Trts[z]	R7 DSI[y]	Yield (kg/ha)		Yield[x]
2017	Timing	MY8N421CLDM	8	3.2	3102	c[w]	High
2017	Timing	MY8N449CLDM	8	2.5	3460	a	High
2018	Timing	N4HM354	8	2.4	3370	ab	High
2018	Timing	Camaro II	8	1.7	3249	bc	High
2018	Efficacy	N4HM354	12	2.8	2633	d	Moderate
2019	Timing	N4HM354	8	1.5	2132	e	Low
2019	Timing	Camaro II	8	1.3	2002	e	Low
2019	Efficacy	N4HM354	12	1.4	1821	f	Low
p-value				0.0717	<0.0001		
CV				77.6	24.0		

[z] No of Trts = the number of treatments in each trial.
[y] DSI = Disease severity index is calculated from a plot as incidence times severity. Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion. Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.
[x] Trials were categorized into low-yielding, moderate-yielding and high-yielding environments based on the statistical groupings of the average yield of each trial.
[w] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

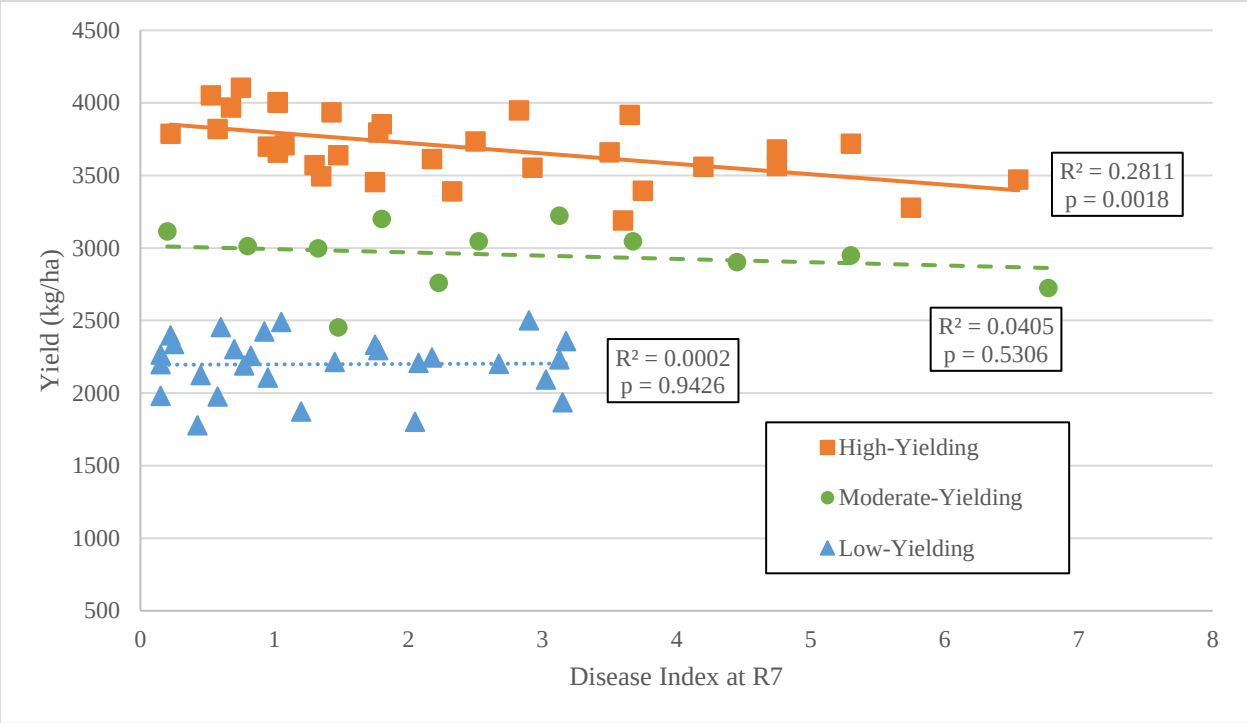

Figure 1.1. Yield loss assessment correlating Phoma black stem disease index values at R7 and yield values of treatment means for high, moderate and low-yielding trials.

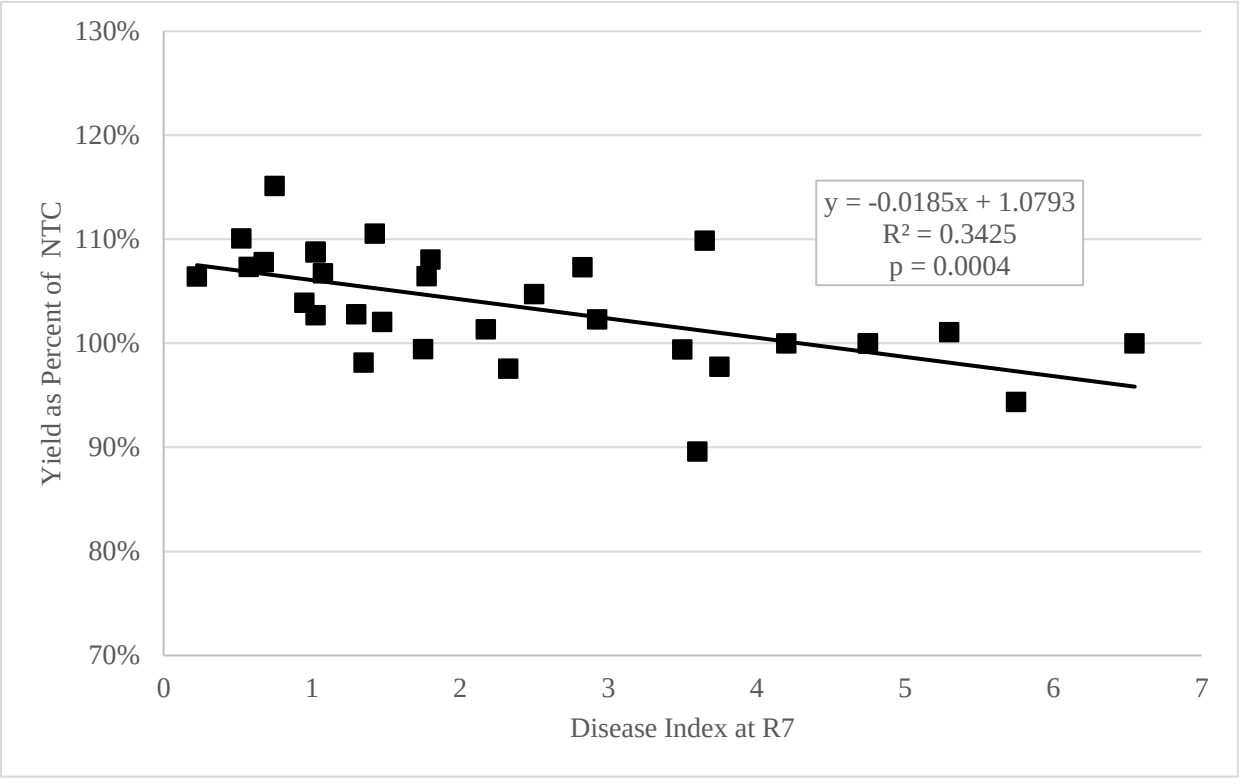

Figure 1.2. Yield loss assessment relating Phoma black stem disease index values at R7 and yield as a percent of the NTC for the high-yielding environment.

Discussion

The results of this study demonstrated that fungicide applications are an effective tool in managing Phoma black stem. Results of efficacy trials demonstrated that nearly all fungicides used in this study reduced DSI, but applications that included an SDHI were the most effective. Results of timing trials demonstrated that the greatest reductions in DSI were observed when fungicide applications, either singly or in combinations, were made at the R1 growth stage. While fungicide applications consistently reduced disease, higher yields were less frequent in those treatments, suggesting that disease losses to Phoma black stem may be uncommon. However, under high yield and high disease pressure environments, losses to Phoma black stem may be prevented by fungicide application.

Results of this study demonstrate that multiple fungicides can manage Phoma black stem, but that efficacy differences among fungicide mode of actions exist. For efficacy trials with high disease pressure (DAV 2018 and DAV 2019), the DSI as a percentage of the NTC ranged from 3-16%, 26-67% and 60-94% for SDHIs, QoIs and DMIs fungicides, respectively. Lower levels of disease were always found in the SDHI and QoI fungicide treatments when compared to the NTC. However, only 62.5% of the DSI ratings on DMI fungicides were significantly lower than the NTC. Variation among fungicide efficacy within fungicide mode of actions was observed. Within the QoIs, numerically lower DSI values were observed with pyraclostrobin in 75% of the ratings. The differences between picoxystrobin and azoxystrobin were not consistent. Within the DMIs, metconazole was the only fungicide with DSI values always lower than the NTC, while tebuconazole DSI values were different than the NTC for only 25% of the ratings.

To our knowledge, this is the first research conducted in the US evaluating the efficacy of fungicides on Phoma black stem of sunflower. In Yugoslavian trials, thiophanate methyl (FRAC 1: methyl benzimidazole carbamate), chlorothalonil (FRAC M: multi-site activity), and mancozeb (FRAC M: multi-site activity) were suggested as effectively reducing Phoma black stem severity in seed production fields (Gulya et al. 1997). While these fungicides were not evaluated in our study, the combination of this study and the Yugoslavian work demonstrate a large selection of fungicides are available as potential management options.

All fungicide timings tested in this study (V8-V10, R1, R5) were found to reduce DSI in at least some cases. Fungicide applications made at V8-V10 or R5 had R7 DSI values lower than the NTC in 83% and 33% of trials with adequate disease pressure, respectively. However, the lowest DSI values of single-timing fungicide applications were frequently observed at the R1 growth stage. These results could be explained in part by both the timing of *P. macdonaldii*

infection and plant structure. When a sunflower is at growth stages V8-V10, approximately only 50% of the leaves have grown. Consequently, a fungicide application at the late vegetative stage (V8-V10) would provide protection from early-season infections, but additional leaves would be left vulnerable. Additionally, these applications may occur before canopy closure, which is a less favorable micro-climate for infection. Applications at R1 occur when the majority of leaves have grown and when sunflowers are canopied. Thus, fungicides have the potential to provide a high level of coverage in a more favorable microclimate for infection. Fungicide applications made at flowering (R5) would protect leaves from new infections, but it occurs after a period of several weeks where leaves are emerged and exposed to a favorable disease microclimate. Our results are generally consistent with Penaud (1996), who examined management of Phoma black stem with single and multiple fungicide applications of carbendazim (FRAC 1: methyl benzimidazole carbamate) that ranged from late-vegetative to late-flowering. From that three-year study, Penaud (1996) concluded that two fungicide applications, timed pre- and post- bud formation (R1), resulted in fewer lesions per stem.

In this study, there was a relationship between DSI and yield in the high-yielding environment ($p = 0.0004$; $R^2 = 0.2811$), but no relationship was observed in low-yielding ($p = 0.9426$; $R^2 = 0.0002$) and moderate-yielding environments ($p = 0.5306$; $R^2 = 0.0405$). Each of the four trials categorized into the high-yielding environment were planted with a different hybrid and were conducted in 2017 or 2018, suggesting that the relationship between DSI and yield was not confined to specific genetics or a growing season. The level of disease found in these high-yielding environments suggests that environments that promote sunflower growth may also facilitate the development of Phoma black stem. This is consistent with a study from 1994 to 2001 that investigated the effect of crop management on Phoma black stem frequency

and severity (Debaeke and Pérès 2003). Through the influence of management factors such as nitrogen, water, and plant density, the intensity of a crop canopy, as measured by leaf area index (LAI) or fraction of photosynthetically active radiation intercepted (fPARi), could be used to indicate the incidence and severity of Phoma black stem. When the fPARi was greater than 85%, a rapid increase in the number of lesions per stem occurred, up to 12 lesions per stem. An increase in crop canopy intensity may have a positive effect on Phoma black stem, but the increase in light interception will also have a positive effect on the yield.

A difference in yield ($\alpha = 0.05$) was only observed in one of eight fungicide-timing trials with disease pressure (2017 DAV PscMR). Significant differences ($\alpha = 0.10$) were also observed in a combined analysis of timing trials, with an approximate 4-8% increase in yield over the NTC observed in three combination treatments (V8-V10+R1, R1+R5 and V8-V10+R1+R5). Importantly, it may be prudent to investigate further the impact of multiple applications on disease management and yield. The decision to use pyraclostrobin for the fungicide timing trials was made prior to the development of fungicide efficacy trials, and it is notable that results of those trials demonstrated that both boscalid and pydiflumetofen more effectively managed Phoma black stem than pyraclostrobin. Thus, it is not impossible that yield differences ($\alpha = 0.05$) could have been observed had one of these more efficacious fungicides been used. Additionally, an increase in replications, larger plot sizes, and/or more locations all could have helped see smaller differences in yield, as could alterations in fungicide application (such as different nozzles, spray pressure, spray volume, adjuvant use or carrier)

Although, Phoma black stem has proven to be a yield-limiting problem in some sunflower growing regions (Penaud 1996; Quiroz et al. 2014; Velazquez and Formento 2003) we believe this study is the first that provides evidence that yield losses could be occurring in the

NGP, even if rarely. Limited work on Phoma black stem in the NGP has been done since Carson (1991) found no statistical difference in yields in artificially inoculated field trials in South Dakota. However, that research did find numerically lower yields in inoculated treatments and seed weights that were statistically lower in some inoculated treatments when compared to checks.

While Phoma black stem can cause yield loss in some high-yield and intense-production situations, active management with fungicides is likely not commonly needed in the NGP. In this study, differences in yield with fungicide application were only observed when yields exceeded 3,400 kg/ha. According to USDA-NASS (2019), this is over twice the average yield in ND and SD, where 78.7% of US sunflower was produced. Additionally, production practices that have led to increased diseases in other locations are not commonly practiced in the US NGP. Over 99% of sunflower in the Dakotas was produced without any irrigation in 2017 (USDA-NASS 2019) and sunflower is commonly grown only once every four years, and rarely every three years. If sunflower growers do expect a high disease pressure environment by pushing yields with nitrogen, supplemental irrigation or tightening rotations, then it is conceivable that a preventative fungicide application at R1 might be considered. Fungicide would need to be used in a preventative manner, and since stem lesions often do not develop until after flowering and effective management relies on applications prior to flowering, decisions should also include field history and the growing conditions of the season.

In addition to a potential, although rare, threat to yield directly, the high prevalence of Phoma black stem presents a possible complicating factor for sunflower growers in the NGP. Phomopsis stem canker, a disease that also infects through the leaves and causes stem lesions has reemerged as one of the top yield-limiting diseases in sunflower (Mathew et al. 2015). The two

diseases appear similar, but differentiating between Phoma black stem and Phomopsis stem canker is a vital factor in management decisions. The exact losses attributed to Phomopsis are difficult to quantify because the variation in lodging caused by weakened stems depends on other factors such as wind and precipitation, but 40% yield losses have been reported in grower fields (Mathew et al. 2015). Between 2009-2015, Phomopsis stem canker was present in 50 and 56% of fields in South Dakota and North Dakota/Minnesota, respectively (Gulya et al. 2019). While Phomopsis is relatively common, it is less common than Phoma black stem. Mis-identification of Phoma black stem for Phomopsis stem canker could lead to an inaccurate field history resulting in the use of unnecessary management tools and input costs. Conversely, if the field is at risk to both Phomopsis stem canker and Phoma black stem, a single fungicide application may help manage both diseases. Fungicide applications at R1 have provided some efficacy for Phomopsis stem canker, particularly QoI fungicides (Olson 2017), and our data indicates that this can effectively reduce the DSI of Phoma black stem.

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CHAPTER 2: DETERMINATION OF THE IMPACT DISEASE HAS ON YIELD ON SUNFLOWER PRODUCED IN NORTH DAKOTA AND MINNESOTA

Introduction

Sunflower (*Helianthus annuus* L.) in an economically important crop, and is produced globally primarily for oil or direct human consumption. Since 2000, the US has planted approximately 1 million hectares of sunflower annually. While sunflower is produced across many US Great Plains states (Texas, Oklahoma, Colorado, Kansas, Nebraska, South Dakota, North Dakota and Minnesota), the highest concentration of production is in the Northern Great Plains (NGP) states of North Dakota, South Dakota and Minnesota. In recent years, approximately 40% of total US production occurs in the states of North Dakota and Minnesota (USDA-NASS 2019). However, sunflower production is impacted by multiple diseases that reduce grain quality and/or quantity.

Crop surveys can be an effective method to determine the impact of abiotic or biotic stresses to crops, including cereal and oilseed crops produced in the US NGP (Gulya et al. 2013; Knodel et al. 2012; Knodel et al. 2018; Markell et al. 2009b). Since 2002, the US crop commodity group, the National Sunflower Association (NSA), has supported a nationwide survey of the US sunflower crop (Gulya et al. 2019). Data is collected by teams of experts who visit sunflower fields to collect data on components of yield (plant population, head diameter, head fill, seed size, and percent good seed), production practices (oilseed/confection, yes/no irrigation, row spacing, previous crop and tillage) and pests (weeds, birds, insects and diseases). Ten diseases that are thought to be common and/or yield-limiting are included in the survey. These diseases are categorized by what part of the sunflower plant that the disease generally occurs; roots, leaves, stem or head (Markell et al. 2014; Markell et al. 2015).

Pathogens that infect through the roots cause the diseases Verticillium wilt (*Verticillium dahliae* Kleb.), charcoal rot (*Macrophomina phaseolina* Tassi.) and downy mildew (*Plasmopara halstedii* Farl.) (Harveson et al 2016; Markell et al. 2015). After infection, the pathogen systemically spreads through the plant causing symptoms to appear on stem and leaves and may cause wilt. When downy mildew occurs early in the season, total yield loss can occur (Humann et al. 2016, 2019). Downy mildew has a very severe impact on yield. Pre-emergent and post-emergent damping-off can occur or, if the plant does survive past the seedling stage, it will be severely stunted and have leaves that are puckered and chlorotic around the veins. Verticillium wilt and charcoal rot infections can occur early in the season but aboveground symptoms usually develop during or after flowering (Harveson et al. 2016; Markell et al. 2014). For Verticillium wilt, symptoms will appear on leaves as interveinal necrotic spots with a distinct chlorotic area dividing the dead tissue and green tissue. The interior of the stem will be colonized and the pith may be compressed by a ring of microsclerotia. Black streaks of microsclerotia may develop along the outside of the lower stem. Signs and symptoms of charcoal rot appear as a gray-black lesion girdling the base of the stem becoming light-gray as the lesion progresses up the plant and an internal colonization of the pith. Plants infected with Verticillium wilt and charcoal rot may wilt, prematurely senesce and/or may be susceptible to lodging (Harveson et. al 2016).

The most important foliar disease of sunflower in the NGP is sunflower rust (*Puccinia helianthi* Schw.) (Markell et al. 2015). The pathogen is macrocyclic and autoecious, and can overwinter in the NGP (Markell et al. 2009a). Red-cinnamon colored uredinia pustules often initially develop on the lower leaves in the sunflower canopy, but under favorable conditions will spread to the upper canopy. A high severity of rust during flowering on the upper four leaves

(the ones contributing the most to grain fill) can result in reduced test weight, oil content, seed size and yield losses up to 80% (Bradley et al. 2007; Friskop et al. 2015; Markell 2009).

Phomopsis stem canker (*Diaporthe/Phomopsis* spp.) and Phoma black stem (*Phoma macdonaldii* Boerma) are two stem diseases that are frequently observed in sunflower fields (Markell et al. 2019a). Initial disease development is similar between the two diseases: necrotic leaf spots will appear on the leaf margin and continue developing over the entire leaf, through the petiole and into the stem (Harveson et al. 2018). However, Phoma black stem lesions are small (5-6 centimeters), black and generally only superficial. In contrast, Phomopsis stem canker lesions are larger (up to 20 centimeters) with a light to dark brown color. Phomopsis lesions also cause extensive pith degradation within the stem, making the sunflower more prone to lodging. Phomopsis stem canker has increased in recent years, and yield loss can be severe (Berglund 2007, Berglund 2010, Kandel and Gulya 2016, Mathew et al. 2015). However, yield losses caused by Phoma black stem are limited and infrequent (Hansen 2020, [Chapter 1]).

Rhizopus head rot (*Rhizopus* spp.) is a head disease that occurs throughout the US, but is more common in Texas, Colorado, Kansas and Nebraska (Harveson et al. 2019). Symptoms include brown, water-soaked lesions on the back of the head, and signs include the presence of white fluffy mycelia and black sporangia inside an infected head. Rhizopus head rot in sunflower is exacerbated by physical damage to the head (such as that caused by hail, birds or insects). When severe, Rhizopus head rot can cause reductions in test weight, oil content, and yield (Harveson et al. 2019).

Diseases caused by *Sclerotinia sclerotiorum* Lib. are unique in that infection occurs through the roots, the stem or the head of sunflower, resulting in the diseases Sclerotinia wilt, Sclerotinia mid-stalk rot and Sclerotinia head rot, respectively (Harveson et al. 2016). The

pathogen overwinters as sclerotia, which can germinate myceliogenically or carpogenically. Sclerotinia wilt occurs when sclerotia germinate and produce mycelia (myceliogenic germination) that infect the roots of sunflower. At the base of the sunflower, a tan-colored lesion will develop and under humid conditions white mycelia and sclerotia can be observed. Sclerotinia wilt infections reduce the plants ability to absorb water, resulting in a wilted plant. Yield losses as high as 43% have been observed from Sclerotinia wilt, with additional reductions in test weight and oil content (Rashid and Dedio 1992). When sclerotia produce apothecia and ascospores (carpogenic gemination), infection of the stem or the head can occur. Symptoms of Sclerotinia mid-stalk rot include a light-tan lesion will develop near the leaf axil, which may rapidly expand up to 20-30 cm in length. The pith and the vascular tissue will deteriorate adjacent to the lesion, leading to an increased chance of lodging. Sclerotinia mid-stalk rot appears similar to Phomopsis stem canker; however, Sclerotinia mid-stalk lesions will be a light-tan color, the stem may appear to shred and sclerotia may form on or near the surface of the stem (Markell et al. 2015). Yield losses vary depending on the degree of lodging that occurs. Symptoms of Sclerotinia head rot initially appear as water-soaked spots on the back of the head. As the disease progresses, the entire head may decay, and appear to shred, leaving behind bleached vascular tissue and sclerotia. Severe Sclerotinia head rot will result in complete loss, but 10-20% yield reductions are commonly reported (Rashid et al. 2016).

Results of all data collected in the NSA survey are presented at the NSA Annual Research Forum (Gulya et al. 2019; Markell et al. 2019a). This data is used by those supporting the sunflower industry to improve the production, profitability and marketing of sunflower produced in the US. In part, survey results contribute to breeding and/or research priorities, the development of disease management strategies and creation of Extension educational materials

for growers (Gulya et al. 2019, Markell et al. 2019a, Markell et al. 2019b). To date, data from over 2,572 sunflower fields spanning 18 years has been acquired in the NSA survey. However, robust analysis of specific diseases on their impact of yield and yield components has not been done. This data could be used to better understand the relationship between disease and yield and improve prioritization of breeding, research and Extension priorities. The objective of this research is to determine the impact of diseases on sunflower yield in North Dakota and Minnesota.

Materials and Methods

The NSA survey began in 2002, and has continued annually or semi-annually over the next 18 years. For the purposes of this study, survey data collected from 2006 through 2017 was used. In these years, the survey was conducted annually except in 2014 or 2016, resulting in 10 years of survey data. Additionally, only data from North Dakota and Minnesota were included in this study, resulting in 1,003 surveyed fields. Disease assessments, field management practices and diseases were reported in all years included in this study; however, yield component data is only available beginning in 2010.

The methodology of the NSA survey is described in detail by Berglund (2006), Gulya et al. (2019) and Ernst et al. (2019), and summarized as follows. The number of fields surveyed were based on the number of hectares planted to sunflower, and separated at the county level: one field was surveyed for every 4,046 hectares in a county. When counties had less than 4,046 hectares of sunflower, an effort was made to combine other low-acreage counties within close proximity and sample one field among them. Within counties, sunflower fields were chosen randomly by a team of surveyors. Once a field was selected, two separate sites within the field were used for data collection. Sites within a field were selected at the discretion of the survey

teams to best represent the entire field. When sunflower plants were highly variable across the field, sites were selected in attempt to capture this variability. Sites were selected away from field margins to limit edge effects, such as compaction, wind damage, animal damage, etc. Each site within a field consisted of two adjacent sunflower rows, 7.62 meters in length. In solid seeded fields, a 1.52 by 7.62 meter area was used to represent a site. The data from both sites were combined and an average was calculated to represent the results of that field.

The field management component of the survey was included because a wide variety of cultural practices occur across North Dakota and Minnesota. This component of the survey included type of hybrid grown (oil or confectionary), previous crop, planting population, row spacing and tillage. Previous crops were categorized into seven groups: small grains (wheat [*Triticum aestivum* L. emend. Thell], durum wheat [*Triticum durum* Desf.] or barley [*Hordeum vulgare* L.]), large grains (corn [*Zea mays* L.], sorghum [*Sorghum bicolor* (L.) Moench.] or proso millet [*Panicum miliaceum* L.]), soybean (*Glycine max* (L.) Merr.), flax (*Linum usitatissimum* L.), alfalfa (*Medicago sativa* L.) or grassland/fallow. Planting population was calculated from the number of plants in two adjacent rows over 7.62 meters or solid seeded area. The row spacing (distance between rows) was split into categories of greater than 50.8 centimeters or less than/equal to 50.8 centimeters. The tillage practices of the field were split between three categories: no tillage (soil surface is undisturbed between crops), minimum tillage (burial of some residue from previous crop) and conventional tillage (burial of most residue from previous crop) (Berglund 2006). Importantly, some management practices could not be determined and recorded based on random field visits, such as fungicide application and disease resistance in hybrid.

Yield estimations were made using harvestable plant population, head diameter, center seed set, seed size, percent good seed and bird damage. Harvestable plant population was calculated in the same manner as planting population, except only plants that would contribute to yield were included. For example, plants that were lodged, failed to produce a head or were severely infected with a head disease would be omitted in the yield calculation. To accurately represent the effect of head size contributing to yield, two measurements were made on each measured sunflower head: the diameter of the entire head and the diameter of the area in the center of the head that failed to produce seed (center seed set or CSS). Head diameter and CSS were each calculated by averaging the diameter of any five consecutive heads within a site, excluding any that would be considered not contributing to yield. Seed samples were collected to determine seed size and percent good seed. Samples were collected by cutting wedges out of three to five random heads and hand threshing the seed into a container. Percent good seed is the percentage of seeds that developed a kernel, calculated using 100 seeds from the sample. To determine seed size, a representative portion of threshed seeds were placed into one of five categories using Table 2.1. Seeds in these categories were described as light, medium-light, medium, medium-heavy and heavy. Birds are a perennial problem in sunflower and can cause millions of dollars in losses (Enrst et al. 2019; Kleingartner 2003; Linz and Hanzel 2007); therefore, they are included in the yield component section of the survey. Bird damage was visually assessed as the percent of seed missing from a head on 10 consecutive plants. Yield components were measured at both sites independently and averaged after surveying at both sites was complete. Multipliers were derived from the yield components from each survey field and used to calculate a field's average yield. Harvestable plant population was used to calculate the population multiplier using

$$P = \frac{\text{harvestable plant population}}{8094} \quad (1)$$

where P is the population multiplier and harvestable plant population is measured in plants per hectare. Head diameter multiplier was calculated using

$$H = 0.071 * (\text{head diameter}) - 0.44 \quad (2)$$

where H is the head diameter multiplier and head diameter is measured in centimeters. The multiplier for seed size was derived from Table 2.1. The CSS multiplier was calculated using

$$C = -0.0019 * (\text{CCS diameter})^2 + 0.0049 * (\text{CCS diameter}) + 0.975 \quad (3)$$

where C is the CCS multiplier and CCS diameter is measured in centimeters. The bird damage multiplier was calculated using

$$B = \frac{100 - \text{percent bird damage}}{100} \quad (4)$$

where B is the bird damage multiplier. Yield was calculated by combining all multipliers using

$$Y = 2746 * P * H * C * S * G * B \quad (5)$$

where Y is the yield in kilograms per hectare, P is the population multiplier, H is the head diameter multiplier, C is the CCS multiplier, S is the seed size multiplier, G is the percent good seed multiplier and B is the bird damage multiplier.

Table 2.1. Seed dimensions determining class and seed size multiplier.

	Seed dimensions		
Side size class	Wide (mm)	Length (mm)	Multiplier
Light	4.2	7.3	0.8
Medium-Light	5.2	7.8	0.9
Medium	5.8	8.9	1
Medium-Heavy	5.8	9.9	1.1
Heavy	5.8	11	1.2

All identification and recording of disease were done by visual examination of plants for key symptoms and signs of ten diseases; Sclerotinia diseases (wilt, mid-stalk rot and head rot),

Rhizopus head rot, downy mildew, Phomopsis stem canker, Phoma black stem, Verticillium wilt, charcoal rot, and rust (Gulya et al. 2019; Markell et al. 2014; Markell et al. 2019a). Plants infected with Rhizopus were identified by a water-soaked lesion on the head and the presence of white fluffy fungal growth with black pin-sized dots within the head (Harveson et al. 2019). Downy mildew was identified by plant stunting, horizontal head development and leaf chlorosis. Importantly, downy mildew was likely underreported because early-season damping-off caused by the disease would result in an absence of plants on which to make identification (Gulya et al. 2013). Phomopsis stem canker, Phoma black stem and Sclerotinia mid-stalk all were differentiated by lesion size, color and structural integrity under the lesion that develops; Phoma black stem lesions are 5-6 centimeters, a jet-black color and superficial, Phomopsis stem lesions are dark- to light-brown color, typically 15-20 centimeters and compromise the integrity of the stem by creating a 'hollow' spot behind the lesion and Sclerotinia mid-stalk lesions are light-tan to manila color, as large as 15 centimeters or more and compromise the integrity of the stem by creating a 'shredding' appearance (Harveson et al. 2018; Markell et al. 2014). Verticillium wilt can be identified by the distinct necrotic and chlorotic leaf mottling that occurs, black streaky lesions on the stem, and pith damage and colonization (Markell et al. 2014). They key diagnostic symptoms for charcoal rot are the dark- to light-silver lesion that develops at the base of the sunflower, and the pith degradation and colonization resulting in an internal stem appearance of horizontal, flattened black disks (Markell et al. 2014). Rust was identified by pustules of telia (black) or uredinia (cinnamon-brown) on leaf tissue (Friskop et al. 2011; Markell et al. 2015).

Incidence was determined for all diseases, except rust. Incidence is defined as the percent of plants that have disease present. Incidence assessments were made on 25 consecutive

plants for each row in the site (50 plants total assessed). For rust, severity was determined by visually estimating the percentage of leaf area with rust on the upper four fully-expanded leaves on five consecutive plants (20 leaves total) with the aid of rust assessment diagrams (Figure 2.2) (Friskop et al. 2011; Friskop et al. 2015).

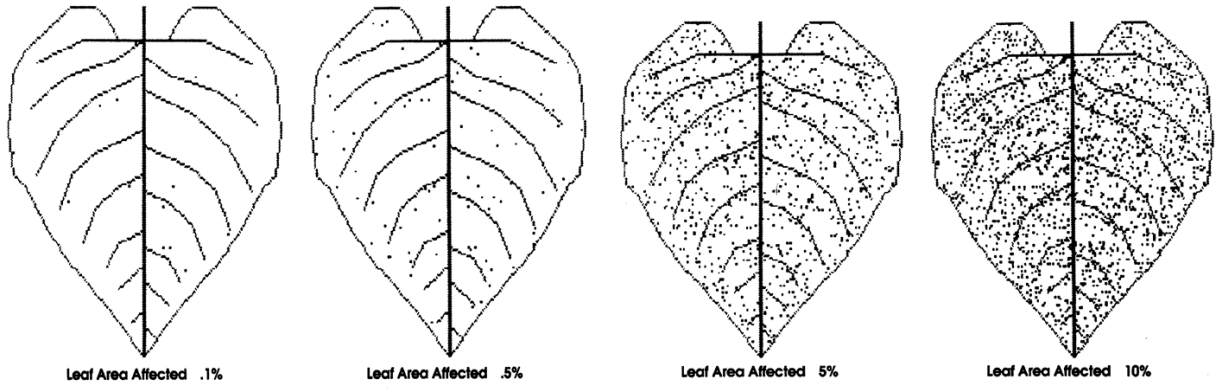

Figure 2.1. Leaf area affected diagram used for rust assessments (Friskop et al. 2009, Friskop et al. 2015).

Once data had been collected surveyors would hypothesize among a set of defined factors the two that had the largest impact on yield potential, specifically; birds, disease, drought, drown-outs, hail, herbicide damage, insects, lodging, plant spacing, weeds, uneven growth, other or none. These yield limiting factors (YLF) were assigned rank based off which one had the larger impact.

The sunflower growing region of ND and MN spans nearly 500 kilometers east to west and 300 kilometers north to south. This results in a wide range of average temperatures and rainfalls between regions within ND and MN. Fields were placed into regions based on USDA-NASS Agricultural Statistics Districts (USDA 2020). The ND fields were split into nine regions (Figure 2.3) and the MN fields were split into two regions (Figure 2.4). North Dakota districts were termed (moving from north to south first, and then left to right in Figure 2.3) NDNW,

NDWC, NDSW, NDNC, NDC, NDSC, NDNE, NDEC and NDSE. Minnesota districts were termed (moving north to south using Figure 2.4) MN10 and MN40.

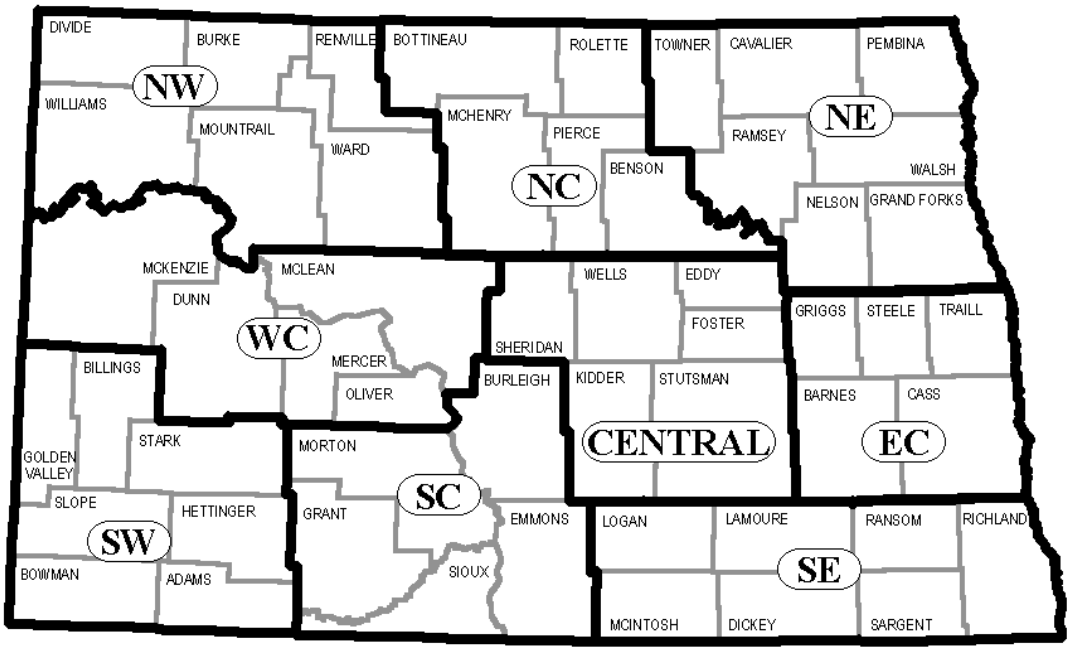

Figure 2.2. North Dakota Agricultural Statistics Districts – Fields were placed into one of nine regions based on district map (USDA 2020).

Figure 2.3. Minnesota Agricultural Statistics Districts – Fields were placed into Minnesota District 10 or District 40 found in the northwest and west-central parts of the state (USDA 2020).

Data analyses was made using SAS (version 9.4; SAS Institute, Cary, NC, U.S.A.). A generalized linear model (PROC GLM) was used to determine differences in yield between yield-limiting factors. Only fields with yield component data were included in the analysis (n = 578). Differences between YLFs were determined using pairwise t-tests of least-square means at $\alpha = 0.05$.

Generalized linear models were created for each individual yield component using all disease incidences as predictor variables. Disease incidences included Sclerotinia wilt, Sclerotinia head rot, Rhizopus head rot, downy mildew, Phomopsis stem canker, Phoma black stem, Verticillium wilt, charcoal rot and rust. After initial disease incidence by yield (component) analyses, an attempt to account for variation across regions and between years was made. Yield from each surveyed field was transformed to be reflected as a percentage difference of the region by year average yield using the following equation:

$$\text{Percent difference in yield} = \frac{(\text{original yield observation}) - (\text{average yield of region by year})}{(\text{average yield of region by year})} \quad (6)$$

For example, if the average yield in the MN40 region in 2013 was 1700 kilograms per hectare, all yield data within MN40 in 2013 would be reflected as a percentage difference from 1700 kilograms per hectare. All yield (component) data for was standardized in this manner for each region by year. Standardized data was analyzed in the same manner as previous yield component by disease incidence models. Additionally, all previous disease incidence by yield (component) models were reanalyzed using data that was filtered to only include fields in which disease was indicated as the first or second yield-limiting factor (n = 332). Disease incidence effects were considered significant at $\alpha = 0.05$.

Generalized linear mixed models (PROC GLIMMIX) were created for each individual yield component using incidence from a single disease as the predictor value. A model was created for all combinations of disease and yield (components). In these analyses, incidence was used as a fixed effect, while region, year, region by year, incidence by region, incidence by year and incidence by region by year were used as random effects. A SOLUTION option in the MODEL statement was used to request incidence parameter estimates. Disease incidence effects were considered significant at $\alpha = 0.05$.

Univariate and multivariate regressions (PROC GLM) were used to determine the effect of each disease incidence on yield components individually and jointly. However, ANOVAs were only conducted on data from the NDSC region in 2015. This region by year combination was chosen because a high number of fields (n = 29) were surveyed, ten of the fields listed diseases as one of the YLFs, and there were no missing values for any of the diseases or yield components. Disease incidence effects were considered significant at $\alpha = 0.05$.

Preliminary Results and Discussion

Of the 578 fields with yield component data, disease was indicated as the number one YLF in 20% of surveyed fields, which was more than any other category (Table 2.1). Differences in yield were observed among YLFs. A higher yield was observed when YLF=none than when compared to all other YLFs, except when YLF=drown-out. A 427 kilogram per hectare difference in yield was observed between YLF=none and YLF=disease. Yield was lower when YLF=drought than when YLF=disease. These results show that surveyors were able to identify when factors, such as uneven plant spacing, birds, insects and disease, were limiting yield. This suggests that significant losses, over 400 kilograms per hectare are occurring from disease; however, there may be an inherent level of bias when surveyors are determining YLFs. The YLFs are determined after a surveyor collects all the data, which can affect the rationale behind their decision since they already are aware of all the yield measurements.

The analyses determining the effect of all disease incidences on each yield (component) were not significant at $\alpha = 0.05$ and had low model fitness ($r^2 < 10\%$) when original data was used (data not presented). When yield component data was standardized on the region by year mean, population and head diameter were significantly affected by disease when all ND and MN data was used (Table 2.2) and when data filtered to represent fields with disease as a yield

Table 2.2. T-values of pairwise comparisons of yield between first yield-limiting factors as determined by survey of 578 sunflower fields in North Dakota and Minnesota from 2006 to 2017.

YLF[z]	Yield[y] (kg/ha)	No of Obs[x]	1	2	3	4	5	6	7	8	9	10	11	12
1. None	2431	66												
2. Birds	1494	51	<.0001**											
3. Disease	2004	118	<.0001**	<.0001**										
4. Drought	1767	66	<.0001**	0.0090**	0.0058**									
5. Drown.	2079	8	0.0934	0.0060**	0.7135	0.1353								
6. Hail	1671	10	<.0001**	0.3596	0.0704	0.6138	0.1237							
7. Herbicide	1259	1	0.0377*	0.6770	0.1843	0.3672	0.1665	0.4820						
8. Insects	1780	15	<.0001**	0.0815	0.1436	0.9325	0.2216	0.6321	0.3665					
9. Lodging	1941	54	<.0001**	<.0001**	0.4929	0.0886	0.5148	0.1600	0.2264	0.3229				
10. Spacing	1939	98	<.0001**	<.0001**	0.3891	0.0536	0.4934	0.1493	0.2264	0.3066	0.9762			
11. Weeds	2071	31	0.0033**	<.0001**	0.5525	0.0125*	0.9713	0.0491*	0.1527	0.0979	0.3022	0.2491		
12. Other	1859	51	<.0001**	0.0010**	0.1214	0.3743	0.3004	0.3301	0.2875	0.6301	0.4511	0.4105	0.0958	
13. Uneven	1644	9	<.0001**	0.4559	0.0629	0.5386	0.1096	0.9181	0.5126	0.5648	0.1403	0.1311	0.0440*	0.2881

[z] YLF = First yield limiting factor. Yield limiting factors are represented by numbers 1 through 13: 1 = No yield-limiting factors; 2 = Birds; 3 = Disease; 4 = Drought; 5 = Drown-outs; 6 = Hail damage; 7 = Herbicide damage; 8 = Insect; 9 = Lodging; 10 = Plant spacing; 11 = Weeds; 12 = Other; 13 = Uneven growth.

[y] Yield was measured in kilograms per hectare

[x] Number of observations for each yield-limiting factor.

* Indicates that the comparison was significant at $\alpha = 0.05$.

** Indicates that the comparison was significant at $\alpha = 0.01$.

Table 2.3. Effect of disease incidence on each yield component standardized on the region by year mean for all fields in North Dakota and Minnesota.

	Yield Component													
	Population[y]		Head Diameter[x]		CSS[w]		Bird Damage[v]		Seed Size[u]		PGS[t]		Yield[s]	
Disease[z]	Parameter Estimate[r]	p-value[q]	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value
S. Wilt	0.05%	0.6731	-0.05%	0.5051	-0.35%	0.1181	-1.04%	0.3038	-0.01%	0.8524	-0.02%	0.5325	0.02%	0.9021
S. Mid-stalk	0.17%	0.3068	0.09%	0.4159	0.02%	0.9502	-1.15%	0.4	-0.10%	0.0839	0.02%	0.7181	0.23%	0.2785
S. Head Rot	-0.53%	0.0022	0.09%	0.4438	-0.33%	0.2958	0.31%	0.8296	0.05%	0.4298	-0.01%	0.8244	-0.40%	0.0701
Rhizopus	-0.28%	0.0267	0.07%	0.3844	0.03%	0.8894	-0.20%	0.8495	-0.06%	0.1504	-0.06%	0.1126	-0.29%	0.073
D. Mildew	-0.64%	0.0124	0.68%	<.0001	-0.55%	0.234	-1.51%	0.4767	0.07%	0.4266	-0.12%	0.0923	-0.01%	0.9745
Phomopsis	0.04%	0.3842	-0.06%	0.0687	0.05%	0.6021	-0.24%	0.5497	0.00%	0.7952	-0.03%	0.058	-0.02%	0.7932
Phoma	0.02%	0.4213	0.00%	0.978	-0.04%	0.3798	0.25%	0.235	0.01%	0.3123	-0.01%	0.3268	0.01%	0.7893
Verticillium	0.19%	0.1979	-0.10%	0.3039	-0.52%	0.0548	0.79%	0.524	0.01%	0.8659	-0.01%	0.754	0.06%	0.7713
Charcoal Rot	-0.17%	0.6167	-0.35%	0.1071	0.24%	0.7007	-0.15%	0.958	0.04%	0.7151	-0.11%	0.2361	-0.60%	0.1627
Rust	-0.32%	0.6019	-0.30%	0.4643	-0.92%	0.4162	3.62%	0.4802	0.49%	0.0197	-0.05%	0.7555	-0.26%	0.7455
Model p-value	0.0196		0.0054		0.2872		0.8621		0.233		0.1577		0.5119	
r^2	0.0375		0.044		0.0213		0.0097		0.0229		0.0255		0.0165	

[z] Diseases included in the survey – S. Wilt = Sclerotinia Wilt (*Sclerotinia sclerotiorum* Lib.); S. Mid-stalk = Sclerotinia Mid-stalk Rot (*S. sclerotiorum*); S. Head Rot = Sclerotinia Head Rot (*S. sclerotiorum*); Rhizopus = Rhizopus head rot (*Rhizopus* spp.); D. Mildew = Downy Mildew (*Plasmopara halstedii* Farl.); Phomopsis = Phomopsis stem canker (*Phomopsis/Diaporthe* spp.); Phoma = Phoma black stem (*Phoma macdonaldii* Boerma); Verticillium = Verticillium wilt (*Verticillium dahliae* Kleb.); Charcoal Rot (*Macrophomina phaseolina* Tassi.); Rust (*Puccinia helianthi* Schw.).

[y] Population = harvestable plant population – measured as the population of plants contributing to yield.

[x] Head Diameter – measured as the diameter of the sunflower head.

[w] CSS = center seed set – measured as the diameter of the area in the center of the head that fails to produce seed.

[v] Bird Damage – measured as the percent area of the head with seed missing from birds.

[u] Seed Size – measured as a multiplier derived from one of five classes of seed size. Seed classes, listed from smallest to largest, include Light, Medium-Light, Medium, Medium-Heavy and Heavy. The seed size multiplier incrementally increases by 0.1 for every increase in seed size class.

[t] Percent Good Seed – measured as the percentage out of 100 seeds that develop a kernel.

[s] Yield – derived using the equation: $Y = 2746 * P * H * C * S * G * B$, where Y is the yield in kilograms per hectare, P is the population multiplier, H is the head diameter multiplier, C is the CCS multiplier, G is the percent good seed multiplier and B is the bird damage multiplier.

[r] Parameter estimates show percent change from the region by year mean for every 1% increase in disease incidence.

[q] P-value associated with each parameter estimate.

Table 2.4. Effect of disease incidence on each yield component standardized on the region by year mean for fields with disease indicated as a yield-limiting factor.

	Yield Component													
	Population[y]		Head Diameter[x]		CSS[w]		Bird Damage[v]		Seed Size[u]		PGS[t]		Yield[s]	
Disease[z]	Parameter Estimate[r]	p-value[q]	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value	Parameter Estimate	p-value
S. Wilt	0.04%	0.767	-0.12%	0.1427	-0.26%	0.2023	0.05%	0.9537	-0.07%	0.0726	-0.03%	0.4977	-0.22%	0.1541
S. Mid-stalk	-0.05%	0.7365	0.05%	0.6024	-0.08%	0.7597	-1.68%	0.1161	-0.12%	0.0182	-0.03%	0.6256	-0.04%	0.8432
S. Head Rot	-0.27%	0.0955	0.11%	0.3038	-0.15%	0.5808	0.44%	0.6895	0.07%	0.1886	0.00%	0.957	-0.17%	0.416
Rhizopus	-0.30%	0.0359	0.08%	0.4161	0.04%	0.8574	-1.83%	0.0645	-0.04%	0.3187	-0.15%	0.0129	-0.35%	0.0594
D. Mildew	-0.81%	0.001	0.60%	0.0004	-0.64%	0.1311	-1.53%	0.3693	0.05%	0.5305	-0.19%	0.0613	-0.37%	0.2451
Phomopsis	0.02%	0.7531	-0.08%	0.018	-0.06%	0.4926	0.03%	0.9374	-0.03%	0.0732	-0.02%	0.3231	-0.14%	0.0276
Phoma	0.00%	0.9228	0.00%	0.9186	-0.07%	0.3104	-0.09%	0.7381	0.01%	0.4116	-0.01%	0.3583	0.01%	0.7933
Verticillium	0.08%	0.5956	0.04%	0.7149	-0.25%	0.339	0.55%	0.6082	-0.02%	0.7384	0.03%	0.6921	0.07%	0.7099
Charcoal Rot	-0.16%	0.6395	-0.15%	0.5313	-0.14%	0.8201	-1.34%	0.5821	-0.10%	0.3899	-0.13%	0.3787	-0.57%	0.2127
Rust	-0.50%	0.5006	-0.38%	0.442	-0.99%	0.4421	4.16%	0.4219	-0.01%	0.9626	0.21%	0.4961	-0.57%	0.5516
Model p-value	0.0486		0.009		0.7659		0.7142		0.2043		0.2733		0.2614	
r^2	0.1026		0.1293		0.0379		0.041		0.0756		0.0691		0.0702	

[z] Diseases included in the survey – S. Wilt = Sclerotinia Wilt (*Sclerotinia sclerotiorum* Lib.); S. Mid-stalk = Sclerotinia Mid-stalk Rot (*S. sclerotiorum*); S. Head Rot = Sclerotinia Head Rot (*S. sclerotiorum*); Rhizopus = Rhizopus head rot (*Rhizopus* spp.); D. Mildew = Downy Mildew (*Plasmopara halstedii* Farl.); Phomopsis = Phomopsis stem canker (*Phomopsis/Diaporthe* spp.); Phoma = Phoma black stem (*Phoma macdonaldii* Boerma); Verticillium = Verticillium wilt (*Verticillium dahliae* Kleb.); Charcoal Rot (*Macrophomina phaseolina* Tassi.); Rust (*Puccinia helianthi* Schw.).

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[s] Yield – derived using the equation: $Y = 2746 * P * H * C * S * G * B$, where Y is the yield in kilograms per hectare, P is the population multiplier, H is the head diameter multiplier, C is the CCS multiplier, G is the percent good seed multiplier and B is the bird damage multiplier.

[r] Parameter estimates show percent change from the region by year mean for every 1% increase in disease incidence.

[q] P-value associated with each parameter estimate.

-limiting factor was used (Table 2.3). Population was negatively affected by Sclerotinia head rot (-0.53% per unit increase in incidence), Rhizopus head rot (-0.28% per unit increase in incidence) and downy mildew (-0.64% per unit increase in incidence) when all ND and MN data was used (Table 2.2). Additionally, head size was positively affected by downy mildew (0.68% per increase in incidence). With data when disease was a yield-limiting factor, population was negatively affected by Rhizopus head rot (-0.30% per unit increase in incidence) and downy mildew (-0.81% per unit increase in incidence). Head size was positively affected by downy mildew (0.60% per unit increase in incidence) and negatively affected by Phomopsis stem canker (-0.08% per unit increase in incidence). Other yield components were not affected by disease incidence when all data or filtered data was used.

The analyses of each individual disease incidence on each individual yield component indicated that some yield components were significantly affected by some diseases (data not presented). However, it may be inappropriate to analyze the data in this manner since low disease incidence values are observed more often than high values resulting in a disproportionate replicated sample size across the continuous scale of disease incidence. Very few observations would be used at some levels, while a very high number of observations would be used at others.

Data from the NDSC region in 2015 was used in univariate and multivariate regression analyses. However, Sclerotinia head rot, Verticillium wilt and charcoal rot did not occur in the region that year and were not included in the analyses. Univariate analyses showed some yield components were affected by disease (data not included). The percent of good seed was negatively affected by downy mildew (-0.13% per unit increase of incidence) and Phoma black stem (-0.001% per unit increase of incidence). Multivariate analyses indicated that downy mildew and Phomopsis stem canker had significantly different effects across yield components

(data not included). Multivariate regression analyses were desired so comparisons of disease incidence could be made across yield components.

The objective of this work was to determine the impact of disease on yield, and additional analysis needs to be done to determine the influence of specific diseases and/or certain levels (incidence or severity) of disease on specific yield components. However, manipulation and preliminary analysis presented here suggests that the NSA survey data could be used to elucidate the levels of yield loss to sunflower caused by many different abiotic and biotic stresses. A better understanding of how each of these factors impact yield could be a great asset to the sunflower community in the future.

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APPENDIX. SUMMARY OF STATISTICAL ANALYSES FOR THE 2017 – 2019 PHOMA BLACK STEM FIELD TRIALS

Table A.1. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately susceptible hybrid in Davenport, North Dakota, 2017.

	MY8N421CLDM			
Growth stage of fungicide application[z]	R7 Severity[y]	R7 Incidence[x]	R7 DSI[w]	Yield (kg/ha)
NTC	6.6 a[v]	100.0 a	6.6 a	3472
V8	3.8 b	100.0 a	3.8 b	3395
R1	2.1 cd	85.0 a	1.8 cd	3453
R5	5.8 a	97.5 a	5.8 a	3277
V8 + R1	1.6 d	67.5 b	1.1 d	3707
V8 + R5	3.4 bc	87.5 a	2.9 bc	3552
R1 + R5	2.7 bcd	87.5 a	2.3 bcd	3389
V8 + R1 + R5	1.9 d	67.5 b	1.3 d	3570
p-value	<.0001	0.0008	<0.0001	0.5639

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.2. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately resistant hybrid in Davenport, North Dakota, 2017.

	MY8N449CLDM			
Growth stage of fungicide application[z]	R7 Severity[y]	R7 Incidence[x]	R7 DSI[w]	Yield (kg/ha)
NTC	4.8 ab[v]	100.0 a	4.8 a	3679 b
V8	5.5 a	97.5 a	5.3 a	3719 b
R1	1.8 d	55.0 b	1.0 c	4001 a
R5	3.8 bc	92.5 a	3.5 b	3658 b
V8 + R1	1.4 d	37.5 b	0.5 c	4050 a
V8 + R5	3.1 c	90.0 a	2.8 b	3948 a
R1 + R5	1.9 d	55.0 b	1.0 c	4004 a
V8 + R1 + R5	1.3 d	50.0 b	0.7 c	3967 a
p-value	<0.0001	0.0002	<0.0001	<0.0001

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.3. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately susceptible hybrid in Davenport, North Dakota, 2018.

	N4HM354						
Growth stage of fungicide application[z]	R7 Severity[y]	R8 Severity	R7 Incidence[x]	R8 Incidence	R7 DSI[w]	R8 DSI	Yield (kg/ha)
NTC	4.8 a[v]	7.9 a	100.0 a	100.0 a	4.8 a	7.9 a	3564
V8	2.7 b	4.2 b	92.5 abc	97.5 a	2.5 b	4.2 b	3734
R1	2.3 bc	2.5 c	95.0 ab	87.5 ab	2.2 b	2.2 cd	3613
R5	3.9 a	5.0 b	92.5 abc	95.0 a	3.7 a	4.7 b	3917
V8 + R1	1.5 c	1.9 c	45.0 d	55.0 c	0.8 c	1.1 cd	4104
V8 + R5	1.8 bc	2.9 c	80.0 abc	85.0 ab	1.5 bc	2.4 c	3639
R1 + R5	2.3 bc	2.5 c	77.5 bc	82.5 ab	1.8 bc	2.2 cd	3852
V8 + R1 + R5	2.3 bc	2.9 c	72.5 c	75.0 b	1.8 bc	2.5 c	3795
p-value	<0.0001	<0.0001	0.0009	0.0014	<0.0001	<0.0001	0.1588

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.4. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately resistant hybrid in Davenport, North Dakota, 2018.

Growth stage of fungicide application[z]	Camaro II												
	R7 Severity[y]		R8 Severity		R7 Incidence[x]		R8 Incidence		R7 DSI[w]		R8 DSI		Yield (kg/ha)
NTC	4.2	a[v]	6.0	a	100.0	a	100.0	a	4.2	a	6.0	a	3558
V8	1.7	cd	3.4	bc	82.5	bc	80.0	b	1.4	c	2.9	c	3934
R1	1.8	c	2.7	cd	75.0	cd	92.5	ab	1.4	cd	2.5	c	3494
R5	3.7	b	4.4	b	97.5	ab	97.5	a	3.6	b	4.3	b	3188
V8 + R1	1.3	de	1.7	de	45.0	e	62.5	c	0.6	ef	1.1	de	3821
V8 + R5	1.4	cde	2.1	de	72.5	cd	92.5	ab	1.0	cd	1.9	cd	3655
R1 + R5	1.5	cde	2.5	cde	65.0	d	80.0	b	1.0	de	2.0	cd	3698
V8 + R1 + R5	1.1	e	1.5	e	20.0	f	52.5	c	0.2	f	0.8	e	3787
p-value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0755

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.5. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately susceptible hybrid in Davenport, North Dakota, 2019.

Growth stage of fungicide application[z]	N4HM354						
	R7 Severity[y]	R8 Severity	R7 Incidence[x]	R8 Incidence	R7 DSI[w]	R8 DSI	Yield (kg/ha)
NTC	3.2 a[v]	5.0 a	100.0 a	100.0 a	3.2 a	5.0 a	2357
V8	2.5 ab	4.5 a	87.5 ab	97.5 a	2.2 b	4.4 a	2246
R1	1.7 bc	2.0 c	62.5 c	70.0 cd	1.1 c	1.5 c	2491
R5	3.2 a	4.2 a	90.0 a	95.0 ab	2.9 a	4.1 a	2500
V8 + R1	0.8 d	1.7 c	22.5 d	65.0 d	0.2 d	1.2 c	2398
V8 + R5	2.7 a	3.0 b	67.5 bc	87.5 abc	1.8 b	2.6 b	2334
R1 + R5	1.8 bc	1.9 c	37.5 d	75.0 bcd	0.6 cd	1.5 c	2456
V8 + R1 + R5	1.3 de	1.3 c	20.0 d	42.5 e	0.3 d	0.6 c	2338
p-value	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.8863

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.6. Disease ratings and yield of Phoma black stem fungicide timing trial conducted with a moderately resistant hybrid in Davenport, North Dakota, 2019.

Growth stage of fungicide application[z]	Camaro II												
	R7 Severity[y]		R8 Severity		R7 Incidence[x]		R8 Incidence		R7 DSI[w]		R8 DSI		Yield (kg/ha)
NTC	3.1	a[v]	6.0	a	100.0	a	100.0	a	3.1	a	6.0	a	2232
V8	2.2	ab	4.6	b	92.5	a	100.0	a	2.1	b	4.6	b	2207
R1	1.4	bc	1.9	cd	35.0	bc	65.0	bc	0.5	cd	1.2	cd	2124
R5	2.9	a	4.2	b	92.5	a	97.5	a	2.7	ab	4.1	b	2201
V8 + R1	0.5	c	1.4	d	15.0	c	47.5	cd	0.2	d	0.7	d	2199
V8 + R5	1.6	b	2.7	c	57.5	b	80.0	ab	0.9	c	2.2	c	2423
R1 + R5	1.4	b	2.2	cd	32.5	bc	57.5	c	0.7	cd	1.4	cd	2303
V8 + R1 + R5	0.5	c	1.6	cd	15.0	c	30.0	d	0.2	d	0.6	d	2266
p-value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.9316

[z] Growth stages are based on Schneiter and Miller (1981). All applications were made with 109.8 g ai/ha of pyraclostrobin (Headline, BASF, Research Triangle Park, NC, USA) in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[x] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[w] DSI = Disease severity index is calculated from a plot as incidence times severity.

[v] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.7. Disease ratings and yield of Phoma black stem fungicide efficacy trials conducted in Davenport, North Dakota, 2018.

	N4HM354										
Fungicide[z]	R7 Severity[w]		R8 Severity		R7 Incidence[v]		R8 Incidence		R7 DSI[u]		R8 DSI		Yield (kg/ha)
NTC	6.8	a[t]	8.7	a	100.0	a	100.0	a	6.8	a	8.7	a	2724
PYR	2.7	de	3.7	efg	67.5	bcd	85.0	abc	1.8	fgh	3.4	ef	3200
AZO	3.5	cd	4.5	def	87.5	af	90.0	ab	3.1	de	4.2	de	3221
PIC	4.2	cd	5.7	cd	85.0	abc	90.0	ab	3.7	cd	5.3	cd	3045
TEB	5.3	b	7.4	ab	100.0	a	97.5	a	5.3	b	7.3	ab	2948
PYD	0.9	g	1.9	h	15.0	e	32.5	e	0.2	i	0.6	h	3115
BOS	1.4	fg	2.3	gh	52.5	d	55.0	d	0.8	hi	1.4	gh	3012
FLU+TEB[y]	2.9	de	4.7	de	85.0	abc	85.0	abc	2.5	def	4.3	de	3045
FLU+TEB[x]	2.8	de	3.7	efg	77.5	abc	87.5	abc	2.2	efg	3.3	ef	2759
PYR+FLX	1.9	efg	3.8	ef	67.5	bcd	75.0	bc	1.5	fgh	3.1	ef	2452
PYR+FLX+MEF	2.1	ef	3.2	fgh	62.5	cd	70.0	cd	1.3	ghi	2.4	fg	2997
MET	4.5	bc	6.7	bc	100.0	a	92.5	ab	4.5	bc	6.3	bc	2902
p-value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.1467

[z] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.

[x] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.

[w] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[v] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[u] DSI = Disease severity index is calculated from a plot as incidence times severity.

[t] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.8. Disease ratings and yield of Phoma black stem fungicide efficacy trials conducted in Davenport, North Dakota, 2019

	N4HM354												
Fungicide[z]	R7 Severity[w]		R8 Severity		R7 Incidence[v]		R8 Incidence		R7 DSI[u]		R8 DSI		Yield (kg/ha)
NTC	3.5	a[t]	6.4	a	90.0	a	90.0	a	3.2	a	5.7	a	1935
PYR	2.9	abc	3.8	cd	47.5	bcd	62.5	bc	1.5	bcd	2.3	de	2214
AZO	2.3	cd	5.2	ab	50.0	bcd	70.0	ab	1.2	cde	3.8	bc	1872
PIC	3.1	abc	3.5	cd	57.5	bc	75.0	ab	1.8	bc	2.7	cde	2296
TEB	3.5	a	5.6	ab	87.5	a	87.5	a	3.0	a	4.9	ab	2092
PYD	0.7	f	1.2	e	10.0	e	17.5	e	0.2	f	0.3	g	1981
BOS	1.3	ef	1.5	e	32.5	d	12.5	e	0.4	ef	0.3	g	1779
FLU+TEB[y]	1.9	de	3.6	cd	42.5	bcd	60.0	bc	0.8	def	2.2	def	2190
FLU+TEB[x]	1.8	de	3.7	cd	32.5	d	42.5	cd	0.6	ef	1.6	ef	1976
PYR+FLX	2.5	bcd	3.1	d	40.0	cd	55.0	bcd	1.0	de	1.7	ef	2106
PYR+FLX+MEF	2.5	bcd	2.5	de	35.0	d	35.0	de	0.8	def	0.9	fg	2257
MET	3.2	ab	4.5	bc	62.5	b	77.5	ab	2.1	b	3.4	cd	1802
p-value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0704

[z] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.

[x] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.

[w] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[v] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[u] DSI = Disease severity index is calculated from a plot as incidence times severity.

[t] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table A.9. Disease ratings and yield of two Phoma black stem fungicide efficacy trials conducted in North Dakota in 2018 and 2019.

	Combined Efficacy Trials												
Fungicide[z]	R7 Severity[w]		R8 Severity		R7 Incidence[v]		R8 Incidence		R7 DSI[u]		R8 DSI		Yield (kg/ha)
NTC	5.1	a[t]	7.5	a	95.0	a	95.0	a	5.0	a	7.2	a	2332
PYR	2.8	bcd	3.7	de	57.5	cde	73.8	bc	1.6	cdef	2.9	def	2710
AZO	2.9	bcd	4.8	cd	68.8	bcd	80.0	abc	2.2	cde	4.0	cd	2549
PIC	3.6	abc	4.6	cd	71.3	bc	82.5	abc	2.7	bcd	4.0	cd	2673
TEB	4.4	ab	6.5	ab	93.8	a	92.5	a	4.2	ab	6.1	ab	2522
PYD	0.8	e	1.6	f	12.5	f	25.0	e	0.2	f	0.5	g	2550
BOS	1.4	de	1.9	f	42.5	e	33.8	e	0.6	ef	0.8	g	2397
FLU+TEB[y]	2.4	cde	4.2	cde	63.8	bcd	72.5	bc	1.7	cdef	3.2	de	2620
FLU+TEB[x]	2.3	cde	3.7	de	55.0	cde	65.0	cd	1.4	def	2.4	ef	2370
PYR+FLX	2.2	cde	3.5	de	53.8	cde	65.0	cd	1.2	def	2.4	ef	2281
PYR+FLX+MEF	2.3	cde	2.8	ef	48.8	de	52.5	d	1.1	def	1.6	fg	2630
MET	3.8	abc	5.6	bc	81.3	ab	85.0	ab	3.3	bc	4.8	bc	2354
p-value	0.0058		<0.0001		0.0001		<0.0001		0.0015		<0.0001		0.3970

[z] Active ingredients of fungicides: NTC = nontreated control, PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), PIC = picoxystrobin (Aproach, DuPont Agricultural Products), TEB = tebuconazole (Folicur, Bayer CropScience), PYD = pydiflumetofen (Miravis, Syngenta Crop Protection), BOS = boscalid (Endura, BASF), FLU + TEB = fluopyram and tebuconazole (Luna Experience, Bayer CropScience), PYR+FLX = pyraclostrobin and fluxapyroxad (Priaxor, BASF), PYR+FLX+MEF = pyraclostrobin, fluxapyroxad and mefentrifluconazole (Revytek, BASF) and MET = metconazole (Quash, Valent USA). All applications were made in a 187 L/ha suspension with a handheld boom pressurized at 275.8 kPa by a CO_2 backpack sprayer.

[y] Applied at a rate of 131.6 g ai/ha and 131.6 g ai/ha for fluopyram and tebuconazole, respectively.

[x] Applied at a rate of 187.2 g ai/ha and 187.2 g ai/ha for fluopyram and tebuconazole, respectively.

[w] Severity is calculated from ten randomly selected plants as the average number of stem lesions per diseased plant.

[v] Incidence is calculated from ten randomly selected plants as the percentage of plants with at least one Phoma stem lesion.

[u] DSI = Disease severity index is calculated from a plot as incidence times severity.

[t] Means with the same letters are not significantly different based on Fisher's protected least significant difference ($\alpha = 0.05$).