MANAGING ECONOMICALLY IMPORTANT DISEASES OF SUNFLOWER AND OILSEED RAPE IN NORTH DAKOTA, CALIFORNIA AND SCHLESWIG-HOLSTEIN

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Rust, caused by *Puccinia helianthi,* and blackleg, caused by *Leptosphaeria maculans,* are economically important diseases on sunflower and oilseed rape, respectively. In order to recommend the most effective disease management practices to growers, management strategies need to be evaluated as new tools are developed and pathogen populations change. The objectives of these studies were to; 1) evaluate the efficacy of foliar fungicides on rust in confection and oilseed sunflowers in the U.S. Northern Great Plains, 2) determine virulence phenotypes of *Puccinia helianthi* in the sunflower seed production region of Northern California, and 3) evaluate the seed applied fungicide adepidyn for efficacy of blackleg on oilseed rape in Northern Germany. Efficacy of 11 foliar fungicides against sunflower rust were demonstrated on both confection and oilseed sunflowers, however, yield differences were only observed in confection experiments. In total, 21 races of *Puccinia helianthi* were identified in the sunflower seed production region of California, of which 18 were reported for the first time. To the best of my knowledge, this work presents the only data generated from wild *Helianthus annuus* populations in the region, and results demonstrate the threat that rust on wild species presents to the commercial seed production industry. Results of experiments conducted to evaluate the suitability of the novel seed treatment adepidyn for management of blackleg demonstrated efficacy under climate-controlled conditions, but efficacy was not observed in field studies. Results of these studies detail relevant and timely information that will help agriculture professionals better manage these economically important diseases of oilseed crops.

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DEDICATION

I dedicate my dissertation to my late father Tim Berghuis. The life lessons, laughs, and your joy for life I still carry with me today.

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LITERATURE REVIEW

Sunflower

Sunflower (*Helianthus annuus* L.) is native to North America and was one of the first crops grown in the United States (Putt 1978). Sunflower was originally cultivated by Native Americans several centuries before European arrival in what is current day the United States. Sunflower was grown by Native Americans for food and used as a dye for basketry and as body paint during ceremonial activities (Heiser et al. 1969; Rogers et al. 1982). Explorers described Native Americans gathering wild sunflowers for food and archaeological digs have found remnants of roasted achenes that date back to as early as 1000 BCE. On Meriwether Lewis and William Clark's western expedition to explore the Louisiana Purchase, they documented sampling sunflower seeds in what is current day North Dakota (Harveson 2016; Putt 1978). These early cultivars and wild sunflowers were brought to Europe by Spanish explorers and soon became a popular ornamental crop throughout Europe (Zukovsky 1950).

The earliest documentation of sunflower being planting in Spain was Madrid in 1510 and the earliest book reference on sunflower was by the author Rembert Dodoens in Florum published in 1568 (Putt 1978). This plant grew in popularity among the aristocracy in Europe and spread throughout the continent (Semelczi-Kovacs 1975). Different types of sunflowers were also introduced to Belgium, England, Germany and Holland from the United States (Harveson 2016; Semelczi-Kovacs 1975). The sunflower plant reached Eastern Europe in the seventeenth century and consumption of the seeds became popular in the 1740s (Harveson 2016; Putt 1978). Peter Romanov, also known as Peter the Great, has been generally attributed with the introduction of sunflower into Russia (Zukovsky 1950).

Peter Romanov traveled the world to look for modern ideas and concepts that he could bring back to his city of St. Petersburg. After seeing sunflower numerous times in Holland, he became quite fond of them and brought them back to his home country (Harveson 2016; Zukovsky 1950). The nutritional value of sunflower was recognized in the middle of the 1800s and Russian farmers began to cultivate this plant as an oil seed crop (Rogers et al. 1982). Sunflower grew in popularity in Russia and by 1880 acreage increased to 370,000. Extensive breeding efforts began in the early 1900s and breeders were able to increase seed size and attain oil contents up to nearly 50% (Harveson 2016).

By the turn of the twentieth century, sunflower became the most important oilseed crop in Europe. During World War II, it was hypothesized that one of the primary reasons that Germany formed a second battle line was to secure the fields of Ukraine and to utilize oil seed crops. During this time an additional utility of sunflower oil was discovered by both the Germans and the Russians (Harveson 2016). Sunflower oil has a lower freezing point than gun oil and thus could be used as a substitute for freezing gun oil on the frigid eastern front. Oil produced by sunflower increased in popularity worldwide proceeding the war and sunflower production expanded to South Africa and South America. Two decades later, cultivation of sunflower began in Australia (Harveson 2016; Putt 1978).

Sunflower is thought to have made its way back to North America by Russian Mennonite immigrants as early as 1875 (Putt 1978). Proceeding the reintroduction of sunflower, the plant was used primarily for a forage and silage crop. Breeding efforts began in Canada for sunflower in 1936 and Mennonite farmers in Manitoba formed the first processing plant for sunflower (Harveson 2016). In 1970, Cytoplasmic male sterility was discovered in wild species of *Helianthus petiolaris* and was breed into cultivars of *Helianthus annuus* to form the first

sunflower hybrids (Leclercq 1968; Harveson 2016). The hybrids improved the crop by increasing disease resistance, uniformity, and yield and thus replacing the old open pollinated varieties (Putt 1978). Today sunflower continues to make history as it served as a test subject for experimentation on the Apollo space shuttle and world production area has grown to 25.41 million hectares in 2016/17 (Harveson 2016; USDA 2018).

Sunflower Production

Sunflower is grown for both industrial and food applications. Due to its short growing season and being highly adaptable to various weather conditions, it has found its way into major production areas worldwide (Harveson 2016). In 2017, the United States sunflower growers produced 1.2 billion kg (2.17 billion pounds). The top producing states in 2017 were South Dakota and North Dakota at 1.04 billion pounds and 697 million pounds respectively (National Sunflower Association 2017). Despite California not being a national leader in sunflower production, more than 90% of all hybrid seed grown in the United States comes from California. The sunflower producing area in California quadrupled between the years 2002 and 2006. By 2014, approximately a quarter of the world's supply of hybrid seed was produced in California (Lazicki and Geisseler 2016).

Taxonomy

There are numerous sunflower species but *Helianthus annuus* is the only one that has been routinely planted and cultivated (Harveson 2016). The genius *Helianthus* comes from the Greek words *helios*, meaning sun and *anthus* meaning flower (Seiler 2016). The species name *annuus* refers to the plant completing its life cycle annually (Harveson 2016). Wild sunflowers, are also classified as *Helianthus annuus*, are a variant of the domesticated sunflowers. These

multiheaded plants are widely dispersed and found throughout the contiguous United States, northern Mexico, southern Canada, Europe, South America, Australia, and south eastern Africa.

Rust of Sunflower

Puccinia helianthi

The fungal pathogen that causes the disease rust on sunflowers was first described by Schweinitz in 1882 and was named *Aecidium helianthi-mollis*. The pathogens name was later changed to *Puccinia helianthi-mollis* and furthermore the suffix was taken out to become the present day name *Puccinia helianthi* (Bailey 1923). Sunflower rust is an economically important disease that can cause a reduction in seed size, oil and yield (Friskop et al. 2011). Yield loss caused by sunflower rust has been recorded as high as 80% (Markell et al. 2016). *Puccinia helianthi* Schwein., has a wide host range which includes all annual and perennial *Helianthus* spp. (Gulya et al. 1997).

Signs and Symptoms

Puccinia helianthi is an autoecious macrocyclic rust with four of the five spore stages visible with the unaided eye (Bailey 1923; Markell 2016). The earliest sign of infection is the pycnia stage and appears as yellow-orange spots on the upper side of the cotyledons or true leaves. Aecia is the next stage and is on the direct opposite of the leaves from pycnia. The aecial stage appears as small orange cups comparable in size to pycnia. Uredinial pustules form approximately one to two weeks after the aecial stage and appear as cinnamon brown pustules. A yellow chlorotic halo might be present around the outside of the rust pustule (Bailey 1923; Friskop et al. 2011). A combination of cool temperatures and host maturity initiate the overwintering spore stage call telia. Telia are black in color and remain attached to plant tissue

until the proceeding season where they serve as an inoculum source (Miah and Sackston 1970; Markell et al. 2016).

Biology and Epidemiology

P. helianthi overwinters as telia or survives as uredinia between growing seasons (Miah and Sackston 1970). In areas that rust overwinters, the teliospores germinate in the spring to initiate the lifecycle. In warmer areas such as a tropical region, the spores will repeatedly cycle in the uredinial spore stage until reaching the telial spore stage. Urediniospores are disseminated by wind and can remain viable for great distances. The optimal conditions for uredinospores are 24 hours of free moisture, temperature of 10-25ºC, and low light intensity. Within these conditions, the asexual urediniospores can cycle in 10-14 days (Markell et al. 2016).

Disease Management

The most economically and environmentally safe option to manage this disease is genetic resistance. However, *P. helianthi* can overcome resistance genes quickly which can make durable disease management quite difficult. Virulence diversity of *P. helianthi* has been reported since the 1920s but with the pathogens ability to undergo sexual reproduction, efforts to survey continue across the world (Friskop et al. 2015b). Distinct races of *P. helianthi* were first established on cultivated sunflowers by Sackston in 1962 (Sackston 1962). Since 1980, in the United States there have been more than two dozen races identified. A few of these isolates are virulent on all differential lines. In China, six different races were identified in the early 2000s. In 2012, six races were also identified in Argentina. Australia has conducted extensive research over 25 years that has compiled information of over 2000 isolates on 21 differential lines of *P. helianthi* that identified 61 pathotypes (Markell et al. 2016). Currently, there are no available

commercial hybrids with resistance to the most virulent race (777) isolated from North Dakota (Friskop et al. 2015b).

Recent research demonstrated that several fungicides were efficacious and economically viable management tools for sunflower rust (Friskop et al. 2015a). Currently, there are numerous fungicides registered for use in North Dakota and these fungicides and their modes of action are FRAC 3, FRAC 7, FRAC 11 (Markell et al. 2012). Timing of application is essential to properly manage disease. Fungicide applications should be made when the severity on the upper four leaves reaches approximately 1% disease severity at or before reproductive stage R5. Applications made at R6 or later have been shown to have little or no effect on yield (Friskop et al. 2011).

Oilseed Rape

Oilseed rape or canola (*Brassica napus* L.) is an economically important oilseed crop grown around the world. The oil is characterized with reduced erucic acid and glucosinolates content, making it valuable for human consumption as well as industrial uses (Howlett 2004). *Brassica napus* was derived from a cross between *B. oleracea* (cabbage) and *B. rapa* (turnip) thousands of years ago (Angadi et al. 2003; Chalhoub et al. 2014). In India, China, and northern Europe, oilseed rape has been grown for centuries primarily for cooking oil and lighting (Busch et al. 1994). In northern Europe, documentation of the crop being grown has been recorded as early as the 13th century and was especially important due to its resiliency to cold temperatures (Angadi et al. 2003; Busch et al. 1994).

In North America, production of industrially grown rapeseed was profoundly expanded when it was discovered that rapeseed oil would cling to water and steam washed metal surfaces in steam power engines and machinery more efficiently than other lubricants (Angadi 2003). It

was this notable quality that led to the expansion of rapeseed production into Canada during WWII (Angadi 2003; Busch 1994). Physiological changes of *Brassica* spp. have been achieved by breeders in Canada through traditional breeding efforts and thus the term "Canada Oil Low Acid" or "canola" has been adopted. Technically, only cultivars of oilseed rape that meet the standards for low erucic acid in the oil and low glucosinolates are classified as canola. However, this term has been loosely adopted and may include unconventional varieties grown for industrial purposes (Angadi et al. 2003). Currently winter oilseed rape is widely cultivated in Asia and Europe and many varieties technically fit the standards for canola with low erucic acid in the oil and low glucosinolates levels (Angadi et al. 2003).

In 2019, the top producing countries of oilseed rape in the world were Canada, China, India, France, Ukraine, Germany, Australia, and Poland. In 2019, Germany produced approximately 2,830,200 tonnes of oilseed rape and was one of the top producing countries within the European Union (FAO 2019). In the northern most German state of Schleswig-Holstein, oilseed rape is the second most important crop only behind winter wheat. Additionally, it is the only broadleaf crop in a typical Schleswig-Holstein crop rotation of winter oilseed rape, winter wheat, and winter barely (Kruse and Verreet 2004). Numerous studies in northern Germany have demonstrated the importance of oilseed rape as an alternate crop in a rotation with increased yield and decreased disease recorded in cereal crops proceeding oilseed rape cultivation (Christen et al. 1992; Kruse and Verreet 2004).

Blackleg of Oilseed Rape

Leptosphaeria maculans

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans*, has been a prevalent disease on oilseed rape in Europe for decades and is widely considered to be the most yield-

limiting and economically important disease of *Brassica napus* (canola, rapeseed, colza) worldwide (McGee and Petrie 1977; Marcroft 2005). Historically, blackleg has had profound economic impacts, and prior to moderately resistant varieties becoming commercially available in the mid 1990's, it was estimated that growers in Canada lost US $500,000,000 in yield as a result of blackleg (Angadi et al. 2003).

Leptosphaeria maculans (Desm.) Ces. & De Not. was first described by Tode (1791) on the stems of red cabbage and named the fungus *Sphaeria lingam* (Williams 1992). Proceeding this classification, Desmazieres (1849) collected this fungus from *Brassica oleracea* plants and reclassified the fungus to the genus Phoma (Desmazieres 1849). Well into the next century, this fungus was also named *Phoma brassicae*, *Phoma oleracea*, *Phoma napobrassicae*, *Plenodomus lingam* and other generic and specific epithets (Boerema 1976). Current classification of the fungus is *Leptosphaeria maculans* (Desm.) Ces. & De Not. for the sexual state and *Phoma lingam* (Tode ex Fr.) for the asexual state. The sexual stage of the pathogen was first identified in New Zealand and later confirmed to be *Leptosphaeria maculans* (Desm.) Ces. & De Not.

Kingdom: Fungi

Phylum: Ascomycota

Class: Dothideomycetes

Order: Pleosporales

Family: Leptosphaeriaceae

Genus: *Leptosphaeria*

Species: *maculans* (Desm.) Ces. & De Not.

Signs and Symptoms

L. maculans can cause disease on cotyledons, leaves, stems, and pods (Söchting and Verreet 2004; Angadi et al. 2003). Signs and symptoms on leaves appear as irregular shaped white/necrotic lesions with small pepper dots known as pycnidia. When weather conditions are moist, a pink spore mass may ooze from the pycnidia (pycnido spores). Blackleg disease signs and symptoms on stems are variable but are typically located at the base of the stem or at the point of leaf attachment. Lesions on stems may appear as white or gray necrotic tissue with a black border and may be several inches in length. Pycnidia typically form in the center of these lesions and under severe infection may result in a canker the base of the stem (Angadi et al. 2003).

Biology and Epidemiology

L. maculans overwinters on infected seed and plant residue. Seed infection is possible, but rare and is not regarded as a major source of inoculum or infection. However, even limited infected seed can lead to pathogen dispersal and result in scattered areas of infection across fields. Planting a seed lot with 1% infected seed can result in disease incidence of 25,000 infected plants per ha (Angadi et al. 2003). In Australia, Europe and North America, epidemics in oilseed rape are caused by inoculum dispersal from infected crop residue. Depending on weather, the pathogen can survive 1-4 years and produce pseudothecia on infected oilseed rape residue (Rimmer and Van Den Berg 2007). The initial source of plant infection is by ascospores formed in pseudothecia and released by wind to adjacent fields or even longer distances (McGee 1977; Rimmer and Van Den Berg 2007). Infection by ascospores creates local lesions on leaf tissue and in these lesions numerous pycnidia are formed (Rimmer and Van Den Berg 2007). Additionally, the pathogen spreads biotrophically from these lesions through the plant vascular

system and to the stem where cankers are formed. The pathogen lifecycle continues as pycnidiospores are spread by rain splash to other parts of the plant or neighboring plants. Secondary infection by pycnidiospores is less damaging as compared to ascospores but may contribute to overall disease severity, especially in fall-sown oilseed rape and other brassica crops (Rimmer and Van Den Berg 2007). In Europe, the vast majority of ascospores are released between September and November which coincides with the sowing of oilseed rape and when seedlings are most susceptible (Rimmer and Van Den Berg 2007; West 2001; Brachaczek et al. 2016).

Disease Management

An integrated pest management approach is recommended to manage disease caused by *L. maculans*. Planting a blackleg resistant or moderately resistant variety is one of the most effective ways to manage this disease. Crop rotation paired with managing weed hosts such as volunteer plants can help reduce inoculum. Additionally, pathogen-free seed, seed treatments and foliar fungicides may be important tools to manage this this disease (Markell et al. 2008; West 2001). Fungicides can be valuable tool for managing *L. maculans* especially in western Europe where high yields traditionally have justified their applications (West 2001). Previous research on canola in Australia has demonstrated that protecting seedlings up to the six-leaf stage is critical to avoid cankers that cause substantial yield loss (Marcroft et al. 2005). A number of studies conducted in northern Germany and Poland that have demonstrated that fungicides applied during peak ascospore dispersal in September has resulted in reduced disease severity and increased yield (Kruse and Verreet 2004; Brachaczek et al. 2016). Traditionally, foliar fungicides (tebuconazole, carbendazim, etc.) have been applied in Germany to manage disease caused by *L. maculans* (Kruse and Verreet 2004; West et al. 2001). Along with foliar fungicides,

multiple studies in other countries have demonstrated the effectiveness of a fungicide seed treatments to reduce disease severity and mitigate yield losses by *L. maculans* in the field and greenhouse (Macroft and Potter 2008; Upadhaya et al. 2019).

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EVALUATION OF FUNGICIDE EFFICACY ON SUNFLOWER RUST (*PUCCINIA HELIANTHI*) ON OILSEED AND CONFECTION SUNFLOWER

Abstract

Sunflower rust, caused by *Puccinia helianthi*, is an economically and globally important disease of sunflower. Two types of sunflowers are produced in the US Northern Great Plains; the oilseed type and the confection type. Although approximately 80% of the acreage in this region is planted as the oilseed type sunflower, fungicide efficacy and timing studies have been conducted primarily on the more rust-susceptible confection type. A total of ten sunflower rust efficacy field experiments were conducted on oilseed type and confectionary type hybrid trials from 2016-2018. Eleven fungicides from three FRAC groups were evaluated for efficacy and protection of yield. Severity differences among fungicide treatments were identified in both confection and oilseed type sunflower trials. A combined analysis of all confection field trials (five) indicated that rust severity was lower in all fungicide treatments as compared to the non-treated control. Despite rust severity levels below the fungicide action threshold for confection sunflower, seven of the eleven fungicide treatments had yield higher than the non-treated control. In oilseed trials, rust severity was lower in all fungicide treatments as compared to the non-treated control, similar to the findings of the confection type. Rust severity was too low to detect yield differences in oilseed trials. Additional work is needed to elucidate yield-loss potential on oilseed type sunflower and refine the fungicide action threshold on confection type sunflower.

Introduction

The common sunflower, *Helianthus annuus* L., is grown around the world for direct human consumption, oil, use as an ornamental, and bird feed. In 2018/19, approximately 27,265,000 ha of sunflowers were harvested in the world with the top producers being Russia,

Ukraine, European Union, and Argentina (FAO 2019). In the United States, approximately 493,000 ha of sunflower was harvested in the 2018/19 season and of that, approximately 75% was in North Dakota and South Dakota (NSA 2021). Two commercial types of row-crop sunflower are grown; oilseed and non-oilseed (confection) (Kandel et al. 2020). The oilseed type produces a small black seed with a high oil content, and is used for sunflower oil, sunflower meal, and bird feed. Confection type sunflower seed is a larger, produces black and white striped seed, and is primarily used in food products (Kleingartner et al. 2015, Dunford et al. 2015). Confection sunflower is considered a specialty-crop (USDA) in the US, and typically commands a higher price than oilseed sunflower with a premium for large seed size (Kandel et al. 2020).

Sunflower rust, caused by the fungal pathogen *Puccinia helianthi* Schwein, is a yield-limiting disease in both oilseed and confection sunflower types (Markell 2016a, Markell et al. 2015, 2016b). *Puccinia helianthi* is an autoecious and macrocylic pathogen with a host range that includes many wild *Helianthus* spp. (Friskop et al. 2015a; Friskop et al. 2015b; and Markell, 2016). The fungus overwinters as telia or as uredinia and survives environmental conditions common in the US Northern Great Plains (Gulya et al. 2019). In the US, rust is among the most common diseases found in sunflower and occurs throughout the entire US Great Plains. In a comprehensive survey conducted between 2002-2015, rust was the most frequently detected disease in sunflower fields with prevalence exceeding 50% in numerous years in the US Great Plains. Yield losses of 80% with unmarketable quality (reduced seed size) have been reported in North Dakota (Gulya et al. 2019, Markell et al. 2009). Research conducted in North Dakota on confection sunflower found a 6.6% reduction in yield for every 1% increase in disease severity at R7 (Friskop et al. 2015a).

Several management tools can be utilized by sunflower growers in the US Northern Great Plains, but they have limitations. Destruction of wild and volunteer sunflowers can reduce inoculum, but this is often impractical due to the high frequency of volunteer and wild plant hosts (Harveson et al. 2016, Kandel et al. 2020). Genetic resistance, conferred by major genes, can be an effective management tool (Qi et al. 2014, Ma et al. 2019, Friskop et al. 2015). However, sexual recombination events occur frequently and often result in new pathogen races that confer virulence on resistance genes in commercial hybrids (Markell et al. 2009, Markell 2016a).

Fungicides are an available and viable management tool for rust and are used to manage the disease (Markell et al. 2016a). Research in Israel on confection sunflower type cultivars demonstrated that tebuconazole applied as a foliar application during early bloom at approximately R5.2 resulted in less disease severity and higher yield (0.86-1.15 t ha) (Shtienberg and Zohar 1992). Additional research conducted by Shtienberg (1995), established an action threshold of 3% disease severity on the upper four leaves if severity is reached prior to 27 days after flowering. More recent research conducted by Friskop et al. (2015a), reported a fungicide application action threshold of 1% disease severity (Figure 1.1.) on the upper four fully expanded leaves at or before R5 in non-irrigated confection sunflower produced in the US Northern Great Plains. Research conducted in both studies found that application of demethylation inhibitor fungicides (FRAC 3, DMI) reduced rust severity, and Friskop et al. 2015 found that quinone outside inhibitor fungicides (FRAC 11, QoI) could effectively manage rust. However, those studies were only conducted on confection sunflower types. Confection sunflowers are widely believed to be more severely impacted by rust, but some research suggests that yield loss on oilseed types may be higher at lower severity levels (Friskop et at. 2011). Additionally, new

fungicides have become available to sunflower growers in the US, and determination of efficacy is critical for the development of management recommendations (Friskop et al. 2020, McMullen and Markell 2010, Mueller et al. 2013). The objective of this study were to evaluate fungicide efficacy on sunflower rust for oilseed and confection sunflower types.

Figure 1.1. One percent disease severity on a fully expanded sunflower leaf. A. Friskop, North Dakota State University, 2011.

Field Experiments

From 2016 to 2018, fungicide efficacy on both sunflower types was evaluated at five locations in total over three years. Specifically, Rothsay, MN and Leonard, ND in 2016, Davenport, ND and Carrington, ND in 2017, and two locations near Davenport, ND in 2018 (Table 1.1). At each location, the two trials (oilseed and confection) were conducted

independently but planted adjacent to one another. All field experiments were arranged in randomized complete block design (RCBD) with four row plots (Figure 1.2). Plot length varied among years but ranged from 4.88 m to 10.67 m long. Row width was 76.2 cm and seed spacing was 21.6 cm. Seeding rate was approximately 9,710 seed/hectare (24,000 seeds/acre). In all experiments, the center two rows were used for inoculation, fungicide application, disease severity data and yield. The hybrids used in the experiment were 8N421CLDM for oilseed trials and 8C451CP for confection trials in 2016 and 2017 (Mycogen, Breckenridge, MN). In 2018, N4HM354 and Jaguar DMR were planted for the oilseed and confection trial, respectively (NuSeed, Breckenridge, Mn). Sunflowers were produced according to the North Dakota State University sunflower production guide (Kandel et al. 2020).

Table 1.1. Year, location, sunflower type, hybrid, planting date, plot length, inoculation, spray date and harvest date for oilseed and confection sunflower type trials conducted from 2016-2018.

Year	Location	Sunflower[x]	Planting date	Plot length	Inoculation[y]	Fungicide[z]	Harvest date
2016	Rothsay, MN	Confection	5/19	6.10	6/23, 7/8	7/28	N/A
2016	Leonard, ND	Confection	5/17	9.75	6/22, 7/8	7/28	10/11
2017	Davenport, ND	Confection	5/18	10.67	6/30, 7/19	8/1	10/18
2017	Carrington, ND	Confection	6/1	4.88	7/10, 7/20	8/11	N/A
2018	Davenport, ND	Confection	5/21	10.67	7/12	7/24	10/17
2016	Rothsay, MN	Oilseed	5/19	6.10	6/23, 7/8	7/28	N/A
2016	Leonard, ND	Oilseed	5/17	9.75	6/22, 7/8	7/28	10/11
2017	Davenport, ND	Oilseed	5/18	10.67	6/30, 7/19	8/1	10/11
2017	Carrington, ND	Oilseed	6/1	4.88	7/10, 7/20	8/7	N/A
2018	Davenport, ND	Oilseed	5/21	10.67	7/12	7/24	10/17

[x]Sunflower type planted between 2016-2018.
[y]Date field trials were inoculated with urediniospores suspended in Soltrol 170 at a concentration of approximately 275,000 spores/mL using a backpack mounted leaf blower at growth stage V8-V16.
[z]Fungicide application date at targeted plant growth stage R5.1-5.2.

Figure 1.2. Aerial photo of Davenport, 2017 sunflower trials. S. Fitterer, BASF, 2018.

Fungicide treatments evaluated in this study were from three Fungicide Resistance Action Committee (FRAC) groups, including demethylation inhibitors (DMI – FRAC 3), quinone outside inhibitors (QoI – FRAC 11) and succinate dehydrogenase inhibitors (SDHI – FRAC 7). Applications were made at growth stages R5.1-R5.2, using a three-nozzle boom with 0.51 m (20”) nozzle spacing, Teejet 8002 flat fan nozzles (TeeJet Technologies, Wheaton, IL) applied at 187 liter/ha (20 gallons per acre) and walking at approximately 4.8 kph (3 mph) (Table 1.2).

Table 1.2. Fungicide product, active ingredients, Fungicide Resistance Action Committee (FRAC) codes, and the application rates applied as a foliar application at R5.1-5.2 using a hand boom, 8002 nozzles, at 187.07 liter per ha (20 gallons per acre).

Product[x]	Active ingredient	FRAC code[y]	Application rate[z]
Onset	Tebuconazole	3	0.29 liter/ha (4 fl oz)
Orius	Tebuconazole	3	0.29 liter/ha (4 fl oz)
TebuStar	Tebuconazole	3	0.29 liter/ha (4 fl oz)
Headline	Pyraclostrobin	11	0.44 liter/ha (6 fl oz)
Quadris	Azoxystrobin	11	0.44 liter/ha (6 fl oz)
Aproach	Picoxystrobin	11	0.44 liter/ha (6 fl oz)
Vertisan (1)	Penthiopyrad	7	0.73 liter/ha (10 fl oz)
Vertisan (2)	Penthiopyrad	7	1.46 liter/ha (20 fl oz)
Aproach Prima	Picoxystrobin + Cyproconazole	11+3	0.25 liter/ha (3.4 fl oz)
Priaxor	Fluxapyroxad + Pyraclostrobin	7+11	0.29 liter/ha (4 fl oz)
Priaxor + Onset	Fluxapyroxad + Pyraclostrobin + Tebuconazole	7+11, 3	0.29 + .145 liter/ha (4+2 fl oz)

[x]Onset (Tebuconazole; WINFIELD solutions, LLC, St. Paul, MN), Orius (Tebuconazole; ADAMA, Raleigh, NC), TebuStar (Tebuconazole; Albaugh, LLC, Ankenny, IA), Headline (Pyraclostrobin; BASF Corporation, Research Triangle Park, NC), Quadris (Azoxystrobin; Syngenta Crop Protection, LLC, Greensboro, NC), Aproach (Picoxystrobin; DuPont, Chestnut Run Plaza, Wilmington, DE), Vertisan (Penthiopyrad; DuPont, Chestnut Run Plaza, Wilmington, DE), Vertisan (Penthiopyrad; DuPont, Chestnut Run Plaza, Wilmighton, DE), Aproach Prima (Picoxystrobin and Cyproconazole; DuPont, Chestnut Run Plaza, Wilmington, DE), Priaxor (Fluxapyroxad and Pyraclostrobin; BASF Corporation, Research Triangle Park), and Priaxor + Onset (Fluxapyroxad, Pyraclostrobin, Tebuconazole; BASF Corporation, Research Triangle Park, NC and BASF Corporation, Research Triangle Park, NC).
[y]Fungicide Resistance Action Committee (FRAC.info).
[z]Application rate in liters per hectare and in parenthesis fluid ounces per acre.

Pathogen Material and Inoculation

To facilitate an epidemic, *P. helianthi* isolate ND07-01 was used to inoculate all experiments. Isolate ND07-01 was selected because it was isolated in ND in 2007 and has a virulence phenotype characterized as race 336, the most prevalent race in a recent survey (Friskop et al. 2015b). Freshly-produced urediniospores of isolate ND07-01 were used for all

field inoculations. To increase pathogen inoculum, the susceptible sunflower hybrid 'Jaguar DMR' (Nuseed, Breckenridge, MN) was planted and grown in the greenhouse at approximately 22ºC with a 16 h photoperiod. When the first true leaves had emerged and elongated (approximately 14 days after planting), urediniospores of ND07-01 were taken out of cryopreservation (-80ºC) and heat shocked in a 40ºC water-bath. Urediniospores were then suspended in Soltrol 170 (Chevron Philips LLC., The Woodlands, TX) at approximately 275,000 spores mL^{-1} and inoculated on true leaves of susceptible plants using a Preval CO2 sprayer (Chicago Aerosol, Coal City, IL). Inoculated plants were left to dry for approximately 60 min and then placed in a misting chamber set to mist every 4 minutes for 20 seconds and left in the dark for 24 hours at 23°C. After 24 hours, plants were taken out of the misting chambers and placed in a greenhouse room and bottom watered daily as before inoculation. Approximately 12-14 days later, urediniospores were collected into gel capsules (Gallipot Inc., St. Paul, MN) using a mini-cyclone collector (G-R Manufacturing, Manhattan, KS) attached to a vacuum pump (Welch, Niles, IL). Collected urediniospores were desiccated (Drierite, W.A. Hammond Company, Xenia, OH), and stored at 3.5ºC for one-two weeks until utilized for field inoculations. Timing of field inoculation was coincided with optimal environmental conditions for urediniospore infection; consequently, sunflowers were inoculated the evening before a forecast overnight/morning dew that could result in prolonged leaf wetness. At each field location, the center two rows of each sunflower plot were inoculated with a suspension of approximately 275,000 spores/mL urediniospores in Soltrol 170 between sunflower vegetative growth stages V8-V16. The spore solution was applied using a backpack mounted leaf blower (model # SR 450, Stihl Incorporated USA, Virginia Beach, VA). Inoculation was repeated if highly favorable environmental conditions for infection did not occur during the 24 hours

following the first inoculation event. Trials in 2016 and 2017 were inoculated twice during vegetative growth stages V8-V10. Trials in 2018 were inoculated once during the vegetative growth stage V10.

Data Collections and Statistical Analyses

Sunflower rust incidence and severity was assessed on the upper four fully expanded leaves from ten arbitrarily selected plants in each research plot (Markell et al. 2018). Severity ratings were conducted with the aid of rust severity diagrams (Friskop et al. 2015a). All ratings occurred at plant growth stages R6 (approximately 14 days after fungicide application) and R7 (approximately 14 days following the first rating) at all trials (Friskop et al. 2011; Gulya et al. 1990).

All data were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using a generalized linear model (PROC GLIMMIX). Data were combined to create a more robust analysis. Prior to analysis, mean rust severity and incidence values were arcsine-square-root transformed and yield data was square-root transformed to meet assumptions of normal residuals and homogenous variances. Rust severity and incidence data were combined across all field experiments for each type of sunflower (oilseed and confection). Data were analyzed using PROC GLIMMIX with fungicide treatments as a fixed effect and location and rep as random factors in the model. Random effects included environment and replication within environment. If significant, LSMEANS for rust severity, rust incidence and yield were compared using pairwise differences with a t test (α=0.05). The LSMEANS were then back transformed for presentation of results.

Fungicide Efficacy on Rust on Confection Sunflower

Sunflower rust was established in all confection sunflower trials conducted in this study. Disease severity was great enough in all trials to evaluate fungicide efficacy, with highest

severity (mean rust severity on non-treated control at growth stage R7) occurring in Leonard, ND in 2016 (15.87%) and the lowest occurring in Carrington, ND in 2017 (0.33%) (data not presented). Across all field experiments all fungicide treatments reduced rust severity at growth stage R6 compared to the non-treated control, with the exception of penthiopyrad at a low rate (0.73 L/ha; 10 oz/A) (Table 1.3). At growth stage R7, disease severity of all fungicide treatments was lower than the non-treated control. All three tebuconazole fungicide treatments were among the most efficacious products tested in this study, suggesting that sunflower growers may expect sufficient and similar management of rust if using less expensive generic tebuconazole formulations. Statistical differences in rust severity at R7 were observed among QoI fungicides, with the highest disease severity occurring with the picoxystrobin fungicide treatment and the lowest occurring with azoxystrobin. A rate response was observed with the single SDHI product tested (penthiopyrad), where disease severity at R7 was significantly lower at the higher rate. Differences in rust severity were also observed among fungicide treatments containing more than one active ingredient, but no premixture or mixture outperformed tebuconazole. The disease severity results are similar to the findings of Friskop et al. (2015a), in that multiple fungicides were efficacious against sunflower rust, and products containing DMI fungicides and/or QoI active ingredients tended to be the most efficacious.

Table 1.3. Sunflower rust incidence and severity percent at plant growth stages R6 and R7 for five confection type sunflower trials conducted in 2016, 2017, and 2018.

Common name	Active ingredient[v]	Disease incidence R6[y]		Disease severity R6[z]		Disease incidence R7		Disease severity R7	
NTC[x]	-	82.50	A	0.57	A	100	A	6.14	A
Onset	Tebuconazole	67.50	CD	0.21	EF	94.50	BC	0.83	F
Orius	Tebuconazole	75.50	ABC	0.18	EF	94.00	C	0.59	F
TebuStar	Tebuconazole	77.00	ABC	0.30	CDE	94.00	C	0.96	EF
Headline	Pyraclostrobin	69.00	BCD	0.30	CDE	98.00	AB	1.74	D
Quadris	Azoxystrobin	61.50	D	0.17	F	93.50	C	1.03	EF
Aproach	Picoxystrobin	67.50	CD	0.29	CDE	99.50	A	2.79	C
Vertisan (1)	Penthiopyrad	78.50	AB	0.46	AB	100	A	4.55	B
Vertisan (2)	Penthiopyrad	75.49	ABC	0.35	BC	99.60	A	2.71	C
Aproach Prima	Picoxystrobin + Cyproconazole	71.21	BCD	0.24	DE	99.40	A	1.55	DE
Priaxor	Fluxapyroxad + Pyraclostrobin	69.50	BCD	0.36	CD	100	A	2.52	CD
Priaxor + Onset	Fluxapyroxad + Pyraclostrobin + Tebuconazole	69.00	BCD	0.19	EF	95.50	BC	0.73	F
P value		<.0.001		<.0.001		<.0.001		<.0.001	

[v]Foliar fungicides were applied at approximately R5.1-5.2 using a three nozzle boom, 0.508 m (20") nozzle spacing, Teejet 8002 flat fan nozzles, applied at 187.07 liter/ha (20 gallons per acre) and walking at approximately 4.82 kph (3 mph).

[w]Data were analyzed using PROC GLIMMIX with fungicide treatments as fixed effect and location and rep as random factors. Mean separations were determined on the least square means test at P = 0.05 level.

[x]NTC represents non-treated control used in the experiment.

[y]Disease incidence percent assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.

[z]Disease severity percent assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.

Due to abiotic influences such as wind, hail, etc, yield was obtained in three of the five confection type trials (Davenport 2017, Carrington 2017 and Davenport 2018). Of those, yield data in Carrington 2017 was not included in the combined analysis due to insufficient rust severity levels to impact yield; specifically, 0.06% and 0.33% in the non-treated control at R6 and R7, respectively (data not presented). Statistical differences in yield were observed in the combined analysis of Davenport 2017 and Davenport 2018. Seven of the eleven fungicide treatments had statistically higher yield than the non-treated control, including all three tebuconazole fungicide treatments, two single ingredient QoI fungicide treatments and two fungicide treatments with multiple modes of action (Table 1.4).

Table 1.4. Percent sunflower rust severity and yield of two sunflower confection type trials conducted in Davenport, ND in 2017 and 2018.

Common name	Active Ingredient	Disease severity R6[y]		Disease severity R7[z]		Yield[u]	
NTC[x]	-	0.18	A	6.10	A	2637.83	D
Onset	Tebuconazole	0.06	C	0.24	C	3035.37	AB
Orius	Tebuconazole	0.07	C	0.19	C	2969.53	ABC
TebuStar	Tebuconazole	0.07	C	0.25	C	2942.79	ABC
Headline	Pyraclostrobin	0.06	C	1.18	BC	3212.35	A
Quadris	Azoxystrobin	0.06	C	0.41	C	3203.05	A
Aproach	Picoxystrobin	0.09	BC	1.91	BC	2889.57	BCD
Vertisan (1)	Penthiopyrad	0.12	B	3.04	B	2826.29	BCD
Vertisan (2)	Penthiopyrad	0.13	B	1.76	BC	2725.05	CD
Aproach Prima	Picoxystrobin + Cyproconazole	0.09	BC	1.57	BC	2816.95	BCD
Priaxor	Fluxapyroxad + Pyraclostrobin	0.08	BC	1.15	BC	3073.29	AB
Priaxor + Onset	Fluxapyroxad + Pyraclostrobin + Tebuconazole +	0.06	C	0.19	C	3221.27	A
P value		.0001		.0001		.0004	

[u]Yield in Kilograms per Hectare
[v]Foliar fungicides were applied at approximately R5.1-5.2 using a three nozzle boom, 0.508 m (20") nozzle spacing, Teejet 8002 flat fan nozzles, applied at 187.07 liter/ha (20 gallons per acre) and walking at approximately 4.82 kph (3 mph).
[w]Data were analyzed using PROC GLIMMIX with fungicide treatments as fixed effect and location and rep as random factors. Mean separations were determined on the least square means test at P = 0.05 level.
[x]NTC represents non-treated control used in the experiment.
[y]Disease severity (percent) at R6 assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.
[z]Disease severity (percent) at R7 assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.

The level of yield loss caused by sunflower rust in this study was remarkable, as only trace levels of rust were found at R5.1-5.2 and severity still did not exceed the established action threshold of 1% at R6. However, a substantial increase in disease severity occurred between R6

and R7, demonstrating that a rust epidemic occurring past bloom stages (R5.1-R5.9) can cause yield loss. While the yield loss was high, it was somewhat less than the yield loss model developed by Friskop et al. (2015), where a 1% increase in disease severity resulted in a 6.6% reduction in yield. In this study, when using the yield loss model presented by Friskop et al. 2015, for every 1% increase in rust severity at R7, yield was reduced by 4.4%. The discrepancy can potentially be explained by at least two factors. First, epidemic progression in studies conducted by Friskop et al. (2015) was relatively gradual, had higher rust severities at the R5 growth stage, and had higher yield responses (as high as 60%) when rust severity was kept at 1% or less. Whereas in this study, the epidemic occurred in very late growth stages, reducing the time the disease could negatively impact the crop. Additionally, the greatest yield response observed in this study was 22%, which is substantially less, but may also be indicative of a late epidemic. Second, fungicide applications in both studies were made at R5.1-5.2, approximately 14 and 28 days before growth stages R6 and R7. However, due to the late epidemic onset in this study, fungicides may have been losing effective residual during the time of greatest epidemic severity increase (14-28 days post application).

Fungicide Efficacy on Rust on Oilseed Sunflower

Sunflower rust was established in all oilseed trials conducted in this study and disease severity was high enough to evaluate fungicide efficacy. The highest disease severity in the non-treated control among trials at growth stage R7 was in Leonard, ND 2016 (2.00%) and the lowest was in Carrington, ND 2017 (0.12%) (data not presented). A combined analysis of all oilseed hybrid trials at R6 demonstrated that all fungicides were able to reduce disease severity as compared to the non-treated control, with the exception of the higher rate (1.46 liter/ha (20 fl oz/a)) of penthiopyrad (Table 1.5). At growth state R7, disease severity of all fungicide

treatments was lower than the non-treated control. Differences among fungicide treatments were observed at both evaluation timings. No fungicide treatment had lower disease severity at R7 than tebuconazole fungicide treatments singly-applied. Further, four out of the five most efficacious multi-fungicide treatments contained the active ingredient tebuconazole. Disease severity at R7 was highest among fungicides containing picoxystrobin and penthiopyrad, which also aligns with the findings of the confection type trials. Results demonstrate that the efficacy of fungicides on rust is generally consistent between the two sunflower types (oilseed and confection).

Table 1.5. Percent sunflower rust incidence and severity percent at plant growth stages R6 and R7 for five oilseed type sunflower trials conducted in 2016, 2017, and 2018.

Common name	Active ingredient[v]	Disease incidence R6[y]		Disease severity R6[z]		Disease incidence R7		Disease severity R7	
NTC[x]	-	54.50	A	0.11	A	98.00	A	0.68	A
Onset	Tebuconazole	38.50	BCD	0.04	CD	66.00	CD	0.13	F
Orius	Tebuconazole	33.00	D	0.05	CD	62.00	D	0.18	EF
TebuStar	Tebuconazole	31.50	D	0.03	D	66.00	CD	0.14	EF
Headline	Pyraclostrobin	45.00	AB	0.05	CD	88.50	B	0.19	DE
Quadris	Azoxystrobin	31.00	D	0.03	D	75.50	C	0.13	EF
Aproach	Picoxystrobin	44.50	ABC	0.05	CD	96.00	AB	0.26	BC
Vertisan (1)	Penthiopyrad	47.50	AB	0.05	BC	97.50	AB	0.37	B
Vertisan (2)	Penthiopyrad	53.50	A	0.08	AB	95.00	AB	0.28	BC
Aproach Prima	Picoxystrobin +Cyproconazole	36.00	CD	0.04	CD	94.00	AB	0.22	C D
Priaxor	Fluxapyroxad + Pyraclostrobin	38.50	BCD	0.04	CD	91.50	AB	0.21	C D
Priaxor + Onset	Fluxapyroxad + Pyraclostrobin, Tebuconazole,	34.00	CD	0.03	D	64.00	D	0.10	F
P value		<.0.001		<.0.0072		<.0.001		<.0.001	

[v]Foliar fungicides were applied at approximately R5.1-5.2 using a three nozzle boom, 0.508 m (20") nozzle spacing, Teejet 8002 flat fan nozzles, applied at 187.07 liter/ha (20 gallons per acre) and walking at approximately 4.82 kph (3 mph).
[w]Data were analyzed using PROC GLIMMIX with fungicide treatments as fixed effect and location and rep as random factors. Mean separations were determined on the least square means test at P = 0.05 level.
[x]NTC represents non-treated control used in the experiment.
[y]Disease incidence (percent) assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.
[z]Disease severity (percent) assessed visually at plant growth stage R6 by rating the top four fully expanded leaves of ten arbitrarily selected plants.

Due to abiotic influences, yield was obtained in four out of the five trials which included Leonard, ND (2016), Davenport, ND (2017), Carrington, ND (2017), and Davenport, ND (2018). No statistical differences were found in a combined analysis of yield from all four locations. The average disease severity at R5.2 was only 'trace' levels, and only 0.11% at R6; substantially lower than the 1% and 3% action thresholds developed for confection sunflower (Friskop et al.

2015; Shtienberg 1995). When trials were individually analyzed, yield differences among fungicide treatments and the non-treated control were observed in one of the five trials. However, in that trial, R7 disease severity in the non-treated control was only 0.33%, the second lowest rust severity among all trials conducted (data not presented).

Conclusions and Recommendations

All fungicides evaluated in this study were found to reduce rust severity on both confection and oilseed type sunflower. Efficacy differences among fungicides were observed throughout the study, with the most efficacious products resulting in an approximate 5-10 fold reduction in disease severity from the non-treated control. Although not evaluated within the same experiments, fungicides were generally observed to be equally efficacious against rust regardless of sunflower type grown. Yield differences were not observed in oilseed type trials, likely due to low disease severity. In confection trials, seven of the eleven tested fungicides had yields higher than the non-treated control, with the most effective fungicide treatments resulting in an approximate 20% increase. Importantly, yield responses to fungicide application occurred despite only trace levels of disease severity occurring at the R5.1-5.2 growth stage (data not presented) and 0.18% recorded at the R6 growth stage; far below the fungicide action thresholds of 1% and 3% at bloom stages established by Friskop et al. (2015) and Shtienberg (1995), respectively (Table 1.5).

Sunflower rust remains a serious economic concern for sunflower growers and I recommend growers use genetic resistance when available, frequently scout fields, and consider applying an efficacious fungicide. Fungicides are a very important tool to manage sunflower rust, and evaluation of fungicide efficacy as new products become available is a necessity to maximize rust management in sunflower. In the US, sunflowers are a relatively small acreage

with a supportive crop commodity group (US National Sunflower Association) field-crop in the US, but the high importance of fungicides, I recommend that a public-private partnership (Markell et al. 2020) may be an optimal strategy for funding and execution of future work.

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PHENOTYPIC DIVERSITY OF *PUCCINIA HELIANTHI* ON SUNFLOWER IN CALIFORNIA

Abstract

The Sacramento Valley of California is a globally-important producer of certified sunflower seed, and maintains stringent certification standards for quarantine-type diseases. Sunflower rust, caused by the fungal pathogen *Puccinia helianthi*, has been identified as the most prevalent quarantine-type disease on sunflower in this region. Hosts for *P. helianthi* include both commercially produced cultivated sunflower and wild sunflower (*Helianthus annuus*) variants, which are commonly found in the Sacramento Valley. Despite the importance of rust, limited information about the prevalence of rust on wild sunflower variants and the virulence diversity of *P. helianthi* on wild variants and commercially produced sunflower exists in the region. To determine rust prevalence, surveys were conducted throughout the Sacramento Valley in 2017 and 2018. Virulence phenotypes and races were determined for pathogen isolates collected during the surveys and additional pathogen samples obtained from certified seed cultivated fields by the California Department of Food and Agriculture Diagnostic Laboratory. During the two years of surveys, rust was identified on 37.5% of wild sunflowers but absent on seed production fields. Twenty-one *P. helianthi* races were identified from the 47 single pustule derived isolates generated during the study. Eleven new races of rust were detected for the first time in California and one isolate was virulent to all genes/alleles occurring in the differential set. The differential plants containing the most effective resistance genes/alleles were HA-R2 (R_5), MC29 ($R_2 + R_{10}$), and HA-R3 (R_{4b}). Results from this study demonstrate that rust commonly exists on wild sunflowers and that virulence diversity is consistent between the wild and cultivated seed production sunflower. This study elucidates the important role that wild sunflowers have in

maintaining the most important quarantine-type pathogen in the Sacramento Valley of California.

Introduction

The common annual sunflower, *Helianthus annuus* L., is native to North America and was one of the first crops domesticated in the United States (Harveson 2016; Lazicki and Geisseler 2016). *H. annuus* is arguably the most diverse species of sunflower in North America, with variants ranging from the wild multiheaded sunflower to the domesticated commercially grown sunflower (Seiler 2016). *H. annuus* is also characterized by its varied morphological characteristics, wide geographic distribution and ability to successfully grow in diverse habitats (Rogers et al. 1982). Commercially, *H. annuus* is grown for oil, direct human consumption, ornamental uses, and bird food. Globally, the leading producers in 2018-2019 include Russia, Ukraine, the European Union, and Argentina (FAO 2019). In the United States, approximately 493,000 ha of sunflower were harvested in the 2018-2019 season and production is primarily located in the Midwestern United States (NSA 2021). In addition to commercial sunflower production, the United States has a robust sunflower seed production industry in California, separated from the commercial production region of the Midwest by a distance of over 1,500 km and the Rocky Mountains.

The California seed industry produces approximately a quarter of the world's total sunflower seed supply and supplies approximately 95% of all sunflower seed commercially planted in the United States (Gulya et al. 2012; Lazicki and Geisseler 2016). The primary sunflower seed production region in California is the Sacramento Valley, located in the North Central part of the state which includes Colusa, Solano, Glenn, Sutter, and Yolo counties (Lazicki and Geisseler 2016; Long et al. 2019). The Sacramento Valley is regarded as a premier

sunflower seed producing region because of seed purity, isolation from commercial sunflower production and a dry and hot Mediterranean climate. The geographic isolation and unconducive climate reduce pathogen prevalence and disease development, which is essential to meet the needs of phytosanitary restrictions for seed exports imposed by importing countries (Long et al. 2019).

The state of California has strict standards for all sunflower certified seed in order to meet export mandates (Long et al. 2019). These standards were created by the University of California Division of Agriculture and Natural Resources California Crop Improvement Association (CCIA) and the California Department of Food and Agriculture (Long et al. 2019). CCIA may refuse certification and reject sunflower fields due to unsatisfactory appearance by weeds, growth, stand, insect damage, and disease. Certification standards for certified hybrid sunflower seed also require a minimum of 2.01 km (1.25 mile) isolation from wild, volunteer, or ornamental *Helianthus* species. Failure to isolate fields within the prescribed tolerance would subject harvest seed to a mandatory grow out and possible rejection (Long et al. 2019). Thus, monitoring for the presence of wild sunflowers, which are very common throughout North America, is critical for successful sunflower seed production and export.

While disease prevalence in the region is low, many diseases are classified under quarantine status and thus would preclude seed from being exported to foreign countries. Thus, any disease occurrence has severe economic consequences for sunflower seed growers and their customers (Gulya et al. 2012; Long et al. 2019). In a 15-year span from 1997 to 2011, 7,231 seed production fields in northern California were inspected and samples were evaluated at California's Department of Food and Agriculture. In that time span, sunflower rust (caused by *Puccinia helianthi* Schwein) was the most prevalent quarantined-disease and found in 4.3% of

fields. Stalk rot (caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) and downy mildew (caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni) were the second and third most frequently identified disease, and found in 2.6%, and in 0.5%, respectively (Gulya et al. 2012).

Sunflower rust, occurs on sunflower *Helianthus* spp, and is commonly found on *H. annuus* (Markell 2016). Sunflower rust occurs on all sunflower producing continents and is the most prevalent economically important foliar disease of oilseed and confectionary sunflower in North America (Gulya et al. 2019; Markell 2016). The pathogen is autoecious, macrocyclic and can overwinter in harsh weather as telia and uredinia (Markell 2016). Urediniospores are capable of long-distance dispersal, and under favorable environments, can reproduce in 10 to 14 days (Friskop et al. 2011; Markell 2016). The pathogen is characterized by frequent sexual recombination events (Markell et al. 2009), which has resulted in many virulence phenotypes/races that frequently overcome resistance genes (Friskop et al. 2015; Qi et al. 2011).

Occurrence and prevalence of *P. helianthi* virulence phenotypes in commercial sunflower production regions is reasonably well understood in both global and US sunflower production regions (Friskop et al. 2015, Gulya and Markell, S. 2009, Kong et al. 1999, Markell et al. 2016, Meyer et al. 2020 and Tan, A.S. 2010). However, virulence information from the California seed-production region is very limited. The virulence phenotypes of nine *P. helianthi* isolates originating from seven seed production fields in the sunflower seed region in California were determined as part of a larger survey in 2011 and 2012 (Friskop et al. 2015). The study postulated that differences in race prevalence occurred among years and geography regions, however, conclusions about the population in California could not be made due to the small sample size. Additionally, from the best of my knowledge, there is no virulence phenotype data available for *P. helianthi* on wild sunflower species in California.

The objective of this study was to determine the virulence and diversity of *P. helianthi* in the sunflower seed production region of California.

Identification and Distribution of Sunflower Rust in California

Rust surveys were conducted throughout the California seed production region from the 20th to 28th of August in 2017 and from the 5th to 13th of August in 2018. The surveys included arranged visits from seed production nurseries, and arbitrary visits to wild populations of sunflower. Due to destruction of wild sunflowers and crop rotations, no single location was visited in both years. Locations where wild populations were sampled included (but were not limited to) road ditches, irrigation channels, floodways, wildlife refuges, and a vineyard (Figure 2.1). In 2017, 17 locations were visited across an 1,100 km loop (Figure 2.2). These locations included 14 roadside ditches, one breeder's field, one vegetable field, and the Vic Fazio Wildlife Area. Rust was detected in nine out of the 17 locations (52.94%). In 2018, the survey was expanded to fifty-five locations across a 1,900 km loop (Figure 2.3). Locations included 40 roadside ditches, 11 wild sunflower stands located in small towns, three urban gardens with ornamental sunflowers, and one wild sunflower location in vineyard. Rust was detected in 18 out of the 55 locations in 2018 (32.72%). In both years, all rust positives were found on only wild sunflowers. In both survey years and all locations that rust was detected, only the urediniospore stage was observed. Disease severity on leaves among surveyed locations varied between trace amounts of rust to approximately 80% disease severity (data not presented). To the best of my knowledge, the percentage of wild sunflowers harboring rust in the region was unknown. Importantly, the prevalence was much higher than 4.3% rust detected in previous surveys on sunflowers in California production fields (Gulya et al. 2012).

Figure 2.1. Wild *H. annuus* plants infected with *P. helianthi* growing next to a drainage ditch Glenn County in 2018.

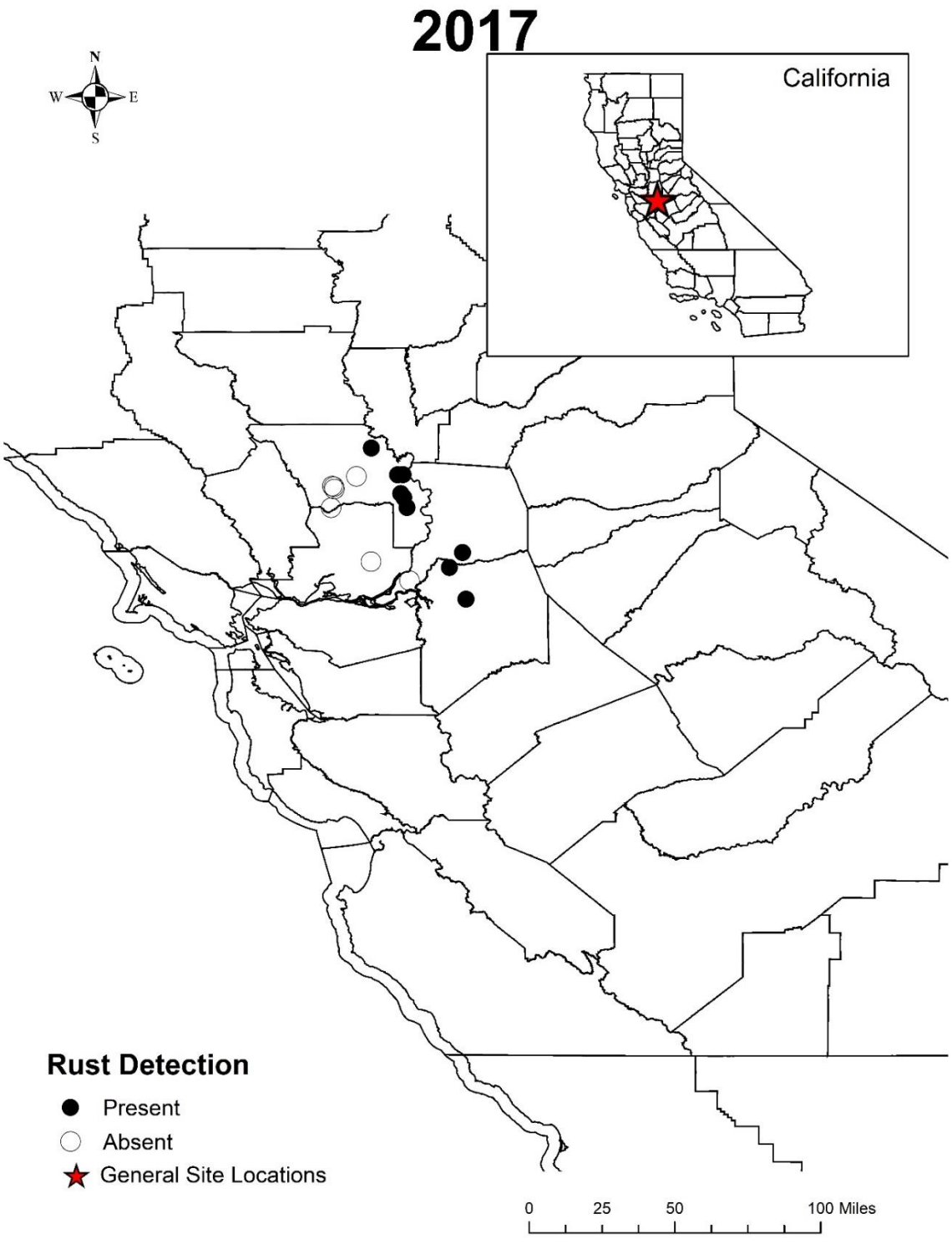

Figure 2.2. Survey locations and distribution of *P. helianthi* on wild *H. annuus* in the Sacramento Valley of California in 2017.

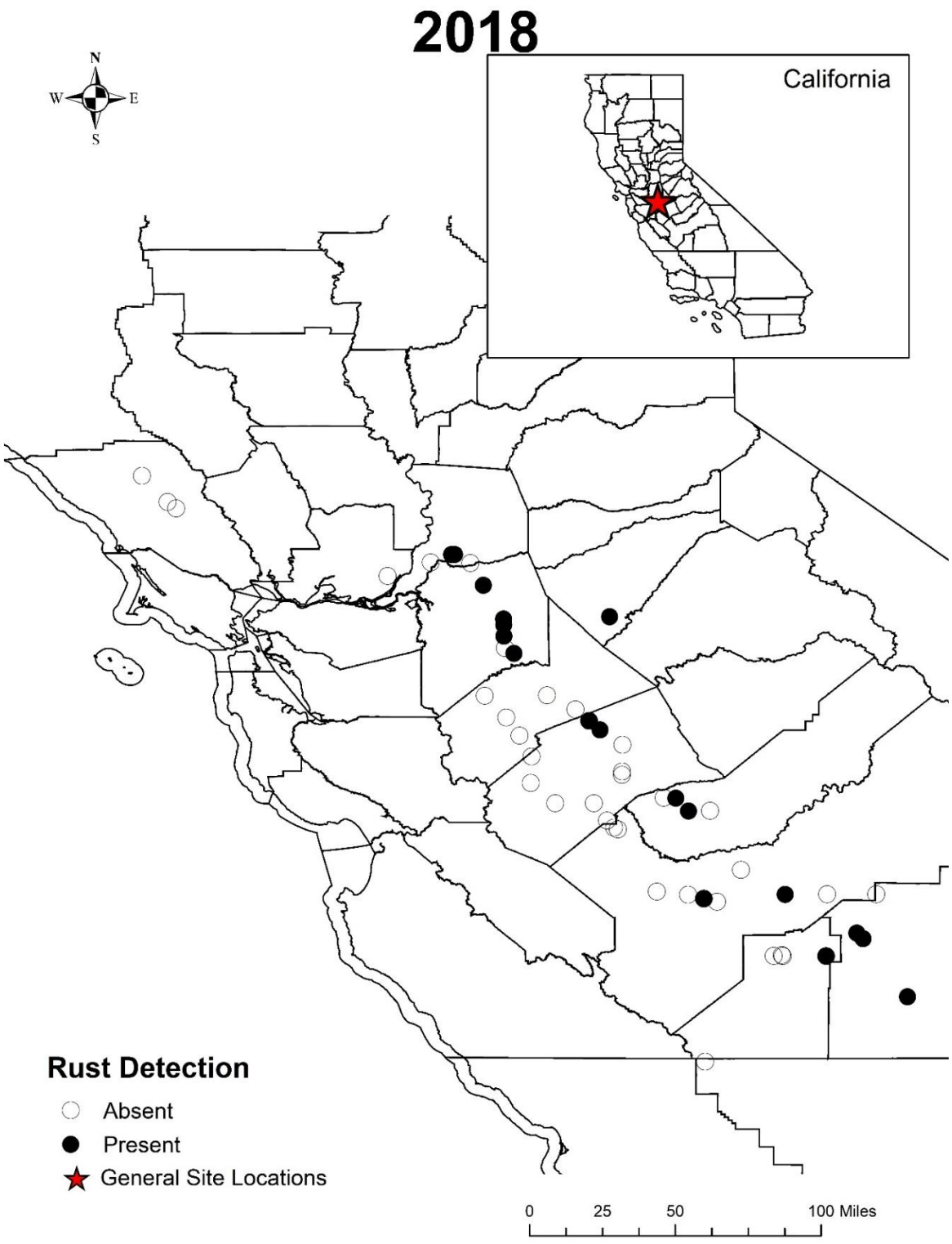

Figure 2.3. Survey locations and distribution of *P. helianthi* on wild *H. annuus* in the Sacramento Valley of California in 2018.

Collection, Isolation and Increase of *Puccinia helianthi*

Pathogen materials used in this study originated from 1) surveys conducted in California and 2) samples submitted by the California Department of Food and Agriculture Diagnostic Laboratory from commercial seed production fields. Notably, all samples originating from wild *H. annuus* were collected during surveys on wild sunflowers conducted in 2017 and 2018 and all samples originating from commercial seed production fields originated from California Department of Food and Agriculture Diagnostic Laboratory submissions. At each location where rust was detected on wild sunflowers, 8 to 15 leaves with visible uredinia were collected and stored between filter paper and cardboard in a plant press (model # 53674, Forestry Suppliers, Jackson, MN) (Figure 2.4). Samples were collected from a total of nine and 18 locations in 2017 and 2018, respectively. Samples were shipped to North Dakota State University for isolation. Additional rust samples from production fields were mailed to North Dakota State University from the California Department of Food and Agriculture Diagnostic Laboratory in Sacramento, CA. This included approximately 300 leaf samples with visible rust from seed production fields in 2017 to 2018. Although the majority of pathogen samples received were telia and could not be used in the study, two samples from Solano county in 2017, one sample Glenn county 2017, four samples Glenn county in 2018 and nine samples Solano county in 2018 were evaluated.

Figure 2.4. Severely infected sunflower leaves collected in a plant press.

To ensure genetic uniformity within *P. helianthi* samples, single-pustule derived isolates were created for determination of virulence phenotypes. Pathogen isolations and increases were made on the susceptible sunflower hybrid 'Jaguar' (Nuseed, Breckenridge, MN). Three seeds of each differential were planted in greenhouse cones 6.4 cm x 25.4 cm (Deepot D40, Stuewe and Sons, Inc. Tangent, OR) filled with potting soil (PRO-MIX BX, Premier Tech Horticulture, Quakertown, PA). Greenhouse conditions included a constant temperature of 22 ± 2ºC with a 14 h photoperiod and 600-watt high pressure sodium lamps (P.L. Light Systems, Inc., Beamsville, Ontario, Canada) to supplement natural light. Approximately 13 to 14 days post planting, the first true and fully-elongated leaves were inoculated with urediniospores, by gently swabbing

and transferring urediniospores from one single pustule on an infected leave sample with a Q-tip (Unilever, London, UK) drenched in Soltrol-170 (Chevron Philips LLC., The Woodlands, TX). Inoculated plants from each sample were physically separated to avoid contaminating and left to dry for approximately 1 h. Plants were then placed in misting chambers (dark for 24 h at 23ºC) set to mist for 20 sec every 4 min with deionized water. After 24 h, plants were transferred into a greenhouse room, isolated within individual containment cages constructed with plexiglass, covered in clothe with clothes pins, and bottom watered daily.

Approximately 13-15 days after inoculation, cinnamon-brown urediniospores from a single uredinia (spatially separated from other uredinia) was carefully collected on a Q-tip with Soltrol-170 and re-inoculated on first true and fully-elongated leaves of a new susceptible plant (as above) (Figure 2.5). Once urediniospores were visible (12-14 days), urediniospores from the single-pustule isolates were collected into gel capsules (Gallipot Inc., St. Paul, MN) using a mini-cyclone collector (G-R Manufacturing, Manhattan, KS) attached to a vacuum pump (Welch, Niles, IL). Gel capsules containing urediniospores were then desiccated (Drierite, W.A. Hammond Company, Xenia, OH) and temporally stored at 3.5ºC for one to two weeks to be used to increase urediniospores until volume was sufficient to conduct virulence phenotyping and preserve samples.

Figure 2.5. Dusty cinnamon-brown urediniospores increased on an inoculated sunflower leaf grown under greenhouse conditions.

In total, the virulence phenotype of 47 single pustule isolates of *P. helianthi* were determined. This included 20 (42.55%) isolates originating from wild sunflowers in 2018, 13 (27.65%) from seed production fields in 2018, 11 (23.40%) from wild sunflowers in 2017 and three (6.38%) from seed production fields in 2017 (Table 2.1). All 16 isolates derived from seed production fields originated from leaf samples from Solano and Glenn counties in 2017 and 2018. Each isolate used in this experiment is derived from a different leaf sample from the diagnostic laboratory in California.

Table 2.1. Unique collection locations of *P. helianthi*, number of single-pustule derived isolates generated, and number of pathogen races characterized from wild and cultivated sunflowers from the Sacramento Valley of California in 2017 and 2018.

Host	Number of	2017	2018
Wild (*Helianthus annus*)	Locations	17	54
	Isolates	11	20
	[z]Races	6	16
Cultivated (*Helianthus annus*)	Locations	11	13
	Isolates	3	13
	[z]Races	3	8

[z]Nomenclature assigned to isolates virulence/reaction on the internationally accepted sunflower rust differential set (Gulya and Masirevic 1996).

Determination of Virulence Phenotypes and Races

Phenotypes of *P. helianthi* isolates were determined on the set of nine internationally recognized differentials (Table 2.2) (Gulya and Masirevic 1996). With the exception of the universal susceptible differential 7350, each differential contains at least one or more resistance gene/alleles.

Table 2.2. Sunflower rust differential line, resistance gene or allele and scoring values for race nomenclature.

Set	Differential	Resistance Gene or Alleles	Scoring Value[z]
One	7350	…	1
	MC90	R_1	2
	MC29	$R_2 + R_{10}$	4
Two	P386	R_{4e}	1
	HA-R1	R_{4a}	2
	HA-R2	R_5	4
Three	HA-R3	R_{4b}	1
	HA-R4	R_{4c}	2
	HA-R5	R_{4d}	4

[z]Scoring value is the numerical value assigned with virulence on a specific differential. The additive score for each set is the digit in the three-digit race name (Gulya and Masirevic 1996).

To determine virulence phenotypes, isolates were inoculated to each of the three reps on each differential and the experiment was repeated once. Three seeds of each differential were

planted in greenhouse cones 6.4 cm x 25.4 cm (Deepot D40, Stuewe and Sons, Inc. Tangent, Oregon) filled with potting soil (PRO-MIX BX, Premier Tech Horticulture, Quakertown, PA) and watered daily with diurnal tempers of 24ºC and 20ºC with a 16 h photoperiod (as above). Due to poor germination of some differentials, cones containing the differential plants were rearranged to complete the differential set. After 14 days, freshly produced single pustule urediniospores were suspended in Soltrol-170 at a concentration of approximately 2.8 x 105 spores/mL and inoculated on the first true and elongated leaves of three reps of all nine differentials using a mini-cyclone collector attached to a piston vacuum pump. Plants were allowed to dry approximately 1 h and placed in misting chambers for 24 h (as described above). Inoculated plants were subsequently placed in the greenhouse (22 ± 2ºC with a 14 h photoperiod).

Phenotypic evaluations were conducted approximately 13 to 15 days post-inoculations. The virulence was determined visually using a modified scale from Yang et al. (1986), whereas; 0 = immune, ; = fleck, 1 = pustule smaller than 0.2 mm, 2 = pustules 0.2 to 0.4 mm, 3 = pustules 0.4 to 0.6 mm, 4= 0.6 to 0.8, and 5 = pustules larger than 0.8mm (Friskop et al. 2015). Ratings over 3 were considered to be a virulent reaction (differential was susceptible to pathogen isolate) whereas 2 or less were considered avirulent (differential was resistant to pathogen isolate) (Friskop et al. 2015).

To assign race names for each isolate, differentials are divided into three sets and each differential within a set is assigned a scoring value (if virulent), whereas the first, second and third differential are scored with a value of one, two and four. After all differential plants from the three sets are evaluated, the scores are added, and a single score for each set is presented in a triplet code (Gulya and Masirevic 1996). For example, the race 326 is virulent on differentials

one (7350) and two (MC90) in the first set (score of 1 + 2 = 3), differential two in the second set (HA-R1) (score of 2) and differentials two (HA-R4) and three (HA-R5) in the third set (score of 2 + 4 = 6). An isolate virulent on all three differentials in all three sets would result in a score and race name of 777.

In total, 21 races of rust were detected throughout the study. Races 336 and 337 were the most prevalent and accounted for nine of 47 (19.15%) and eight of 47 (17.02%) of isolates tested in this study, respectively (Table 2.3). The least virulent isolate detected in this study originated near Cortez, California (Merced County) in 2018 and was race 100, only virulent on the universal susceptible (7350). The most virulent isolate detected originated 3.2 km east of Stockton, California (San Joaquin county) in 2018 and was phenotyped as 777, conferring virulence to all differentials. These isolates were located 80.95 kilometers (50.3 miles) apart and both originated from a wild sunflower. Eight races were identified from the 16 isolates originating from seed production fields and 17 races were identified from the 31 isolates originating from wild sunflowers (Table 2.4). Only four races, comprising five total isolates, were found only on seed production fields and not in wild sunflowers. While those four races had unique virulence combinations, no unique virulence was identified on seed production fields that was not also identified on wilds. My data suggests that virulence of *P. helianthi* is consistent between the wild and seed production sunflower in California.

Table 2.3. Number and frequency of *P. helianthi* races detected in 2017 and 2018 from wild and cultivated sunflowers.

	2017		2018		Total	
[z]Race	n	%	n	%	n	%
100	0	0	1	3.0	1	2.1
102	0	0	1	3.0	1	2.1
137	2	14.3	0	0	2	4.3
317	1	7.1	0	0	1	2.1
323	0	0	1	3.0	1	2.1
325	0	0	1	3.0	1	2.1
330	0	0	1	3.0	1	2.1
332	2	14.3	1	3.0	3	6.4
333	1	7.1	1	3.0	2	4.3
335	1	7.1	2	6.06	3	6.4
336	1	7.1	8	24.2	9	19.2
337	5	35.7	3	9.1	8	17.0
700	0	0	3	9.1	3	6.4
704	0	0	1	3.0	1	2.1
716	0	0	1	3.0	1	2.1
724	0	0	1	3.0	1	2.1
732	0	0	1	3.0	1	2.1
734	0	0	1	3.0	1	2.1
735	0	0	1	3.0	1	2.1
736	1	7.1	3	9.1	4	8.5
777	0	0	1	3.0	1	2.1
Total	14	29.8	33	70.2	47	100

[z]Nomenclature assigned to isolates virulence/reaction on the internationally accepted sunflower rust differential set (Gulya and Masirevic 1996).

Table 2.4. Number and frequency of *P. helianthi* races detected in 2017 and 2018 from wild and cultivated sunflowers.

	Wild sample 2017-2018[x]		Production sample 2017-2018[y]		Total (47)	
Race[z]	n	%	n	%	n	%
100	1	3.2	0	0	1	2.1
102	1	3.2	0	0	1	2.1
137	2	6.5	0	0	2	4.3
317	1	3.2	0	0	1	2.1
323	1	3.2	0	0	1	2.1
325	0	0	1	6.3	1	2.1
330	1	3.2	0	0	1	2.1
332	3	9.7	0	0	3	6.4
333	0	0	2	12.5	2	4.3
335	2	6.5	1	6.3	3	6.4
336	4	12.9	5	31.3	9	19.2
337	6	19.4	2	12.5	8	17.0
700	3	9.7	0	0	3	6.4
704	0	0	1	6.3	1	2.1
716	1	3.2	0	0	1	2.1
724	1	3.2	0	0	1	2.1
732	0	0	1	6.3	1	2.1
734	1	3.2	0	0	1	2.1
735	1	3.2	0	0	1	2.1
736	1	3.2	3	18.8	4	8.5
777	1	3.2	0	0	1	2.1
Total	31	66.0	16	34.0	47	100

[x]Rust isolates derived from diseased leaf tissue samples on the wild sunflower variant from road ditches, in-town, wildlife refuge, irrigation channel, floodways, vegetable fields, urban gardens, vineyard and more.

[y]Rust isolates derived from diseased leaf tissue samples on the cultivated sunflower from seed production fields.

[z]Nomenclature assigned to isolates virulence/reaction on the internationally accepted sunflower rust differential set (Gulya and Masirevic 1996).

My research expands the knowledge of *P. helianthi* virulence diversity in the United States, but also identifies questions that should be addressed by future research. To the best of my knowledge, virulence of the *P. helianthi* populations occurring on wild sunflowers in the

California seed production region was unknown. Further, only races 704, 776, 736, 376, 724, and 337 were identified in California from any host (Friskop et al. 2015). In this study, one isolate was virulent on every differential and 18 races were identified for the first time in California. The high virulence diversity identified on wild sunflowers is of interest to the seed production industry, particularly because wild sunflowers are very common in California and rust found on approximately 1/3 to 1/2 of all wilds in the years of this study. It is likely that the pathogen would have been exposed to diverse resistance genes in seed production fields, and selection for increased virulence may have resulted in those fields. The virulence diversity on wilds suggest that virulence is maintained in those wild sunflower populations, presenting a constant threat to the seed production. However, an additional possibility that could explain the high virulence diversity in California is that pathogen populations in the Sacramento Valley and the commercial production region of the US Great Plains are able to move between regions, or the populations are one in the same. However, there is substantial geographic separation between regions, and the idea is not strongly supported by previous virulence research. In 2011 and 2012, 29 races were identified from 238 *P. helianthi* isolates collected primarily in the US Great Plains (Friskop et al. 2015). Of those, 10 were identified in California in this study. However, I identified eleven races in this study not identified in Friskop et al. (2015) (Race 100, 102, 137, 317, 323, 325, 333, 335, 700, 716, and 735).

Effectiveness of Resistance Genes

The most effective resistance gene in this study was R5 (Differential HA-R2) with only one isolate virulent to this differential set (race 777) (Table 2.5). Additionally, only 14 (28%) isolates conferred virulence to R2 + R10 (Differential MC29) and 20 (40%) to R4b (Differential HAR3). This is consistent with the findings of Friskop el al. (2015) with the three most effective

genes/alleles being R5, R2 + R10 and R4b. Similarly, when 284 bulk (non-single pustule) samples from the US Great Plains were tested for virulence, pathogen virulence was least frequently identified on R5, R2 + R10 and R4b (Gulya and Markell 2009).

Table 2.5. Number and frequency of *P. helianthi* isolates virulent on differentials in 2017 and 2018.

Differential plant	Resistance gene/alleles	Virulence in 2017[y]	Virulence in 2018[z]
7350	…	14	33
MC90	R_1	12	31
MC29	$R_2 + R_{10}$	1	13
P386	R_{4e}	14	24
HA-R1	R_{4a}	13	26
HA-R2	R_5	0	1
HA-R3	R_{4b}	10	10
HA-R4	R_{4c}	13	21
HA-R5	R_{4d}	11	23

[y]Number of *P. helianthi* isolates virulent on differential line derived from wild and production field collections in 2017.
[z]Number of *P. helianthi* isolates virulent on differential line derived from wild and production field collections in 2018.

Importantly, several identified resistance genes/alleles were not included in this study as they were not recognized in differential sets, namely; R_{11}, R_{12}, and R_{13} (Bulos et al. 2013; Gong et al. 2013; Qi et al. 2011). I agree with Moreno et al. (2011) and Friskop et al. (2015) with the inclusion of HAR-6 (R_{13a}) into the differential set proposed by Mareno et al. (2011). Additionally, I support the inclusion of Rf ANN-1742 (R_{11}) and RHA 464 (R_{12}) into an expanded differential set proposed by Friskop et al. (2015). I agree with Meyer et al. (2020) that pathogen diversity and virulence needs to be monitored utilizing additional sources of resistance in order for pathologist and breeders to strategically incorporate the most effective resistance genes and to disseminate the most effective management.

Conclusions and Management Recommendations

This study demonstrates that a large percentage (37.5%) of wild sunflowers in the Sacramento Valley harbor rust, the most commonly found quarantined-disease on sunflower seed production. Similarly, the similarity of virulence diversity and races on wilds sunflower and cultivates seed production sunflower indicates that the rust occurring sunflower seed production fields originate from wilds. Given that *P. helianthi* can travel great distances by wind, I recommend (if possible) growers actively find and destroy wild sunflowers beyond the 2.01 km (1.25 mile) radius created by CCIA and the California Department of Food and Agriculture. Similarly, it would be in the best interest of the industry if ornamental sunflowers were not planted in the seed production region. An Extension education campaign targeting homeowners, gardeners and professionals who utilize ornamental sunflowers in the region may be prudent near seed production fields.

I recommend an expansion of the sunflower rust differential set to include additional differential lines containing recently identified/released resistance genes (Bulos et al. 2013; Friskop et al. 2015; Gong et al. 2013; Moreno et al. 2011; Qi et al. 2014). I recommend that periodic virulence surveys be conducted in the sunflower seed production region of California. Given the concentration of diverse genetics in the seed nurseries and high virulence diversity identified in this study, assessment of virulence in the region may be an indicator of how quickly the pathogen may evolve to overcome resistance genes in other sunflower producing regions globally.

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EFFICACY OF AN ADEPIDYN FUNGICIDE SEED TREATMENT ON *LEPTOSPHAERIA MACULANS* IN OILSEED RAPE IN NORTHERN GERMANY

Abstract

Blackleg of oilseed rape is the most economically important disease on oilseed rape in Northern Germany. Infections that occur in the seedling stage are the most yield-limiting, and fungicides have been demonstrated to reduce infection. The objective of this study was to evaluate the effectiveness of adepidyn fungicide for managing blackleg when applied as a seed treatment. Results of an inoculated seedling study conducted in climate-controlled conditions demonstrated that adepidyn conferred efficacy against blackleg at all five application rates tested. To evaluate adepidyn in the field, four winter oilseed rape field trials were established in the 2019-2020 growing season in Northern Germany. Treatments included seed-treatment applied adepidyn, seed applied acibenzolar-s-methyl, foliar applied difenoconazole and adepidyn in combination with acibenzolar-s-methyl or difenoconazole. The study was conducted on a moderately-resistant and susceptible variety at all locations. At growth stage BBCH 17, only difenoconazole treatments had statistically lower disease severity than the non-treated controls. No differences among treatments were observed at growth stage BBCH 85, but a lower disease severity index was observed on the moderately resistant variety than the susceptible variety. Yield difference among treatments were not observed. Results of this study demonstrate efficacy of adepidyn in climate-controlled studies, but a reduction in blackleg was not observed in field studies. Adepidyn is a promising new fungicide that may provide an additional tool to manage blackleg of oilseed rape, and further studies that evaluate its efficacy and suitability in commercial oilseed rape field conditions are warranted.

Introduction

Oilseed rape or canola (*Brassica napus* L.) is an economically important oilseed crop grown around the world with reduced erucic acid and glucosinolates content (Howlett 2004). In 2019, the top producing countries of oilseed rape in the world were Canada, China, India, France, Ukraine, Germany, Australia, and Poland. In 2019, Germany produced approximately 2,830,200 tonnes of oilseed rape and was one of the top producing countries within the European Union (FAO 2019). In the northern most German state of Schleswig-Holstein, oilseed rape is the second most important crop only behind winter wheat. Additionally, it is the only broadleaf crop in a typical Schleswig-Holstein crop rotation of winter oilseed rape, winter wheat, and winter barely (Kruse and Verreet 2004). Numerous studies in northern Germany have demonstrated the importance of oilseed rape as an alternate crop in a rotation with increased yield and decreased disease recorded in cereal crops proceeding oilseed rape cultivation (Christen et al. 1992; Kruse and Verreet 2004).

The most economically important disease of winter oilseed rape in Germany is blackleg (Kruse and Verreet 2004). Blackleg, also known as Phoma stem canker, is caused primarily by the dothideomycete *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. (anamorph: *Phoma lingam* (Tode:Fr.) Desm.). Oilseed rape residue serves as the primary overwintering substrate for inoculum the pathogen, which survives as pseudothecia for 1-4 years (Howlett 2004). Ascospores are produced inside pseudothecia and are disseminated to adjacent plants or up to 5 km (3 miles) by wind (Rimmer and Van Den Berg 2007; Angadi et al. 2003). In Europe, disease epidemics by *L. maculans* are initiated by disseminating ascospores from infected crop residue to fall sown oilseed rape plants (Angadi et al. 2003). Additionally, secondary infection by pycnidiospores can occur and may contribute to overall disease severity, especially in fall-sown

oilseed rape, however, infections are less damaging as compared to ascospores (Rimmer and Van Den Berg 2007). *L. maculans* develops a gene-for-gene interaction with its host and numerous genes (*Avr*) have been characterized (Balesdent et al. 2005; Ghanbarnia et al. 2018).

Genetic resistance can be an effective way to manage disease caused by *L. maculans* and multiple resistance genes have been incorporated into commercial oilseed rape varieties (Markell et al. 2008; Ghanbarnia et al. 2018). However, frequent evolution by *L. maculans* to confer virulence on resistance genes has resulted in a significant challenge for phytopathologists and breeders to develop durable resistance (Howlett 2004). Results of a recent virulence survey of 644 isolates of *L. maculans* from northern Germany found that the majority conferred virulence to all resistance genes with the exception of *Rlm7* (Winter and Koopman 2016). Additional management strategies for blackleg include strategic crop rotations and managing volunteer plants. However, the effect is limited in Northern Germany as oilseed rape is the only broadleaf crop commonly grown. Fungicides can be an effective tool to manage blackleg, and seed and foliar application are used commonly. Fungicides are particularly effective in regions with higher yields, such as Western Europe (West et al. 2001). Application timing is critical, and a study conducted in Australia demonstrated that plants are most susceptible in the early seedling growth stages and early infections up to the six-leaf stage can result in yield-reducing cankers (Marcroft et al. 2005). Consequently, protection of plants in early growth states, whether by applied as seed treatments or foliar applications, is economically important. Numerous seed treatment fungicides have been shown to be effective in managing disease by blackleg in the field and greenhouse (Macroft and Potter 2008; Upadhaya et al. 2019). Recently, the fungicide adepidyn (Saltro, Syngenta, Basel, Switzerland) has been registered in numerous countries as a Fungicide Resistance Action Committee (FRAC) Group 7 fungicide. This fungicide has shown promising

results in other crops and pathosystems (Adee 2020). However, the efficacy of this new seed treatment to manage blackleg on oilseed rape, especially in northern Europe, remained largely unknown.

The objective of this study was to evaluate the efficacy of the novel seed treatment adepidyn for managing blackleg on oilseed rape in northern Germany.

Climate-Controlled Evaluations

Climate-controlled studies were conducted to evaluate adepidyn for the management of blackleg. Six treatments consisting of a non-treated control (NTC) and five adepidyn rates (0, 2.5, 3.75, 5.00, 7.50, 10.00 g a.i./1 Mio. Seeds) were evaluated on the susceptible oilseed rape variety Fortis (Syngenta, Basel, Switzerland) and the moderately resistant variety SY Florida (Syngenta, Basel, Switzerland), that latter containing the resistance gene *Rlm7*. Notably, neither variety is commercially available in Germany. The climate-controlled experiments were arranged in a randomized complete block design. Varieties and their respective treatments were randomized together in each of the four replications. The experiment was repeated once for validation.

Two fungicide treated seeds and the non-treated control were planted approximately 2 cm deep in each pot (17x17x16.5 cm) filled with potting soil Einheitserde-Classic (Einheitserdewerke Werkverband, Sinntal-Altengronau, Germany). Plants were randomized in a climatic chamber set to 17°C at night and 21°C at daytime with 16 h photoperiod and lightly watered daily. To avoid lodging, seedlings were carefully supported with a splint of wood and parafilm (Bemis Company Inc., Neenah, United States). Plant stand was thinned to one plant per pot upon emergence.

Two *L. maculans* isolates used in this study were collected from the field trials near the German cities of Rohlstorf and Dorf Mecklenburg. Isolates were cultured separately in petri plates on Tomaten-Gemüsesaft (A. Dohm & Timm, Diedersdorf, Germany) agar (pH 7.2) in an incubation chamber set to 21ºC. Inoculations and ratings were performed by a modified methodology developed by Williams and Delwiche (1979). Pycnidiospores were harvested from 14-day-old cultures, to be used in inoculations. Mycelia was carefully removed from the surface of the culture using a sterile cover slip. Pycnidia were then dislodged from the agar using a sterile pipette tip. 5 ml of distilled sterile water was added and the suspension (pycnidiospore and water) and sieved through a 200 µm gaze into a sarstedt-tube (Sarstedt AG & Co. KG, Nümbrecht, Germany). The spore solution was vortexed (Eydam KG, Kiel Germany), and the concentration adjusted to $2x10^7$ spores/ml with a Thoma-Chamber (GmbH & Co. KG, Wertheim, Germany), and promptly chilled by placing on ice.

Inoculations (*in vivo*) took place on seven-day-old seedlings by puncturing the cotyledon with a sterile needle and inoculating a 10 µm pycnidiospore solution ($2x10^7$ spores/ml) using a sterile pipette tip (Eppendorf Research, Hamburg, Germany) on top of the punctures in cotyledons (Figure 3.1).

Figure 3.1. Cotyledons of an oilseed rape seedling after inoculation of pycnidiospores at a concentration of to $2x10^7$ spores/mL.

To ensure a conducive environment for infection, inoculated seedling and containers were misting with deionized water by hand every hour and covered for the following 24 h. After 24 h, seedlings were uncovered and were lightly watered daily at the base of the pot for the next twelve days.

Seedlings were evaluated twelve days after inoculation using the 0-9 disease severity class rating scale by Williams and Delwiche (1979), where 0 = pinprick only, no blackening, 1 = limited blackening around wound; lesion diameter 1-1.5 mm, 2 = blackening with some chlorosis around wound; lesion diameter 1-1.5 mm, 3 = blackening around wound; lesion diameter 1.5-3 mm, 4 = large (3-5 mm) lesion, irregular darkening, 5 = tan-centered lesion, variable in size; sharp black margin, 6 = greyish-green tissue collapse, sharp black margin; lesion size variable, 7 = greyish-green tissue collapse, sharp margin; lesion size variable, 8 = spreading tissue collapse,

few pycnidia; lesion diameter greater than 5 mm, 9 = spreading tissue collapse, many pycnidia; lesion diameter greater than 5 mm.

Seedling data were analyzed in SAS 9.4 (SAS Institute, Cary, NC) by the methods of Upadhaya et al. (2019). The average disease severity class rating of all non-treated controls from both repetitions on SY Florida and Fortis was 2.8 and 3.2 respectively. While consistent disease pressure on the non-treated control demonstrated that sufficient disease pressure to evaluate efficacy, no disease occurred on either variety at any adepidyn rate (data not presented). Further, a total absence of any signs or symptoms were observed on any plant containing any concentration of adepidyn. Results demonstrate the adepydin was very effective at a wide range of rates in climate-controlled conditions.

Field Trial Establishment and Confirmation of Infection by *Leptosphaeria maculans*

Four fungicide trials were established during the 2019-2020 growing season to evaluate efficacy of adepidyn (Table 3.1). Two trials were located in each German state of Schleswig-Holstein and Mecklenburg-Vorpommern. All trials were arranged in a randomized complete block design (RCBD) with four replications. The experiment consisted of six treatments (including non-treated control) each conducted on the susceptible variety Fortis and moderately resistant variety SY Florida. Germination tests conducted before planting determined the germination rate for Fortis and SY Florida were 96% and 98%, respectively (data not presented). The six fungicide treatments used in this experiment included a non-treated control, seed treatment (adepidyn), growth regulator acibenzolar-s-methyl (bion, Syngenta, Basel, Switzerland), seed treatment and a growth regulator (adepidyn + acibenzolar-s-methyl), adepidyn seed treatment plus a foliar application difenoconazole (Score, Syngenta, Basel, Switzerland) (adepidyn + difenoconazole), and a treatment with only a foliar application (difenoconazole)

(Table 3.2). The rate of adepidyn used throughout the field study was 50 grams of adepidyn per 100 kg and is equivalent to the lowest rate evaluated in the growth chamber studies (2.5 g a.i./1 Mio. Seeds). Difenoconzole was used as the foliar application in this experiment because it does not have any known growth regulator effects and was applied as a foliar application at each trial when the first signs/symptoms of *L. maculans* were observed.

Table 3.1. Agronomic and trial information for all experimental locations.

Trial	City[z]	German state	Planting date	Infected residue placed[x]	Summer rating	Harvest date
1	Veelböken	Mecklenburg-Vorpommern	August 22nd, 2019	August 22nd, 2019	July 15th, 2020	July 31st, 2020
2	Neuengörs	Schleswig-Holstein	August 26th, 2019	August 27th, 2019	July 18th, 2020	August 3rd, 2020
3	Rohlstorf	Schleswig-Holstein	August 29th, 2019	August 30th, 2019	July 14th, 2020	August 4th, 2020
4	Dorf Mecklenburg	Mecklenburg-Vorpommern	August 31st, 2019	September 2nd, 2019	July 17th, 2020	July 27th, 2020

[y]Ten infected stems were arbitrarily placed in each plot within two days trials being planted.
[z]Location of nearest German city.

Table 3.2. Treatment number, product common name, active ingredient, and treatment rate for seed treatment and foliar application.

Treatment	Common name[x]	Active ingredient	Treatment rate	
			Seed treatment[y]	Foliar[z]
1	NTC[w]	…		
2	Saltro	Adepidyn	50	…
3	Bion	Acibenzolar-S-Methyl	37.5	…
4	Saltro + Bion	Adepidyn + Acibenzolar-S-Methyl	50 + 37.5	…
5	Saltro + Score	Adepidyn + Difenoconazole	50 +	125
6	Score	Difenoconazole	…	125

[w]NTC = non-treated control
[x]Adepidyn (Saltro; Syngenta, Basel Switzerland), Acibenzolar-S-Methyl (Bion; Syngenta, Basel Switzerland), Difenoconazole (Score; Syngenta, Basel Switzerland)
[y]Seed treatment rate in grams of active ingredient per 100 kg
[z]Foliar application in grams active ingredient per hectare. A single foliar application was made when first signs and symptoms of *L. maculans* was observed in trials.

Trials were planted in late August 2019, at a sowing density was on 50 seeds/m^2 in ten rows per plots. Plots were planted three meters wide and twelve meters long. The first eight meters of the plot were used for harvest data and the remaining four meters was used for destructive sampling for plant cross section evaluations. In order to facilitate an epidemic, ten naturally infested oilseed rape stems were arbitrarily placed within each plot within two days of each trial being planted. Infected stem residue originated from a recently harvested breeders field located near Blekendorf, Germany on July 31st, 2019 (Figure 3.2). Upon collection, residue was mixed, wrapped in bundles of 50 stems, placed outside, and watered daily to promote pathogen development.

Figure 3.2. Severe disease signs and symptoms (blackleg canker) caused by *L. maculans* collected from a recently harvested breeders field in Blekendorf, Germany on July 31st, 2019.

To confirm infection by *L. maculans*, 15 leaves with signs and symptoms of blackleg were arbitrarily collected from the edge of each trial within the designated destructive sampling area at approximately growth stage BBCH 17 (October 20-25, 2019). Infected leave tissue samples were brought to Christian-Albrecht University of Kiel for evaluation. Using a sterile scissor, lesions with pycnidia were cut from leaves from each sample and separated in petri plates containing sterile filter paper and deionized water. After 24 hours under a laminar flow hood (Prozeßtechnik GmbH, Bempflingen, Germany), a sterile needle was used to transfer pycnidiospores from a single pycnidia to a petri plate containing Tomaten-Gemüsesaft (A. Dohm & Timm, Diedersdorf, Germany) agar (pH 7.2) and penicillin (Carl Roth GmbH + Co. KG,

Karlsruhe, Germany). Petri plates were sealed with parafilm (Bemis Company, Neenah, United States) wrapped in aluminum foil and stored in the incubation chamber set to 21ºC for five to seven days. Under a laminar flow hood, fungal growth was transferred to petri plates containing medium without antibiotics. Plates were sealed with parafilm, wrapped in aluminum foil, and stored in incubation chamber for 10-12 days until mycelia had fully colonized the media. Mycelial was scrapped from the agar surface and DNA was extracted using a modified CTAB (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) technique. A polymerase chain reaction (PCR) was conducted using a Biometra Thermocycler (Analytik Jenna AG, Jena, Germany) with specific primers for *L. maculans* and *L. biglobosa* (Mahuku et al. 1996). All samples and a positive and negative control were evaluated on a gel via electrophoresis.

Out of the of the 60 leaf samples collected (15 each trial), 28 total single-pycnidium isolates were created. One-hundred percent of those cultures (28 isolates) were confirmed to be *L. maculans*. Specifically, six of eight, eight of ten, six of seven, and eight of ten isolates were confirmed as *L. maculans* at Veelböken, Neuengörs, Rohlstorf, and Dorf Mecklenburg. No sample tested in this experiment tested positive as *L. biglobosa*. These data confirm that efficacy data are derived from infection and disease caused by *L. maculans*.

Evaluation of Efficacy in Field Experiments

Throughout the course of this study, efficacy was evaluated by visual assessments that included; stand counts, autumn incidence and severity ratings at BBCH 17 on true leaves, and a cross section rating at physiological maturity between growth stages BBCH 81-85. Evaluation of full emergence was conducted approximately 20-22 days after planting (Table 3.3). Emergence was evaluated by placing a 2-m ruler arbitrarily between rows (total 8 meters), and counting the number of emerged seedlings. Emergence data was analyzed in SAS 9.4 (SAS Institute, Cary,

NC) using a generalized linear model (PROC GLIMMIX). Data were combined across all locations, and no statistical difference were observed among treatments. During the emergence evaluations, plants were also assessed for phytotoxicity by visually examining emerged seedlings for symptoms consistent with chemical injury, including yellowing, burning or physical malformation as compared to non-treated control. No phytotoxicity was observed from any treatment or at any location.

Table 3.3. Percent average plant stand count taken at full emergence in four field trials in northern Germany.

Treatment	Active ingredient[x]	Stand (1.8m^2) SY Florida[y]	Stand (1.8m^2) Fortis
1	…	78.2 A	83.1 A
2	Adepidyn	83.7 A	84.2 A
3	Acibenzolar-s-methyl	74.6 A	74.6 A
4	Adepidyn + acibenzolar-s-methyl	73.5 A	72.7 A
5	Adepidyn + difenoconazole	82.7 A	79.0 A
6	Difenoconazole	78.3 A	81.4 A
P value < 0.01		.01	.01

[x]Adepidyn (Saltro; Syngenta, Basel, Switzerland), Acibenzolar-s-methyl (Bion; Syngenta, Basel, Switzerland), difenoconazole (Score; Syngenta, Basel, Switzerland).
[y]Stand counts were taken at growth stage BBCH 11 (approximately 20-22 days after planting) with a 2-m ruler arbitrarily placed between rows (total 8 meters), and counting the number of emerged seedlings on both sides of the ruler.

Autumn disease incidence and severity ratings were conducted at BBCH 17 by arbitrarily selecting 25 plants per plot and visually evaluating the true leaves on each plant for symptoms consistent with blackleg (Figure 3.3). The percent area expressing signs and symptoms (chlorosis, necrosis, pycnidia) was recorded by visually estimating the percent of true leaf area infected.

Figure 3.3. Disease signs and symptoms caused by *L. maculans* on true leaves of an oilseed rape plant during an autumn assessment at the field trial in Rohlstorf, Germany.

Autumn disease severity data was analyzed in SAS 9.4 (SAS Institute, Cary, NC) using a generalized linear model (PROC GLIMMIX). Prior to analysis, disease severity values were arcsine-square root and yield was square-root transformed to meet the assumptions of normal residuals and homogenous variances. All data were combined using PROC GLIMMIX with fungicides as a fixed effect and location and rep as random factors in the model. If significant, LSMEANS for disease severity were compared using pairwise differences with a (α=0.05). The LSMEANS were then back transformed for presentation of results.

Blackleg severity of the non-treated control at BBCH 17 was 7.5 and 8.3% for SY Florida and Fortis, respectively (Table 3.4). No seed treatment had statistically different disease severity as compared to the non-treated control. However, treatments that include the foliar

fungicide application of difenoconazole had statistically lower disease severity, ranging from 3.7 to 4.1% on SY Florida and 4.1 to 4.7% on Fortis.

Table 3.4. Efficacy of foliar and seed applied fungicides on blackleg severity in four field trials in northern Germany at growth state at BBCH.17.

Treatment	Active ingredient[y]	Severity SY Florida[z]	Severity Fortis
1	…	7.5 A	8.3 A
2	Adepidyn	6.9 A	8.2 A
3	Acibenzolar-s-methyl	7.0 A	7.8 A
4	Adepidyn + acibenzolar-s-methyl	6.4 A	7.3 A
5	Adepidyn + difenoconazole	3.7 B	4.1 B
6	Difenoconazole	4.1 B	4.7 B
P value < 0.0001			

[y]Adepidyn (Saltro; Syngenta, Basel, Switzerland), Acibenzolar-s-methyl (Bion; Syngenta, Basel, Switzerland), Difenoconazole (Score; Syngenta, Basel, Switzerland).
[z]Disease severity ratings were conducted by arbitrarily selecting 25 plants per plot and rating the percentage of true leaves with disease at BBCH 17.

Summer disease evaluations were conducted between plant growth stage BBCH 81-85, approximately two weeks prior to harvest. Evaluations were conducted by arbitrarily selecting 25 plants per plot in the destructive sampling area. Plants were cut at the base of the plant just above the crown region (ground level) and the resulting cross section was evaluated for disease. Disease severity was evaluated using a 0-5 scale (Canola Council of Canada), based on the percent of diseased tissue visible on the cross section, where: 0 = no diseased tissue, 1 = <25% disease tissue, 2 = 26-50% diseased tissue, 3 = 53-75% diseased tissue, 4 = >75% disease tissue, and 5 = 100% diseased tissue. Using the 0-5 values, a disease severity index (DSI) was calculated to analysis treatment differences using the formula (Chiang et al. 2017),

$$DSI(\%) = \sum \left[\left(\frac{P\ X\ Q}{125} \right) x\ 100 \right]$$

Where DSI = Disease severity index, P = disease score and Q = score frequency.

Prior to analysis, DSI values were arcsine-square root transformed to meet the assumptions of normal residuals and homogenous variances. Combined DSI analyses were conducted using a generalized linear model (PROC GLIMMIX). Random effects include location and replication within location. Fixed effects were treatment and variety. If significant, LSMEANS for disease severity were compared using pairwise differences with a (α=0.05). The LSMEANS were then back transformed for presentation of results.

The DSI of the non-treated control was 37.5 across all locations and both varieties, and no seed or foliar fungicide treatment was significantly different (P = 0.8825) (Table 3.5). However, significant differences in disease severity index (DSI) values were observed between varieties (P < 0.0001) with the moderately resistant variety SY Florida (29.5 DSI) having statically lower DSI values as compared to the susceptible variety Fortis (42.5 DSI) (Table 3.6).

Table 3.5. Efficacy of foliar and seed applied fungicides on blackleg severity in four field trials in northern Germany at physiological maturity for Fortis and SY Florida.

Treatment	Active ingredient[y]	Disease severity index DSI[z]
1	…	37.5 A
2	Adepidyn	38.1 A
3	Acibenzolar-s-methyl	36.7 A
4	Adepidyn + acibenzolar-s-methyl	36.6 A
5	Adepidyn + difenoconazole	35.5 A
6	Difenoconazole	38.6 A
P value < 0.0001		

[y]Adepidyn (Saltro; Syngenta, Basel, Switzerland), Acibenzolar-s-methyl (Bion; Syngenta, Basel, Switzerland), difenoconazole (Score; Syngenta, Basel, Switzerland).
[z]Disease severity index (DSI) ratings were conducted by arbitrarily selecting 25 plants per plot within the destructive sampling area and cutting and rating the cross section of plant roots (base of plants) two weeks prior to harvest.

Table 3.6. Disease severity index values (DSI) calculated from evaluation of destructively sampled stems on the moderately resistant variety SY Florida and susceptible variety Fortis at BBCH 81-85 for four trials in northern Germany.

Variety[y]	Disease Severity Index (DSI)[z]
SY Florida	44.49 A
Fortis	29.91 B
P value < 0.001	

[y]Susceptible oilseed rape variety Fortis (Syngenta, Basel, Switzerland) and the moderately resistant variety SY Florida (Syngenta, Basel, Switzerland) that contains the resistance gene *Rlm7*.
[z]Disease severity index values was calculated by arbitrarily selected 25 plants within the destructive sampling and evaluating the cross section of each plant.

To evaluate treatment efficacy on yield, all trials were harvested at plant maturity, in late July or early August. Data was combined, transformed and analyzed as using generalized linear model. Data was back transformed for presentation purposes. No statistical differences in yield were observed in treatment on either variety (Table 3.7).

Table 3.7. Yield for SY Florida and Fortis varieties in four field trials in northern Germany.

Treatment	Active ingredient[y]	Yield SY Florida[z]		Yield Fortis	
1	…	2124.6	A	1916.3	A
2	Adepidyn	2166.3	A	1958.0	A
3	Acibenzolar-s-methyl	1999.6	A	1874.7	A
4	Adepidyn + acibenzolar-s-methyl	2041.3	A	1833.0	A
5	Adepidyn + difenoconazole	2124.6	A	2041.3	A
6	Difenoconazole	2249.6	A	1916.3	A
P value < 0.05					

[y]Adepidyn (Saltro; Syngenta, Basel, Switzerland), Acibenzolar-s-methyl (Bion; Syngenta, Basel, Switzerland), difenoconazole (Score; Syngenta, Basel, Switzerland).
[z]Yield kg per hectare.

Conclusion and Recommendations

Under climate-controlled conditions, adepidyn was able to prevent any visual signs and symptoms of disease development caused by *L. maculans* at every concentration evaluated in this study. However, disease reduction using the seed treatment was not observed under field conditions. My results are consistent with fungicide efficacy evaluations on blackleg of spring

canola in North America (Upadhaya et al. 2019), where efficacy was observed under controlled conditions but not under field conditions. Upadhaya et al. (2019) proposes that the primary reason for the inconsistency between controlled and field environments is the timing and frequency of inoculum arrival. They postulate that ascospores arrived after the beneficial activity of seed treatment was past, which commonly lasts for only 2-4 weeks after planting (Mueller et al. 2013). While ascospore dispersal was not directly measured in this study, their rationale may also fit in this study, although the experiments were conducted in very different growing conditions. Their research was conducted using spring canola, which is planted in spring when soil and air temperatures are typically cool (often 5-15^{o}C), whereas the winter canola in this study was planted in mid-summer following harvest of a cereal crop, where soil temperatures often exceed 25^{o}C; neither conditions being optimal for infection.

A number of studies conducted in northern Germany and Poland have demonstrated that temperature and precipitation in September strongly influenced ascospore dispersal (Kruse and Verreet 2004; Brachaczek et al. 2016). Results of a study conducted between 2009 and 2012 in Poland, which is adjacent to Northern Germany and is very similar climatologically and agronomically, demonstrated that in three out of the four years, the greatest number of ascospores were detected in the air from the 22-29th of September, and that ascospores arrived every year as late as November (Brachaczek et al. 2016). In this study, plants were between growth stages BBCH 10-13 (cotyledon to three leaves unfold) on September 20th, 2019 (approximately one month after planting), and thus, it is likely they were exposed to ascospores after seed treatment efficacy had diminished. In three of the four trials in this study, the foliar fungicide treatment(s) was not applied until the first week in October. This left the seedlings exposed to ascospores for the period of time after seed treatment residual was gone and before

the foliar fungicide was applied. However, the foliar fungicide would have provided protection longer in the season and potentially at a critical time for infection, which may explain why the only treatments in this study with statistically lower disease levels in the fall included a foliar fungicide. Despite the effect of a foliar fungicide on disease in the fall, reduction in disease at the end of the season was not observed. Results emphasize the importance of using resistant varieties for season-long management of blackleg, as a significantly lower DSI during the destructive sampling/evaluations conducted at BBCH 81-85 was observed in the moderately resistant SY Florida (conferred by the *Rlm7* resistance gene) when compared to the susceptible variety Fortis.

Adepidyn efficacy has been demonstrated on other diseases and crops (Adee 2020), and results of this study demonstrate efficacy against blackleg of oilseed rape in greenhouse studies. However, field data suggests that optimizing the use of the new chemistry is needed before seed application can be widely recommended in Northern Germany. Several variables influence the risk and timing of infection from blackleg in Northern Germany, including variable planting dates and weather conditions. Consequently, research to optimize usage is warranted, and adepidyn may provide an effective management tool in the future. I recommend growers continue to manage blackleg with tools available, including genetic resistance, lengthening crop rotations and considering a foliar fungicide.

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WORK CONTRIBUTION

Evaluation of fungicide efficacy on sunflower rust (*Puccinia helianthi*) on oilseed and confection sunflower - My role included experimental design, field and greenhouse inoculations, fungicide applications, disease ratings, analysis, composition and completion. The roles played by additional colleagues and collaborators include; Scott Fitterer, David Carruth, Bob Benson, Mark Halvorson, Blaine Schatz (planting, plot maintenance, and harvest), Dr. Andrew Friskop (isolate donation and statistical consultation), Dr. Ryan Humann (contribution to original grant proposal and teaching me spray methodology), Michelle Gilley, Bryan Hansen and Jessica Halvorson (technical support as needed for field and greenhouse work), and Dr. Samuel Markell (major advisor and advised throughout).

Phenotypic diversity of *Puccinia helianthi* on sunflower in California - My role included conducting field surveys in California in 2017 and 2018, pathogen collections, experimental design, greenhouse inoculation, evaluations, composition and completion. The role for additional colleagues and collaborators include; Tom Gulya (organizing meetings with cooperators in California and assisting with pathogen collections), Andrew Friskop (contribution to teaching me greenhouse and inoculation methodology), Marc Michaelson (Greenhouse assistance), Suzanne Rooney Latham and Cheryl Blomquist (identified and mailed production-field pathogen-isolates), Tom Heaton and Bill Vaccaro (assisted with locating wild populations of *H. annuus*).

Efficacy of an adepidyn fungicide seed treatment on *Leptosphaeria maculans* in oilseed rape in northern Germany - My role included pathogen collections, greenhouse and field inoculations, collaborator with field technical support, emergence evaluations, disease evaluations in autumn, field and greenhouse/laboratory project lead, greenhouse experimental

design, increasing inoculum in laboratory, molecular identification of pathogens, statistical analysis, composition and completion. Professor Dr. Joseph-Alexander Verreet (Fulbright host and advisor), Dr. Melanie Goll, Dr. Monika Joss and Franz Brandl (assistance with field trial designs and technical support), Ketel Prahl (assisted with pathogen collection), Dr. Jannika Drechsel, Susanne Kleingarn, Bettina Bastian (PCR and laboratory assistance), Dr. Holger Klink, Dr. Tim Birr, Dr. Jens Aumann (coordinating and organizing personnel/equipment and technical support), Finn Großmann (greenhouse/laboratory assistance and summer evaluations), Albrecht Buschmann (field assistance), Bjoern Kaiser and Bjoern Kuhle (planting, plot maintenance, and harvest).