DEVELOPING MANAGEMENT TOOLS FOR SUNFLOWER RUST (*PUCCINIA HELIANTHI)*

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Developing Management Tools for Sunflower Rust (*Puccinia helianthi*)

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Sunflower rust, caused by *Puccinia helianthi* (Schwein), is an economically important disease in North Dakota. Since 2008, sunflower rust incidence and severity have increased. Therefore, a four year study was initiated to examine the management strategies for the disease. A total of 19 fungicide efficacy and timing trials were conducted from 2008-2011. Results indicated that DMI and QoI chemistries can be used effectively to manage the disease. Timing trials indicated that disease control was highest when fungicide applications were made at R5.0-R5.8. Results indicate a fungicide application is warranted when rust severities at approximately 1% are found on the upper-four leaves at R5. To observe phenotype variability in the pathogen, a *P. helianthi* survey was completed in 2011 and 2012. Single-pustule isolates were obtained and virulence phenotypes were evaluated on a set of nine differentials. Race characterization was assigned based on virulence phenotypes. In 2011, the most commonly detected races were 300 and 304, while the most virulent was 776. In 2012, races 304 and 324 were the most commonly detected and the most virulent was 777. To identify new sources of rust resistance, the core-set of *Helianthus annuus* germplasm was obtained from the USDA-North Central Regional Plant Introduction Station. The accession lines were screened both in the greenhouse and the field. The accessions were screened in the greenhouse individually to races 300, 304, 336, 337, and 777. Lines were screened in the field to a mixture of *P. helianthi* isolates coding to 300, 304, 336, and 337. The majority of lines were susceptible in both the field and greenhouse. At both field locations, PI 431538, PI 432512, and PI 650362 had year-end severities under 1%. Similarly, PI lines 432512 and 650362 had resistant infection types across all five races. The results of these studies provide information and tools that are being used currently to manage rust and will contribute to management in the future.

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DEDICATION

This dissertation is dedicated to my late father Marcus Friskop. He was the greatest father, mentor, visionary, educator, and friend a son could ask for. My father initiated my curiosity for science as a young adolescent and was the biggest enthusiast of my college education.

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LITERATURE REVIEW

Sunflower Origin

Sunflower (*Helianthus annuus*) is one of the few commercially grown crops native to North America. Native Americans used sunflowers primarily as a food source and are given credit for the cultivation and domestication of the crop (Heiser, 1955). Numerous tribes across the United States have been associated with growing sunflowers in their culture, including the Hopi, Mandan, Arikara, Hidatsa, and Algonquin (Heiser, 1955; Putt, 1997). The specific location of sunflower domestication is suggested to be in the eastern United States (Harter et al. 2004) although disagreement has existed. Recently, it was suggested that the earliest record of documentation was in Mexico (Lentz et al. 2001). However, it was later refuted due to the misidentification of gourd seed as sunflower achenes (Heiser, 2008).

Sunflower as a Crop

Although sunflower is native to North America, the value of sunflower as a cash crop was realized overseas. Spanish explorers are believed to have brought sunflowers to Europe during the 16th century (Putt, 1997). Original cultivation of sunflower was primarily for use as an ornamental or for novelty. As sunflower spread into Russia during the 18th century, its use as an oilseed crop was recognized (Seiler and Riesenberg, 1997). Traditional selection methods were used in small garden plots for high oil varieties with many developed by 1880 (Putt, 1997). By the 20th century breeding efforts were initiated and sunflower was considered a major crop in Russia with one of the most successful breeding programs developed at Krasnodar by Pustovoit (Putt, 1997). Sunflower was reintroduced back to North America during the 19th century. However, most sunflowers were used for silage throughout Canada and the USA (Putt, 1997). The transition from silage to oilseed largely happened in Canada. In the 1930's, it was

recognized that the Mammoth Russian was not suitable for oilseed production. Mennonite farmers possessed shorter statured sunflowers that were better adapted to the environmental conditions in Canada (Putt, 1997). This material provided the framework for the development of the first breeding nursery in Canada in 1937. A cross between the Mennonite cultivar and the Russian S-490 (Russian high oil cultivar) created one of the first available cultivars for growers in North America (Putt, 1997). When profitable economic returns materialized in Canada, sunflower garnered interest in North Dakota and Minnesota in 1948 (Putt, 1997). A continued effort to increase disease resistance and oil content in sunflower in Canada and Russia strengthened the economic interest in the U.S. The early hybrid 'Advent' from Canada and the cultivar Peredovik from Russia both were varieties that made the crop attractive to U.S. farmers in the 1960's (Putt, 1997).

Sunflower Types

Two types of sunflower are commonly grown in the US; oilseed and non-oilseed. Oilseed sunflower is widely used for vegetable oil production. Non-oilseeds (also termed confectionary) are used for human consumption. Oilseeds tend to be black, small seeded, and occupy more acreage (approximately 80%) in North Dakota (Berglund, 2007). Non-oilseeds usually are striped, large seeded, and are grown on less acreage (Berglund, 2007).

Sunflower Production

The first reported production of sunflowers for seeds in the US was in 1966 at 6,000 acres. Sunflower acreage rapidly increased in the 1970's climaxing in 1979 at 5.5 million acres. Throughout the 1970's, the U.S. was the second largest producer of sunflower globally, behind the former Soviet Union. However, in subsequent decades, Russia and Argentina became the largest producers of sunflower, while the US is still considered to be a major producer with

approximately 2 million acres of the crop. Within the US, the majority of production lies in North Dakota, South Dakota, Minnesota, Kansas, Colorado, Nebraska, and Texas (Sandbakken and Kleingartner, 2007). North Dakota typically produces 40-50% of the total crop in the US, with approximately 1 million planted acres (NASS, 2012).

***Puccinia helianthi* (Sunflower Rust)**

Puccinia helianthi was first described in 1882 on the host *Helianthus mollis* by Schweinitz (Bailey, 1923). However, before Schweinitz's description, previous reports of sunflower rust were reported from the countries of Canada, Germany, Austria, Italy, Romania, Serbia, Sweden and Russia (Bailey, 1923). Schweinitz initially named the pathogen *Aecidium helianthi-mollis*. Eventually, the pathogen was renamed to *Puccinia helianthi-mollis* and subsequently the suffix *mollis* was dropped to form the present pathogen name. Life cycle studies began in the late 1800's and discrete confirmations were made in the early 1900's. Woronin (1872) obtained the aecial stage of the pathogen on sunflower, indicating it was an autoecious rust (Woronin, 1872). The macrocyclic nature of the pathogen was confirmed in 1900 and 1903 (Arthur, 1903; Kellerman, 1905). In the 1920's, Craigie completed studies on the sexual patterns of rust fungi using *P. helianthi*. Based on his conclusions, he determined pycnia were the sexual stage of rust fungi, and *P. helianthi* was a heterothallic fungus (Craigie, 1927).

Another significant figure in *Puccinia helianthi* studies was Waldemar E. Sackston, a plant pathologist who was stationed at Macdonald College of McGill University in Canada. Sackston published a series of articles entitled "Studies on Sunflower Rust." These publications covered a breadth of topics ranging from sources of rust resistance to the biological nature of the pathogen (Hennessey and Sackston, 1972a; Hennessy, et al., 1972; Hennessy and Sackston, 1972b; Hennessy and Sackston, 1970; Putt and Sackston, 1963; Putt and Sackston, 1957; Sackston,

1962; Sackston, 1960; Sood and Sackston, 1972; Sood and Sackston, 1970; Sodd and Sackston, 1969). In addition to these publications, Sackston was involved with numerous articles on *P. helianthi* and generated a foundation of knowledge for this pathogen. Sackston studies answered some basic questions regarding protocols for studying the pathogen in laboratory settings and provided insight on basic breeding principles.

The importance of sunflower rust in a given year is related to the time of disease onset and environmental conditions during the growing season. Severe yield losses in amounts of 80% can occur in severely infected fields (Markell et al., 2009). In the U.S., inoculum sources are attributed to both distant and local events. Distant inoculum sources follow the *Puccinia* pathway and often will result in later infections in the Northern Great Plains. In 2008, the aecial stage of *P. helianthi* was observed in North Dakota and Minnesota, thus indicating the pathogen had completed its sexual cycle (Markell et al., 2009). This event indicates a local inoculum source exists in these states resulting in earlier infection and increasing the likelihood for rust epidemics. The aecial stage was also reported in 2008 in Nebraska and Manitoba (Harveson, 2010; NSA of Canada, 2011).

Taxonomy of *P. helianthi*

Kingdom: Fungi

Phylum: Basidiomycota

Class: Pucciniomycetes

Order: Uredinales

Family: Pucciniaceae

Genus: *Puccinia*

Species: *helianthi*

The disease name rust refers to the reddish-orange pustules that appear on host plants during the uredinial stage of the pathogen at some point in the life cycle. Formation of basidiospores on a basidum define the reproductive cycle of rust fungi, thus they are placed in the phylum Basidiomycota (Cummins and Hiratsuka, 2003). The class Pucciniomycetes is rather difficult to associate morphologically but molecular methods have revealed the separation of Pucciniomycetes from other classes. The order Uredinales is often the name reserved for rust fungi. The morphological identification of a rust species is often based on teliospore shape and structure (Cummins and Hiratsuka, 2003). There are numerous families represented in the order Uredinales and morphological differences can be seen among them. *P. helianthi* is grouped in the family Pucciniaceae (Cummins and Hiratsuka, 2003). A major determinate of this family is teliospores are borne on stalks (Cummins and Hiratsuka, 2003). The genera of rust fungi are further identified by other teliospore characteristics. The *Puccinia* genus has a specific set of distinguishing characteristics according to the "Illustrated Genera of Rust Fungi." Following the dichotomous outline, the genus *Puccinia* have two-celled teliospores, with external basidiospores, both uredinia and telia lack a peridium, teliospores are not produced in columns, and teliospores have one pore per cell. The species identification of *P. helianthi* correlates to the host it infects (Sunflower – *Helianthus*) (Cummins, 1978; Cummins and Hiratsuka, 2003).

Biology of *P. helianthi*

P. helianthi is an autoecious macrocyclic rust pathogen indicating all five spore stages of the fungi occur on a *Helianthus* host. Additionally, the sunflower rust pathogen (like all rusts) are obligate parasites, therefore can only grow on a living host and cannot be cultured on artificial medium. The life cycle of the pathogen has been well characterized and all spore stages have been documented. Most spore structures can be seen with the unaided eye, the exception

being the basidium. In the spring, teliospores germinate and produce a promycelium producing four basidiospores. The four basidiospores are the result of a meiotic event and are classified as either (+) or (-). The basidiospore has a thin cell wall and therefore cannot travel great distances before losing viability. Basidispores enter the host plant cells and produce a monokaryotic mycelium. After 10-12 days, the mycelium produces a flask shaped pycnia possessing pycniospores and receptive hyphae. Pycnia are found on the upper side of the sunflower leaf, appear as yellow-orange spots, and are less than a ¼ inch wide. Insects cross-fertilize the pycniospores and the receptive hyphae and result in the production of a mycelium that gives rise to aecia. Aecia are found on the underside of the sunflower leaf and are arranged in orange cup-like clusters. Aecial cups release dikaryotic aeciospores. Aeciospores are ellipsoid with a thick colorless wall, measuring 20-25x16-21 µm. Aeciospores are able to survive several weeks and travel great distances. Aeciospores eventually will land on the host germinate and penetrate the leaf stomata and produce a dikaryotic mycelium giving rise to dikaryotic urediniospores. Uredinial pustules can be found on the both the upper side and bottom side of leaves, bracts, stems, and petioles. Urediniospores are cinnamon brown, ellipsoid, with lateral pores, and measure 26-33x18-28 µm and often have a cholortic halo surrounding the pustule. Urediniospores are the repeating stage of the fungus and cycling will occur until adverse conditions stimulate the fungus to convert the dikaryotic urediniospores into diploid teliospores. Teliospores are the overwintering stage and are characteristically black and remain on the plant's surface when agitated. Teliospores are oblong, black, pedicellate, and measure 38-60x21-30 um (Gulya et al., 1997; Cummins and Hiratsuka, 2003).

Although the aecial stage has been documented to occur in nature, it is infrequently detected. Putt and Sackston documented aecial infections in mid-June of 1951 on volunteer

seedlings. Coincidently, a major sunflower epidemic resulted during that same year (Putt and Sackston, 1957). Since 2008, widespread documentation of the aecial stage was observed in North Dakota, Minnesota, Nebraska and Canada. From a management point of view, this situation presents two challenges for producers. The early appearance of the aecial stage indicates that the uredinial stage of sunflower rust may occur earlier, thus increasing the risk for substantial yield loss. Secondly, the aecial stage indicates the sexual cycle has been completed. This event may lead to an increase in pathogen diversity and result in the formation of new races (Kong et al., 1999).

All 61 North American *Helianthus* species can be hosts to *P. helianthi* (Gulya et al., 1997). With the abundance of wild sunflowers across the U.S., wild sunflower species can be a substantial source of inoculum for sunflower rust epidemics and sexual recombination. Wild sunflowers can be found along roadsides, gravel pits, agronomic fields, botanical gardens, and other locations where wild sunflowers thrive (Friskop et al., 2011)

The infection process has been well documented for sunflower rust. A research study was completed on the infection process of *P. helianthi* for both resistant and susceptible sunflowers (Sood and Sackston, 1969). Results indicated that haustorium formation was completed within 24 hours after inoculation and differed morphologically on susceptible and resistant sunflowers. Haustoria were elongated and plentiful on susceptible sunflowers and round and few on resistant sunflowers. Additionally, mycelial growth was more progressive on susceptible sunflowers than resistant sunflowers (Sood and Sackston, 1969).

Although *P. helianthi* is the most common rust found on sunflowers, four other species of *Puccinia* and *Coleosporium helianthi* can cause infection on *Helianthus* hosts. However, the distribution of the other rust species is limited and has only been reported on selected *Helianthus*

hosts. *Puccinia encelia* and *P. massalis* cause similar signs and symptoms as *P. helianthi* but teliospore characteristics can differentiate these species. Additionally, *P. encelia* has only been identified on ornamentals and desert shrubs in the western U.S. through South America. *P. massalis* has been documented along the Rio Grande River but has been naturally limited to Texas blueweed. Greenhouse tests have confirmed that *P. massalis* can infect cultivated sunflowers. *Puccinia xanthii* has caused infrequent infections on sunflower, but is characteristically diagnosed by large telial brown pustules. *P. canaliculata* has only been reported once on cultivated sunflower in Kansas and is a heteroecious rust with *Helianthus* serving as the aecial host. *C. helianthi* is also a heteroecious rust with the *Helianthus* host serving as a host for the uredinial stage. Uredinial pustules are bright orange, which is easily differentiated from the cinnamon-brown *P. helianthi* uredinia (Gulya, et al., 2011). Differentiation between the rust species that infect sunflower can be done by observing teliospore morphology, identification of spore stage represented, and uredinia.

Management

Management of sunflower rust is primarily accomplished with resistant hybrids and fungicides. Another management option that may offset early disease onset is eliminating wild sunflowers in close vicinity to production fields (Friskop et al., 2011).

Resistance. Host resistance has been demonstrated in sunflowers since its appearance as a silage crop in the 1920's (Baily, 1923). In 1955, the first rust resistant material was grown commercially in North America. The sunflower variety 'Beacon' was derived from crosses of wild sunflowers in Renner, TX with 'Advent' and 'Admiral' (Putt and Sackston, 1957). Subsequently, the resistance genes R_1 and R_2 were first identified by Canadian researchers in 1963 (Putt and Sackston, 1963). Since then, numerous resistance genes have been detected and

some have been genetically characterized (Sendell et al., 2006; Qi et al., 2011; Bulos et al., 2013). Due to the pathogen's ability to evolve, susceptibility is observed in most sunflower hybrids. In 2008, commercially available hybrids were evaluated for their reaction in a naturally-occurring rust epidemic at the Carrington Research Extension Center. Results indicated that approximately 80% of the hybrid entries were susceptible to local sunflower rust pathogen races, and rust severity and yield were inversely correlated (Friskop et al., 2010). This indicates that more emphasis is needed on the introgression of rust resistance genes into commercial hybrids.

Identification of novel sources of resistance is a critical tool for managing a disease. Germplasm and commercial hybrid screenings have been conducted by USDA-ARS in Fargo, ND and by North Dakota State University Research Extension Centers (Gulya et al., 1997; Gulya and Brothers, 2000; Gulya, 2006; Friskop et al., 2011; Qi et al., 2011). A majority of the germplasm screened are susceptible to local rust isolates. Sources of resistance have been identified in sunflower germplasm and have been released by the USDA-ARS, Fargo, ND (Miller and Gulya, 2001; Jan et al., 2004; Jan et al., 2006). Although numerous accessions have been screened for rust resistance, to our knowledge the core-set of *Helianthus annuus* germplasm has not been screened.

Fungicides. Like many diseases, management of sunflower rust with copper based fungicides was investigated. However, one year field trials in the 1920's indicated copper sprays did not satisfactorily reduce rust infections (Bailey, 1923). The majority of subsequent studies evaluating the performance of fungicides were conducted overseas. Efficacy trials conducted in Kenya and Turkey demonstrated fungicides increased yield component values compared to non-treated plots (Singh, 1975; Thakore et al., 1980). Another efficacy trial was performed in North Dakota in 1989 evaluating chemistries belonging to the demethylation inhibitors and multisite

activity groups (Gulya and Lamey, 1990). Results indicated that demethylation inhibitors significantly lowered rust severities compared to the multisite activity fungicides. One of the most recent studies on fungicide efficacy and timing was done in Israel. Shtienberg et al. 1995 reported an action threshold determined by both leaf severity and growth stage. The authors indicated a fungicide application should be made if rust severity of 3% on the upper leaves is reached prior to 27 days after flowering. However, this recommendation was based on growing conditions in Israel and exclusive to triazole chemistries (Shtienberg, 1995). Since this study, new classes of fungicides (ie: QoI) have been developed and labeled on sunflowers (McMullen and Markell, 2010).

Removal of Wild Sunflowers. No research has been done to observe the value of removing wild sunflowers from around field margins. However, since *P. helianthi* can infect all *Helianthus* species, all wilds can serve as an inoculum reservoir. Also, with the appearance of aecial in major production states, removing wilds may reduce the potential for recombination events (Kong et al., 1999).

Race Surveys

Understanding the biology of the pathogen is necessary for interpreting the effectiveness of resistance (Arthur, 1903). Bailey (1923) performed inoculation studies on multiple sunflower species using multiple *P. helianthi* isolates and noticed a differential response. He further described his observations as two "forms" of the pathogen. Characterization of sunflower rust races was accomplished using three Canadian derived differentials and races were named North American race 1, 2, 3, and 4 (Sackston, 1962). Numerous sunflower rust surveys have been completed in the U.S. since 1989 (Gulya, 1990; Gulya and Viranyi, 1994; Gulya et al., 1996, Gulya, 2003; Gulya and Markell, 2009). During this time, the four differentials were expanded to

nine internationally accepted sunflower differentials. Race nomenclature was established using a triplet coding system. Virulence phenotypes were assessed on the nine differentials and virulence formulas could be converted to a three digit race designation (Gulya and Masirevic, 1996). Recent rust surveys have obtained virulence phenotypes from bulk collections, rather than single pustules. The most recent survey, conducted in 2008, determined that the predominant races from bulk isolates were race 334 and 336 (Gulya and Markell, 2009). During this same time frame, a total of 25 and 31 bulk virulence phenotypes were detected from location samples obtained in 2007 and 2008 respectively (Gulya and Markell, 2009).

Phylogenetics

Limited studies have been performed on the phylogeny of *P. helianthi*. One of the more elaborate analyses of pathogen diversity was conducted by Sendall et al. (2006). Included in their analysis was the construction of a dendrogram comparing isolates from Australia, the Americas, Asia, Africa, and Europe. Seventy-six isolates were obtained and analyzed using virulence phenotypes and molecular data. The selected isolates were molecularly assessed using randomly amplified polymorphic DNA fingerprints amplified from 11 primers. Based on the dendrogram, three groups (A, B, and C) were created. Group A included isolates solely of Australian nature. Groups B and C contained isolates from Australia and the other countries represented in the study. The study concluded that the Australian isolates had higher levels of genetic diversity based on the following reasons: Australian isolates were represented in all three groups and some isolates were represented in more than one group. The occurrence of Australian pathotypes being placed into multiple groups suggests recombination events have occurred in Australia. No thorough study has been completed evaluating the genetic diversity of

P. helianthi in North America. Given that sexual recombination has been documented in the United States, it is possible that genotypic diversity is continually changing.

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CHAPTER ONE: EVALUATION OF FUNGICIDE EFFICACY AND TIMING FOR THE MANAGEMENT OF SUNFLOWER RUST

Introduction

Sunflower rust, caused by *Puccinia helianthi* Schwein., is an important yield-limiting disease in United States sunflower production areas. The pathogen is macrocyclic, autoecious and can overwinter in all areas of sunflower production in the United States (Gulya et al., 1997; Markell et al., 2009; Harveson, 2010). Thus the pathogen has potential for frequent sexual reproduction which can result in race changes, early disease onset, and multiple urediniospore cycles under appropriate environmental conditions. Recently, earlier onsets of rust in producers' fields lead to the development of higher year-end severity levels and yield loss. For example, in a ND grower's field in 2008, a 5% to 10% disease severity at the reproductive growth stage resulted in an 80% yield reduction. (Markell, et al. 2008; S. Markell *personal communication*). Also, most commercially grown hybrids are susceptible to rust, and the impact of crop rotation on rust is limited because of long-distant spore dispersal, leaving fungicides as one of the only effective management tools.

The majority of previous studies evaluating the performance of fungicides were conducted in areas outside the United States. Results from efficacy trials conducted in Kenya and Turkey demonstrated that fungicides increased yield-component values, when compared to non-treated plots (Singh, 1975; Thakore et al., 1980). In the 1990's in Israel, Shtienberg et al., (1995) developed a fungicide action threshold based on both leaf severity and growth stage and recommended a fungicide application when leaf severity of 3% on the upper four leaves is reached prior to 27 days after flowering. However, development of the threshold was based solely on research conducted with demethylation inhibitors, and in a production system different

from the United States (irrigation, day length, and temperature). Since then, quinone outside inhibitors have been labeled (McMullen and Markell, 2010), and recommended for management of other rust pathosystems (Mueller et al., 2009; Wanyera et al., 2009; Olson et al., 2011), but efficacy and optimal application timing in sunflower rust is unknown. The objectives of this study were to evaluate fungicide efficacy and timing on management of sunflower rust and yield.

Materials and Methods

Locations and Experimental Design. Experimental sites were at four locations in North Dakota; Casselton (CASS), the NDSU Carrington Research Extension Center (CREC), Grandin (GRAN), and the NDSU Langdon Research Extension Center (LREC). Trials were conducted in a randomized complete block design with four replications at CASS in 2008, CREC from 2008-2011, GRAN from 2009-2011, and LREC from 2008-2011. CREC was under center-pivot irrigation, all other locations were non-irrigated. Four row plots were seeded with a susceptible confection hybrid 'Jaguar' (Seeds2000). Row width was 76.2 centimeters (cm) and seed spacing was 21.6 cm at each location. Four row plots were used at all locations; the middle two rows of each plot were used for data collection, while the other plot rows were used as borders. Planting date, inoculation date, and plot length varied at each location (Table 1.1). Agronomic practices appropriate for sunflower production were followed for each location and year (Berglund, 2007).

Artificial Inoculation. In order to facilitate adequate disease pressure, plots were artificially inoculated at each location. Inoculum was produced by increasing urediniospores of *P. helianthi* isolate ND07-01 (race 336) on a susceptible sunflower hybrid to ensure a fresh viable source of inoculum. Collected spores were suspended in a Soltrol 170 suspension and quantified to approximately 275,000 spores/ml. Application of the spore suspension was done using a modified leaf blower. In 2008 and 2009, only border rows of the plots and internal

spreaders plots were inoculated, while in 2010 and 2011, the treatment rows were inoculated. Timing of inoculation was completed to coincide with disease onset objectives. Inoculation was completed during early sunflower reproductive stages in 2008 and 2009; and in late vegetative stages during 2010 and 2011.

Table 1.1. Agronomic and inoculation information across all experiment locations in each year.

Trial	Planting Date	Row Length (in m)	Row Width (in cm)	Seed Spacing (in cm)	Inoculation Date	Rust Detection Date
CASS 2008	June 19	7.6	76.2	21.6	August 7	August 21
CREC 2008	May 22	7.6	76.2	21.6	July 11	August 7
LREC 2008	May 20	4.6	76.2	21.6	July 15	August 7
CREC 2009	June 1	7.6	76.2	21.6	July 16	July 30
GRAN 2009	June 1	7.6	76.2	21.6	July 16	July 30
LREC 2009	May 11	4.6	76.2	21.6	July 1	July 21
CREC 2010	May 27	7.6	76.2	21.6	June 21	July 7
GRAN 2010	May 28	7.6	76.2	21.6	June 29	July 28
LREC 2010	May 19	4.6	76.2	21.6	Jun 24	July 7
GRAN 2011	May 25	7.6	76.2	21.6	July 8	July 22
LREC 2011	May 30	4.6	76.2	21.6	June 29	July 11

Fungicide Efficacy. Efficacy trials were established in 2008 and 2009. Locations varied in the total number of fungicides evaluated (Table 1.2) but all trials included the demethylation inhibitors (DMI – FRAC 3); prothioconazole (Proline, Bayer CropScience, Research Triangle Park, NC), prothioconazole + tebuconazole (Prosaro, Bayer CropScience, Research Triangle Park, NC), tebuconazole (Tebuzol, United Phosphorous Inc., King of Prussia, PA), and the quinone outside inhibitors (QoI – FRAC 11); pyraclostrobin (Headline, BASF, Research Triangle Park, NC) and azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC). Other DMI fungicides included at some locations were; metconazole (Quash, Valent, Walnut

Creek, CA) and tebuconazole (Folicur, Bayer CropScience, Research Triangle Park, NC). Succinate dehydrogenase inhibitors (SDHI – FRAC 7) included at some locations were boscalid (Endura, BASF, Research Triangle Park, NC) and penthiopyrad (Vertisan, DuPont Agricultural Products, Wilmington, DE). Additionally, a non-treated control (NTC) was used at each location. All fungicides were applied between R5.0 to R5.5 (start of flowering to mid-flowering) (Schneiter et al., 1998).

Table 1.2. The number of fungicides used, application timing, and obtainable yield for each trial.

Type of Fungicide Trial	Location Year	Number of Fungicides	Growth Stage Applications[a]	Yield Obtained[b]
Efficacy	CASS 2008	7	R5	Yes
	CREC 2008	8	R5	Yes
	LREC 2008	8	R5	Yes
	CREC 2009	5	R5	Yes
	GRAN 2009	10	R5	No – Stem Lodging
	LREC 2009	7	R5	Yes
Timing – Normal Onset	CASS 2008	2	R3, R5, R6	Yes
	CREC 2008	1	R3, R5, R6	Yes
	LREC 2008	2	R3, R5, R6	Yes
	CREC 2009	2	R3, R5, R6	Yes
	GRAN 2009	2	R3, R5, R6	No – Stem Lodging
	LREC 2009	2	R3, R5, R6	Yes
Timing – Early Onset	CREC 2010	1	V8-V12, R1, R5	No – Sunflower Midge
	GRAN 2010	2	V8-V12, R1, R5	No – Stem Lodging
	LREC 2010	1	V8-V12, R1, R5	Yes
	CREC 2011[b]	…	…	…
	GRAN 2011	2	V8-V12, R1, R5	Yes
	LREC 2011	1	V8-V12, R1, R5	Yes

[a] Sunflower growth stages according to Schneiter et al., 1998.
[b] Indicates if yield was obtained or indicates the agronomic issue if yield was not obtained.

Fungicide Timing – Normal Onset. Timing trials conducted in 2008 and 2009 were designed to develop timing recommendations in a "normal" disease onset scenario. A normal

disease onset is defined as when rust is first found on the upper leaves of sunflowers during the reproductive stages of development. This approximately simulates the time of disease onset in production fields when the source of inoculum are urediniospores from nearby fields or long distance dispersal. To simulate this, spreader rows were inoculated with urediniospores approximately one to two weeks before the reproductive growth stages began in the trials. Fungicide applications were made at sunflower growth stages (Schneiter, 1998) R3-4 (mid-bud elongation), R5 (flowering), and R6 (flowering complete) using single or multiple applications of pyraclostrobin or tebuconazole. A NTC was used at each location as well. The number of treatments assessed varied among location and years (Table 1.2).

Fungicide Timing – Early Onset. Timing trials conducted in 2010 and 2011 were designed to develop fungicide timing recommendations in an "early" disease onset scenario. Early disease onset is defined as when occurred when rust is found on the upper portion of the plant prior to growth stage R1. This approximately simulates the time of disease onset in production field when the source of inoculum originates from within the field as a result of completion of the sexual stage. To simulate this, all plots were first inoculated with urediniospores between V6 and V12 growth stages, and re-inoculated periodically until uredinia were visually observed. Fungicide applications were made at growth stages (Schneiter et al., 1998) V8-V12 (late vegetative), R1 (bud formation), and R5 using single and multiple applications. Experimental timings were developed, in part, to coincide with sunflower production practices. Herbicide applications are usually initiated at late vegetative (V8-V12), fungicide applications for non-disease yield impacts have been suggested to be applied at R1, and the R5 timing was deemed the most important fungicide timing of the three tested in 2008-

2009. Pyraclostrobin was used at all locations, while tebuconazole was also evaluated at GRAN (Table 1.2).

Fungicide Application. Fungicides were applied at 241.3 kilopascals with CO_2 powered backpack sprayers at GRAN, CASS, and LREC and with a tractor sprayer at CREC. Applications were made at 187 liters per hectare (L/ha) at GRAN and CASS, 86 L/ha at LREC, and 121.6 L/ha at CREC. Teejet 8002 flat fan nozzles were used at all locations. Nozzle spacing was 76.2 cm at CASS, GRAN, and LREC and 38.1 cm at CREC. The target area of application was the upper-four leaves of the sunflower plant.

Data Collection. Disease evaluations were conducted visually, with the aid of rust assessment diagrams (Gulya et al., 1990; Shtienberg, 1995; Friskop et al., 2011). The sunflower rust assessment diagrams are more conservative than other rust assessment tools. For example, a 1% rust severity on sunflower rust diagrams translates to approximately a 20% severity for wheat rust using the modified Cobb scale (Peterson et al., 1948). For all trials, the mean percent leaf area covered by pustules on the upper four leaves of ten randomly plants in the center two rows of each plot were evaluated (Shtienberg, 1995). Assessments were conducted at approximately two week intervals beginning at disease onset and ending at season's end (Table 1.1). Disease severity ratings were used to calculate the Area Under Disease Progress Curve (AUDPC) values and relative Area Under Disease Progress Curve (rAUDPC) (Madden et al., 2007). AUDPC was calculated as: AUDPC = $\sum_{i=1}^{n}\left((y_i + y_{i+1} / 2)\ (t_{i+1} - t_i)\right)$ where yi = rust severity at the i^{th} observation, ti = time (days) at the i^{th} observation, and n = total number of observations. The rAUDPC was calculated as: rAUDPC = AUDPC / $\left(t_f - t_0\right)$ *100 where t_f = the duration of days at the final rating and t_0 = the time of disease onset. Additionally, percent disease control (hereafter referred to as disease control) was calculated at each location using the formula [((1 –

(rAUDPC of a treatment / rAUDPC of the NTC) * 100). Yield was collected from the center two plot rows at season's end. Yield loss assessment was evaluated by correlating R7 leaf severities to percent change in yield from the non-treated control. Only trials where rust severity on the NTC was greater than 5% at R7; and had significant yield differences were used for yield loss assessment. Namely, CREC 2008-Normal Onset, CREC 2009 Efficacy, CREC 2009-Normal Onset, and LREC 2010.

Statistical Analysis. Data were analyzed at each location separately due to differences in the establishment and development of disease in the trials. Analysis of variance (ANOVA) was used in the general linear models procedure within the SAS 9.2 (SAS Institute, Cary, NC) program. Fisher's protected least significant differences at $\alpha = 0.05$ were used to determine significance difference among treatment means. Pearson's correlation coefficient and simple linear regression procedure within the SAS 9.2 program was used to assess yield loss.

Results

Fungicide Efficacy. Rust developed at all locations in 2008, and R7 severity was high enough on the NTC at CREC 2008 and CASS 2008 to see differences in disease control with fungicide applications (Table 1.3). At CREC 2008, disease control provided by all DMI fungicides was statistically the same and higher than applications of SDHI fungicides. Disease control differences among QoI treatments were observed. At CASS 2008, rust severity at R5 and R7 was relatively low, but year-end severities were among the highest at physiological maturity (data not shown). At CREC 2008 and CASS 2008 disease control provided byDMI fungicides was statistically higher than that of the only SDHI tested at that location, penthiopyrad. Due to the low amount of disease pressure at LREC 2008, no levels of disease control were found among the fungicides tested.

In 2009, rust developed at all locations with great enough severity that disease control differences among treatments were observed (Table 1.4). DMI fungicide applications resulted in higher levels of disease control than a boscalid application at locations where it was tested. Greater disease control was achieved with tebuconazole and prothioconazole + tebuconazole applications than azoxystrobin at CREC and GRAN in 2009. At LREC 2009, levels of rust control did not significantly differ between applications of pyraclostrobin and the DMI fungicides tested. At CREC 2009, applications of DMIs and pyraclostrobin resulted in a statistically higher yield than the NTC. However, yield between the azoxystrobin treatment and the NTC was the same.

Fungicide Timing – Normal Onset. Rust developed at all locations in 2008, but severity was great enough at only CASS 2008 and CREC 2008 to observed meaningful differences in disease control, and yield differences were observed only at CREC 2008 (Table 1.5). At CASS 2008, disease control from fungicide applications ranged from 97.3 to 71.6 percent, and statistically differences were observed among treatments. Yield was not statistically different than the NTC for any fungicide application. At CREC 2008, significant differences in disease control and yield were observed among fungicide timings. Levels of rust control and yield were statistically similar between a single application of pyraclostrobin at R5 and three fungicide applications of pyraclostrobin. Two treatments, PYR at R5 and PYR at R3 + R5 + R6 had statistically higher yield than the NTC.

Rust developed at all locations in 2009, and severity was high enough to see meaningful statistical difference in disease control (Table 1.6). In CREC 2009 and LREC 2009, disease

Table 1.3. Sunflower rust severity, rAUDPC, percent disease control, and yield for efficacy trials conducted in 2008.

Treatments		CASS 2008					CREC 2008					LREC 2008				
Fungicide[a]	**Timing[b]**	**R5 (% Sev)[c]**	**R7 (% Sev)**	**Relative AUDPC[d]**	**% Disease Control[e]**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**
NTC	…	0	1.12	0.0558	0.0	2245	2.61	9.92	0.0659	0.0	2558	0.32	0.24	0.0023	0.0	1715
PRO	R5	0	0.22	0.0070	83.6	2005	0.42	0.47	0.0042	93.6	2765	0.29	1.30	0.0066	-224.2	2271
TEB	R5	0	0.26	0.0058	86.1	2344	0.40	0.69	0.0049	92.6	2780	0.13	0.39	0.0027	-29.0	2042
MET	R5	…	…	…	…	…	0.78	1.15	0.0094	85.4	3003	…	…	…	…	…
PRO + TEB	R5	0	0.28	0.0042	90.8	2070	0.39	0.46	0.0048	92.3	2541	0.21	0.37	0.0026	-29.3	1890
PYR	R5	0	0.31	0.0141	66.1	2144	0.70	2.23	0.0157	74.3	2765	0.10	0.31	0.0020	-2.5	1895
AZO	R5	0	0.35	0.0197	53.4	2268	1.23	4.55	0.0345	43.8	2621	0.11	0.52	0.0028	-30.4	1920
PEN	R5	0	0.44	0.0261	43.6	2215	1.03	5.40	0.0365	44.3	2768	0.19	0.48	0.0028	-46.6	1988
LSD (*P* = 0.05)		N/A	0.39	0.0193	17.9	ns	0.77	2.14	0.0121	18.5	ns	0.10	ns	ns	ns	ns

[a] Fungicides tested at each location: NTC = non-treated control, PRO = prothioconazole (Proline, Bayer CropScience), TEB = tebuconazole (Tebuzol, United Phosphorus Inc.), MET = metconazole (Quash, Valent), PRO + TEB = prothioconazole + tebuconazole (Prosaro, Bayer CropScience), PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), and BOS = Penthiopyrad (Vertisan, DuPont)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $(t_f - t_0)$ *100, AUDPC = $\sum_{i=1}^{n}((y_i + y_{i+1} / 2)(t_{i+1} - t_i))$ where y*i* = rust severity at the i^{th} observation, t*i* = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100]

Table 1.4. Sunflower rust severity, rAUDPC, percent disease control, and yield for efficacy trials conducted in 2009.

Treatments		CREC 2009					GRAN 2009					LREC 2009				
Fungicide[a]	**Timing[b]**	**R5 (% Sev)[c]**	**R7 (% Sev)**	**Relative AUDPC[d]**	**% Disease Control[e]**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**
NTC	…	0.20	6.93	0.0528	0.0	1742	0.02	1.53	0.0159	0.0	N/A	0.06	16.75	0.0281	0.0	1817
PRO	R5	0.16	1.16	0.0176	65.2	2375	0.01	0.22	0.0022	86.3	N/A	0.04	3.90	0.0067	75.7	2066
TEB	R5	0.19	1.05	0.0104	80.1	2372	0.02	0.31	0.0028	82.1	N/A	0.03	5.15	0.0090	68.2	1895
MET	R5	…	…	…	…	…	0.03	0.13	0.0015	90.6	N/A	0.04	3.65	0.0072	74.1	2022
PRO + TEB	R5	0.18	1.05	0.0117	77.8	2484	0.03	0.14	0.0017	89.0	N/A	0.03	3.90	0.0068	75.6	2115
PYR	R5	0.21	0.9	0.0249	51.5	2333	0.02	0.46	0.0068	58.4	N/A	0.06	5.63	0.0094	66.2	1661
AZO	R5	0.19	2.03	0.0302	36.5	2043	0.02	0.43	0.0078	47.8	N/A	0.05	7.35	0.0114	57.7	1910
BOS	R5	…	…	…	…	…	0.03	0.88	0.0078	50.5	N/A	0.06	9.73	0.0158	42.1	1798
LSD (*P* = 0.05)		ns	0.86	0.0141	28.4	316	ns	0.55	0.0033	24.6	N/A	ns	4.21	0.0058	19.6	ns

[a] Fungicides tested at each location: NTC = non-treated control, PRO = prothioconazole (Proline, Bayer CropScience), TEB = tebuconazole (Tebuzol, United Phosphorus Inc.), MET = metconazole (Quash, Valent), PRO + TEB = prothioconazole + tebuconazole (Prosaro, Bayer CropScience), PYR = pyraclostrobin (Headline, BASF), AZO = azoxystrobin (Quadris, Syngenta Crop Protection), and BOS = boscalid (Endura, BASF)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $(t_f - t_0)$ *100, AUDPC = $\sum_{i=1}^{n}((y_i + y_{i+1} / 2)(t_{i+1} - t_i))$ where y*i* = rust severity at the i^{th} observation, t*i* = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100

Table 1.5. Sunflower rust severity, rAUDPC, percent disease control, and yield for normal onset timing trials conducted in 2008.

Treatments		CASS 2008					CREC 2008					LREC 2008				
Fungicide[a]	**Timing[b]**	**R5 (% Sev)[c]**	**R7 (% Sev)**	**Relative AUDPC[d]**	**% Disease Control[e]**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**	**R5 (% Sev)**	**R7 (% Sev)**	**Relative AUDPC**	**% Disease Control**	**Yield (kg/ha)**
NTC	…	0	1.46	0.0710	0.0	2505	1.75	8.73	0.0645	0.0	1613	0.36	0.37	0.0029	0.0	1168
PYR	R3	0	0.03	0.0023	96.4	2570	0.83	4.05	0.0414	35.4	1849	0.51	0.34	0.0034	-22.9	1235
PYR	R5	0	0.75	0.0205	71.6	2563	0.77	1.28	0.0133	78.6	2042	0.11	0.22	0.0015	46.2	1218
PYR	R6	0	0.49	0.0108	85.5	2426	2.65	6.00	0.0365	43.3	1549	0.29	0.36	0.0027	-1.1	988
TEB	R3	0	0.29	0.0152	78.9	2758	…	…	…	…	…	0.11	0.31	0.0020	23.1	1260
TEB	R5	0	0.48	0.0086	88.5	2670	…	…	…	…	…	0.25	0.24	0.0022	19.6	989
TEB	R6	0	0.61	0.0057	92.1	2405	…	…	…	…	…	0.30	0.24	0.0022	17.0	969
PYR	R3, R5, R6	0	0.05	0.0018	97.3	2893	0.37	0.42	0.0042	93.3	2086	0.08	0.34	0.0019	30.2	1154
LSD (P = 0.05)		N/A	0.48	0.0131	5.3	ns	0.98	2.35	0.0139	17.8	329	0.15	ns	0.0011	38.7	ns

[a] Fungicides tested at each location: NTC = non-treated control, PYR = pyraclostrobin (Headline, BASF), and TEB = tebuconazole (Tebuzol, United Phosphorus Inc.)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $\left(t_f - t_0\right)$ *100, AUDPC = $\sum_{i=1}^{n}\left(\left(y_i + y_{i+1} / 2\right)\left(t_{i+1} - t_i\right)\right)$ where yi = rust severity at the i^{th} observation, ti = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100]

Table 1.6. Sunflower rust severity, rAUDPC, percent disease control, and yield for normal onset timing trials conducted in 2009.

Treatments		CREC 2009					GRAN 2009					LREC 2009				
Fungicide[a]	Timing[b]	R5 (% Sev)[c]	R7 (% Sev)	Relative AUDPC[d]	% Disease Control[e]	Yield (kg/ha)	R5 (% Sev)	R7 (% Sev)	Relative AUDPC	% Disease Control	Yield (kg/ha)	R5 (% Sev)	R7 (% Sev)	Relative AUDPC	% Disease Control	Yield (kg/ha)
NTC	…	1.16	12.00	0.0925	0.0	1729	0	0.55	0.0057	0.00	N/A	0.35	22.13	0.0400	0.00	1167
PYR	R3	0.41	4.95	0.0514	44.5	2225	0	0.20	0.0034	34.6	N/A	0.10	12.75	0.0243	28.2	1754
PYR	R5	0.85	1.05	0.0275	69.6	2571	0.01	0.16	0.0024	48.8	N/A	0.03	4.13	0.0072	79.4	1729
PYR	R6	0.86	5.85	0.0443	50.5	2006	0.01	0.79	0.0057	-14.1	N/A	0.07	8.43	0.0141	57.0	1969
TEB	R3	0.71	6.88	0.0577	35.3	1888	0	0.01	0.0006	86.8	N/A	0.13	6.85	0.0123	65.8	1475
TEB	R5	0.86	2.15	0.0224	75.5	2387	0	0.22	0.0021	52.1	N/A	0.26	5.68	0.0126	59.7	1905
TEB	R6	0.95	7.18	0.0450	51.2	1767	0.01	0.65	0.0056	-17.0	N/A	0.08	9.28	0.0167	41.0	1588
TEB, PYR, TEB	R3, R5, R6	0.90	1.03	0.0129	85.5	2692	0	0.01	0.0006	86.4	N/A	0.03	2.63	0.0055	84.0	1675
LSD (P = 0.05)		ns	3.01	0.0189	18.6	252	Ns	0.23	0.0021	38.9	N/A	ns	5.85	0.0102	26.6	ns

[a] Fungicides tested at each location: NTC = non-treated control, PYR = pyraclostrobin (Headline, BASF), and TEB = tebuconazole (Tebuzol, United Phosphorus Inc.)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $(t_f - t_0)$ *100, AUDPC = $\sum_{i=1}^{n}((y_i + y_{i+1} / 2)(t_{i+1} - t_i))$ where yi = rust severity at the i^{th} observation, ti = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100]

control with an R5 application of pyraclostrobin or tebuconazole was statistically the same as three applications of fungicides. Statistical differences in disease control were observed among single application timings in both trials. Five fungicide treatments in CREC 2009 resulted in statistically higher yield than the NTC, and difference in yield among treatments was observed. At GRAN 2009, rust was not effectively controlled by singular applications made at R6.

Fungicide Timing – Early Onset. An early disease onset was not achieved at any locations in 2010; however rust severities were high enough to observe significant differences among treatments (Table 1.7). At CREC 2010, disease control was significantly higher than the NTC when single applications of pyraclostrobin were made. Also, disease control levels were the same with pyraclostrobin applications made at R1, R5, and V8-V12 + R1 + R5. No differences were observed among treatments made up of multiple applications of pyraclostrobin. At GRAN 2010, disease control from single fungicide applications at V8-V12 and R1 was statistically the same as the NTC. At LREC 2010, disease control levels of four fungicide treatments were significantly lower than the NTC. Disease control values were among the highest for treatments containing multiple applications of pyraclostrobin when a R5 timing application was included. However, yield differences were not observed among treatments with multiple applications.

An early disease onset in 2011 did not occur at any location. However, significant differences in disease control were observed among treatments (Table 1.8). At GRAN 2011, disease was not effectively controlled with a single V8-V12 pyraclostrobin application. The highest levels of disease control were achieved with multiple applications of a fungicide, which often included a R5 application. At LREC 2011, rust control was significantly higher for all fungicide applications than the NTC. Levels of disease control and yield were statistically the

Table 1.7. Sunflower rust severity, rAUDPC, percent disease control, and yield for early onset timing trials conducted in 2010.

Treatments		CREC 2010					GRAN 2010					LREC 2010				
Fungicide[a]	Timing[b]	R5 (% Sev)[c]	R7 (% Sev)	Relative AUDPC[d]	% Disease Control[e]	Yield (kg/ha)	R4-R5 (% Sev)	R6-R7 (% Sev)	Relative AUDPC	% Disease Control	Yield (kg/ha)	R4-R5 (% Sev)	R6-R7 (% Sev)	Relative AUDPC	% Disease Control	Yield (kg/ha)
NTC	…	0.52	7.33	0.0226	0.0	N/A	0.10	4.65	0.0113	0.0	N/A	0.41	8.25	0.0202	0.0	1270
PYR	V8-V12	0.14	5.43	0.0127	38.4	N/A	0.16	3.75	0.0095	15.8	N/A	0.43	5.88	0.0165	16.8	1314
PYR	V8-V12, R1	0.10	3.99	0.0096	49.8	N/A	0.02	1.44	0.0034	68.8	N/A	0.19	5.93	0.0165	17.1	1500
PYR	V8-V12, R5	0.16	1.48	0.0052	73.9	N/A	0.14	1.10	0.0032	70.7	N/A	0.33	4.28	0.0119	39.8	1734
PYR	V8-V12, R1, R5	0.04	1.63	0.0049	75.5	N/A	0.01	0.71	0.0017	84.6	N/A	0.16	2.90	0.0086	58.4	1675
PYR	R1	0.13	3.38	0.0082	56.3	N/A	0.04	3.35	0.0080	23.8	N/A	0.25	6.35	0.0153	23.1	1505
PYR	R1, R5	0.08	1.33	0.0051	75.4	N/A	0.02	0.68	0.0017	85.0	N/A	0.52	5.13	0.0161	18.8	1470
PYR	R5	0.30	2.18	0.0082	56.9	N/A	0.21	0.93	0.0032	72.1	N/A	0.40	6.73	0.0171	11.8	1055
TEB	V8-V12	…	…	…	…	…	0.09	4.63	0.0113	0.5	N/A	…	…	…	…	…
TEB	V8-V12, R1	…	…	…	…	…	0.00	0.80	0.0019	82.6	N/A	…	…	…	…	…
TEB	V8-V12, R5	…	…	…	…	…	0.07	0.85	0.0023	79.3	N/A	…	…	…	…	…
TEB	V8-V12, R1, R5	…	…	…	…	…	0.00	0.50	0.0012	89.5	N/A	…	…	…	…	…
TEB	R1	…	…	…	…	…	0.00	0.59	0.0014	87.6	N/A	…	…	…	…	…
TEB	R1, R5	…	…	…	…	…	0.00	0.59	0.0014	87.5	N/A	…	…	…	…	…
TEB	R5	…	…	…	…	…	0.16	1.00	0.0031	72.5	N/A	…	…	…	…	…
LSD (P = 0.05)		0.29	2.24	0.0067	20.0	N/A	0.10	1.15	0.0028	24.5	N/A	0.16	1.43	0.0035	18.1	343

[a] Fungicides tested at each location: NTC = non-treated control, PYR = pyraclostrobin (BASF, BASF), and TEB = tebuconazole (Tebuzol, United Phosphorus Inc.)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $(t_f - t_0)$ *100, AUDPC = $\sum_{i=1}^{n}\left((y_i + y_{i+1} / 2)\ (t_{i+1} - t_i)\right)$ where yi = rust severity at the i^{th} observation, ti = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100]

Table 1.8. Sunflower rust severity, rAUDPC, percent disease control, and yield for early onset timing trials conducted in 2011.

Treatments		GRAN 2011					LREC 2011				
Fungicide[a]	Timing[b]	R5 (% Sev)[c]	R7 (% Sev)	Relative AUDPC[d]	% Disease Control[e]	Yield (kg/ha)	R4-R5 (% Sev)	R6-R7 (% Sev)	Relative AUDPC	% Disease Control	Yield (kg/ha)
NTC	…	0.48	2.85	0.0088	0.0	1777	1.30	3.73	0.0091	0.0	1211
PYR	V8-V12	0.29	2.98	0.0081	3.2	1828	0.57	2.20	0.0047	41.3	1719
PYR	V8-V12, R1	0.03	1.35	0.0032	50.0	2351	0.25	1.53	0.0028	67.5	2052
PYR	V8-V12, R5	0.08	0.74	0.0020	65.2	2120	1.04	1.55	0.0054	37.9	1534
PYR	V8-V12, R1, R5	0.01	0.70	0.0016	75.6	2624	0.18	0.64	0.0014	82.1	2455
PYR	R1	0.09	1.96	0.0049	32.7	2108	0.46	2.43	0.0046	42.4	1866
PYR	R1, R5	0.02	0.74	0.0018	71.2	2521	0.40	0.86	0.0024	71.6	2389
PYR	R5	0.13	0.95	0.0028	58.6	1681	1.40	1.65	0.0067	29.3	1319
TEB	V8-V12	0.18	1.73	0.0047	25.8	1890	…	…	…	…	…
TEB	V8-V12, R1	0.04	0.94	0.0023	63.0	1991	…	…	…	…	…
TEB	V8-V12, R5	0.26	0.51	0.0025	60.0	2229	…	…	…	…	…
TEB	V8-V12, R1, R5	0.03	0.51	0.0013	81.5	2197	…	…	…	…	…
TEB	R1	0.12	0.94	0.0027	56.7	2067	…	…	…	…	…
TEB	R1, R5	0.08	0.41	0.0013	78.0	2235	…	…	…	…	…
TEB	R5	0.54	0.64	0.0042	41.8	1994	…	…	…	…	…
LSD (P = 0.05)		0.23	1.00	0.0031	22.6	411	0.74	0.83	0.0030	18.9	525

[a] Fungicides tested at each location: NTC = non-treated control, PYR = pyraclostrobin (BASF, BASF), and TEB = tebuconazole (Tebuzol, United Phosphorus Inc.)

[b] Timing of fungicide application according to sunflower growth stages defined by Schneiter et al., 1998

[c] % Sev = mean severity on upper four leaves of sunflower using assessment diagrams (Friskop et al., 2011)

[d] Relative AUDPC = AUDPC / $(t_f - t_0)$ *100, AUDPC = $\sum_{i=1}^{n}\left((y_i + y_{i+1} / 2)\ (t_{i+1} - t_i)\right)$ where yi = rust severity at the i^{th} observation, ti = time (days) at the i^{th} observation, and n = total number of observations.

[e] % Disease control = [((1 – (rAUDPC of a treatment / rAUDPC of the NTC)) * 100]

same among multiple pyraclostrobin applications at V8-V12 and R1, R1 and R5, and at all three timings.

Yield Loss Assessment. To determine the impact of rust on yield, trials CREC 2008-Normal Onset, CREC 2009 Efficacy, CREC 2009-Normal Onset, and LREC 2010 were selected for analysis because R7 severity values on the NTC were greater than 5% and significant differences were observed in yields. A negative correlation of r = -0.7756 was found between percent change in yield from the NTC and fungicide treatment at R7 severity values (Figure 1.1). Specifically, for every 1% increase in severity at R7, yields decreased by 6.6%.

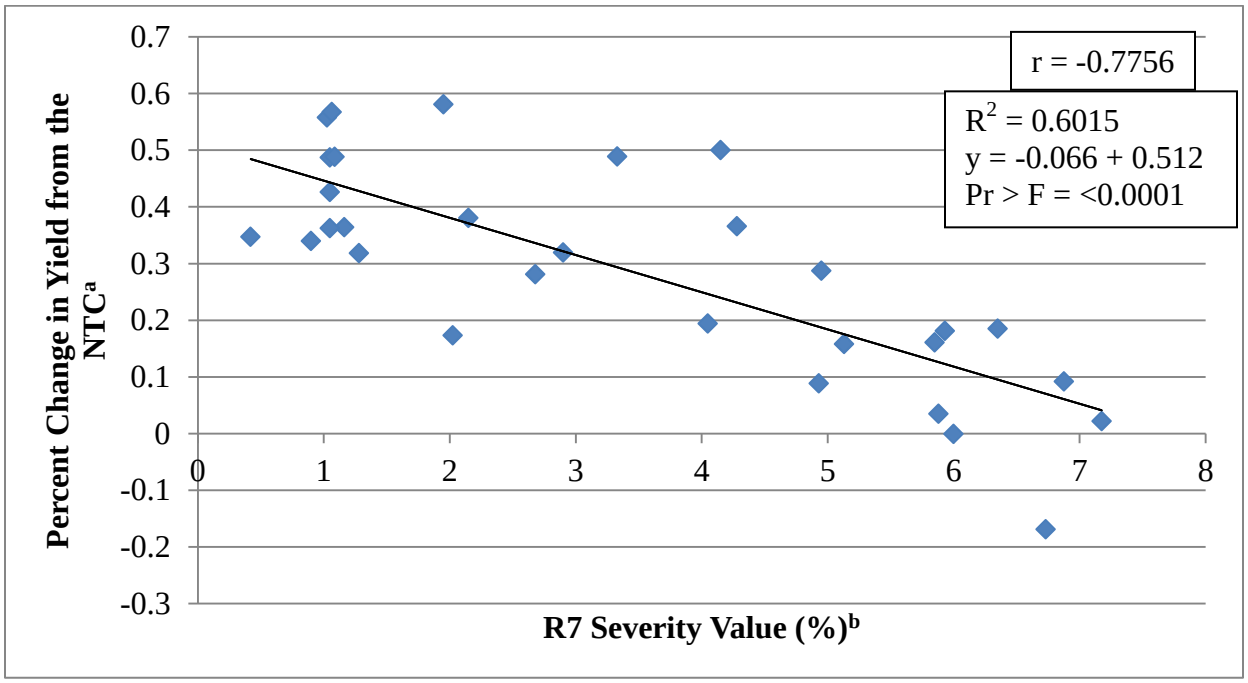

Figure 1.1. Yield loss assessment of R7 severity values on yield loss.

[a] Percent change in yield from NTC = (fungicide treatment mean yield – NTC mean yield) / NTC mean yield
[b] R7 severity value = mean rust severity on upper four leaves according to rust assessment diagrams (Friskop et al., 2012) during R7 growth stage (Scheiter et al., 1998)

Discussion

Results from this study demonstrated that fungicide applications can reduce sunflower rust severity, and with few exceptions, DMIs and QoIs reduced disease greater than SDHIs. Results also demonstrated management of rust depended greatly on timing, and an R5 application, which

typically corresponded to a severity value of 1% or less, was more efficacious for reduction of disease severity than any other timing.

In most trials, levels of disease control were statistically the same among QoI and DMI fungicide treatments. Few differences in disease control among the three fungicides labeled for sunflower rust in the United States (azoxystrobin, pyraclostrobin, and tebuconazole) were observed. However, when differences were observed, disease control from tebuconazole applications was higher than pyraclostrobin or azoxystrobin applications. Fungicides not labeled for sunflowers generally controlled rust as well as labeled fungicides in their respective FRAC groups.

Fungicide timing proved to be a critical component in disease management. Of all single timings evaluated throughout this study, disease reductions appeared most frequently with a single application at R5. Applications at earlier or later growth stages tended to control disease less than applications made at R5. This could be because applications made earlier than R5 may have not provided enough protection to the top leaves prior to leaf expansion, or that the duration of protection was inadequate for the length of growing season remaining. Conversely, an application later than R5 may have had a more limited impact on disease control because a high severity had been reached by the time of application. In four trials, disease control from a single R5 fungicide application reduced rust as much as three applications (CREC 2008-Normal Onset, CREC 2009-Normal Onset, GRAN 2009-Normal Onset, and LREC 2009-Normal Onset). With the exception of GRAN 2009, it is notable that these trials had the highest R7 rust severities of the six 2008 and 2009 timing trials. This indicates that reduction of rust with a single application was as effective as three applications, under the highest disease pressure situations.

Although one objective of the research project was to evaluate fungicide application timing in and 'early onset' disease scenario, rust did not develop early enough to simulate an 'early onset' epidemic. However, some trends were noticed. Based on disease control values, single applications made at V8-V12 do not provide satisfactory disease management and multiple applications of a fungicide including a R5 application often were among the highest for disease control. Prophylactic fungicide applications for yield gain independent of disease control and have been suggested on sunflower at the R1 growth states. Trials established in 2010 and 2011 included a R1 application, but no correlation between single R1 applications and yield were observed.

Yield data in this study was limited, largely because sunflowers are sensitive to a variety of yield-limiting and hard to control problems such as insects, other diseases, bird damage, and lodging. Further, many of these other yield limiting factors tend to be highly localized in edges or 'hot-spots' of plots (i.e. bird damage, sunflower midge, lodging), which limits the impact of statistically design. This is a common problem with sunflower trials for many pests, and consequently, very little yield loss data under rust pressure exists in the literature. However, statistically different yields were observed in several trials, and a negative correlation ($r = -0.7756$) was observed between R7 severity values and percent change in yield from the NTC. Also, it was found that for every 1% increase in severity, yield is reduced by 6.6%. This supports the importance of protecting the upper-four leaves at R5 to reduce the disease severity that may occur at R7.

Based on previous work, an action threshold of 3% severity was recommended to reduce disease pressure and limit yield loss (Shtienberg, 1995). Data from these studies indicate that an application at a lower disease severity is more appropriate, but choice of DMI or QoI product

infrequently matters. As a result of these studies, a threshold of 1% disease severity on the upper four leaves, when occurring at or before R5 has been suggested and widely accepted in the US by industry and producers in North Dakota.

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CHAPTER TWO: PHENOTYPIC DIVERSITY OF *PUCCINIA HELIANTHI* (SUNFLOWER RUST) IN THE UNITED STATES FROM 2011 AND 2012

Introduction

Sunflower rust, caused by *Puccinia helianthi* Schwein., occurs on cultivated and volunteer sunflowers as well as wild *Helianthus* species in North America (Gulya et al., 1997). *Puccinia helianthi* is an autoecious and macrocyclic rust, and it is presumed that the pathogen is native to North America (Gulya et al. 1997). An increase in sunflower rust incidence and severity has been observed from 2007 to 2011 in North Dakota (Kandel, 2012), and most recent data suggest that over 50% North Dakota production fields have had some level of rust in recent years. Yield loss potential is very high, and a near total yield and quality loss was reported in North Dakota in 2008 (Markell et al., 2009). Widespread sexual events of *P. helianthi* have been recently documented in North Dakota and Nebraska in 2008 (Markell et al., 2009; Harveson, 2010). The occurrence of the sexual cycle may lead to an increase in incidence and severity of the disease (Putt and Sackston, 1957; Sendall et al., 2006), which may also explain the increase in recent sunflower rust epidemics in the Northern Great Plains.

Rust can be managed with genetic resistance, and several resistance genes have been identified (Sendall et al., 2006; Qi et al., 2011). However, periodic evaluations of rust resistance in sunflower hybrids in the United States indicate that a majority of them are susceptible to the *P. helianthi* population (Friskop et al., 2011; Gulya, 2006; Gulya and Viranyi, 1994). For resistance to be effective, information from both the host and pathogen are needed. Information about the pathogen can be determined by virulence phenotype. As described by Hovmoller et al. (2008) is the phenotypic data from the presence of virulence and/or avirulence genes in the pathogen. For *P. helianthi*, virulence phenotype is determined on a set of nine internationally accepted

differentials: susceptible hybrid, MC90, MC29, P386, HA-R1, HA-R2, HA-R3, HA-R4, and HA-R5 (Gulya and Masirevic, 1996). Differential MC90 contains the R_1 gene, while differential MC29 is the only line with two resistance genes R_2 and R_{10} (Sendall et al., 2006). Differentials P386, HA-R1, HA-R3, HA-R4, and HA-R5 possess alleles of the R_4 gene (Sendall et al., 2006). The R_5 resistance gene is found in the differential HA-R2 (Sendall et al., 2006). Once a virulence phenotype is obtained on a set of differentials, race nomenclature is used to describe the pattern of resistance and susceptibility in the host (Hovmoller et al., 2011). Race nomenclature for sunflower rust uses a numerical triplet coding system (Gulya and Masirevic, 1996). Differentials are arranged into three sets of three differentials. Within each set, an additive value is assigned to each differential and the aggregate virulence phenotype from all differentials is converted to a three digit race name (Gulya and Masirevic, 1996).

Phenotypic diversity of a rust population is often examined in one of two ways. First, virulence phenotypes can be assessed from bulk pathogen collections. This information can be obtained quickly, and can provide a useful snapshot of aggregate virulence in a population. This may be particularity interesting for breeders who are attempting to incorporate resistance to all known races. A second way to examine phenotypic diversity is to determine virulence phenotypes from single-pustule isolates of the pathogen. Single pustule isolates are clonal, and determination of virulence phenotypes from them provides information about virulence diversity of individuals within a population and allows scientists to make assessments about virulence combinations within the pathogen. This method is more robust generates data on the actual diversity of the pathogen population, and has been extensively used in other rust species (Kolmer, 1999; Markell and Milus, 2008; Kolmer and Liu, 1999). While infrequent surveys of the virulence phenotype of *P. helianthi* from bulk collections have been conducted in the United

States (Gulya, 1990; Gulya and Viranyi, 1994; Gulya, 2006; Gulya and Markell, 2009), virulence diversity using single pustule isolates has not been done. Virulence phenotype information of *P. helianthi* from bulk collections have demonstrated that phenotypic variation exists in the pathogen population (Gulya, 1990; Gulya and Viranyi, 1994; Gulya, 2006; Gulya and Markell, 2009). Survey data from the 1990's, and 2000's identified more than two dozen virulence phenotypes from bulk collections, however, the amount of virulence phenotypes that were present in one bulk sample is not known. Furthermore, it is unclear if all individuals in the population had the same virulence pattern, or if many different patterns exist in the population.

Recent work suggested that evolution of *P. helianthi* phenotypes could be attributed to the frequency of sexual recombination occurring in the pathogen's life cycle (Sendall et al., 2006). Prior to 2008, completion of the sexual cycle had not been confirmed in North Dakota (Markell et al., 2009). In 2008, the widespread occurrence of aecia was observed throughout the sunflower growing areas in Minnesota and North Dakota. Further, aecia were observed from early vegetative stages until the reproductive stages of the host (Markell et al., 2009). Since that occurrence, evidence of completion of the sexual cycle (presence of aecia) has been annual and widespread throughout the region (S. Markell, *personal communication*). Frequent sexual recombination can rapidly lead to the development of new virulence combinations in the pathogen population. A complete lack of virulence phenotype data obtained from single pustule isolates presents a critical need for assessment of pathogen diversity. The objective of this study was to assess the phenotypic diversity of *P. helianthi* from the United States using single pustule isolates.

Materials and Methods

2011 Collections. A week-long trip to collect *P. helianthi* samples was completed during the last week of August in North Dakota. The survey route covered approximately 1000 miles and stops were made periodically at both wild populations and cultivated fields. At each sampling site, a "W" pattern was walked, and field derived single-pustule isolates were collected. Field-derived single pustule isolates are defined as pustules that were distinctly isolated on a sunflower leaf. In North Dakota, two to twenty field-derived single pustule isolates were obtained from thirty-seven locations.

Nine bulk collections from nine discrete locations in Nebraska (Robert Harveson, University of Nebraska-Lincoln Panhandle Research and Extension Center), and one bulk sample from one location was received from South Dakota (Seeds 2000) were received from collaborators. To obtain single pustules isolates from bulk collections, bulk samples were inoculated (below) onto 14 day old plants of the susceptible sunflower hybrid 'Jaguar' (Seeds 2000) in the greenhouse). Approximately 14 days later, individual uredinia pustules were collected separately. Two to three single pustules from each bulk sample were randomly selected to represent diversity at that location. In 2011, a total of 129 single-pustule isolates from 47 locations were increased for assessment of virulence phenotypes (Table 2.1, Figure 2.1).

2012 Collections. Rust samples from wild and cultivated sunflowers were obtained from survey trips, industry collaborators, and USDA-ARS personnel. Field derived single pustule isolates were collected during two rust survey trips in North Dakota, using the same sampling procedures in 2011. The first survey conducted in July covered the north east and north central portion of ND, and the second survey trip was conducted in the central and western half of ND. Bulk collections from California (Suzanne Latham, California Department of Food and

Figure 2.1. *Puccinia helianthi* sampling locations in 2011 and 2012, by host.

Table 2.1. *Puccinia helianthi* sampling locations, number of isolates collected, and number of races identified from sunflower host types and geographic locations in 2011.

Host-type	Number	NE[a]	ND[b]	SD[c]
Oil	Locations[d]	0	27	0
	Isolates[e]	0	79	0
	Races[f]	0	13	0
Confection	Locations	0	4	1
	Isolates	0	12	3
	Races	0	6	2
Wild	Locations	0	2	0
	Isolates	0	2	0
	Races	0	1	0
Not Known Cultivated	Locations	9	1	0
	Isolates	20	2	0
	Races	6	2	0

[a] NE = Nebraska
[b] ND = North Dakota
[c] SD = South Dakota
[d] Locations = number of locations *P. helianthi* isolates were collected from, with respect to host-type and state
[e] Isolates = number of *P. helianthi* isolates collected, with respect to host-type and state
[f] Races = number of races detected according to virulence phenotypes of *P. helianthi* isolates, with respect to host-type and state

Agriculture and Nick Oliver, Sutter County Department of Agriculture), Manitoba -Canada (Claire Kincaid, National Sunflower Association of Canada), Iowa (Charles Block, USDA-ARS Plan Introduction Station), Minnesota (Tom Gulya, USDA-ARS Sunflower and Plant Biology Research Unit), Nebraska (Tom Gulya), South Dakota (Tom Gulya), and Texas (Joseph Legako, Triumph Seed Company) were sent in from collaborators, and single pustule isolates were generated as previously described. Up to six single pustule isolates were selected from each

location. An additional rust survey trip was conducted in sunflower production areas in Vermont (Heather Darby and Hannah Harwood, University of Vermont), but rust was not detected in any of the eleven fields surveyed. In 2012, a total of 109 single pustule isolates from 56 locations were increased (described below) for virulence phenotype assessment (Table 2.2, Figure 2.1).

Table 2.2. *Puccinia helianthi* sampling locations, number of isolates collected, and number of races identified from sunflower host types and geographic locations in 2012.

Host	Number	CA[a]	IA[b]	MN[c]	NE[d]	ND[e]	SD[f]	TX[g]
Oil	Locations[h]	0	0	0	0	9	0	0
	Isolates[i]	0	0	0	0	29	0	0
	Races[j]	0	0	0	0	12	0	0
Confection	Locations	0	0	2	0	6	0	0
	Isolates	0	0	6	0	19	0	0
	Races	0	0	3	0	10	0	0
Wild	Locations	0	3	2	2	2	0	0
	Isolates	0	5	4	3	2	0	0
	Races	0	3	3	3	1	0	0
Not Known Cultivated	Locations	7	0	5	4	3	7	1
	Isolates	9	0	8	5	4	9	2
	Races	6	0	7	4	4	7	2

[a] CA = California
[b] IA = Iowa
[c] MN = Minnesota
[d] NE = Nebraska
[e] ND = North Dakota
[f] SD = South Dakota
[g] TX = Texas
[h] Locations = number of locations *P. helianthi* isolates were collected from, with respect to host-type and state
[i] Isolates = number of *P. helianthi* isolates collected, with respect to host-type and state
[j] Races = number of races detected according to virulence phenotypes of *P. helianthi* isolates, with respect to host-type and state

Isolate Inoculation and Urediniospore Increase. The susceptible confection hybrid 'Jaguar' (Seeds 2000) was planted in 7.62 cm cone-tainers filled with potting soil (Sunshine mix, SunGro Horticulture Distribution Inc., Bellevue, WA). After 14-days, single pustule isolates were inoculated onto the first true leaves by suspending urediniospores in Soltrol 170 (ConocoPhillips Inc., Houston) at approximately 275,000 spores/mL and sprayed onto susceptible plants. The oil suspension was allowed to dry for 30-40 minutes then placed into misting chambers for 18-20 hours at 22 ± 2°C with a photoperiod of 16 hours, then moved to a greenhouse at 22 ± 2°C with a photoperiod of 16 hours. Light was supplemented by a 400 watt halogen bulb (Phillips, Royal Philips of the Netherlands) using the P. L light systems model PL2000 HPS Super (P. L. Light Systems, Beamsville, ON, Canada). Urediniospore collections were made 14, 16, and 18 days after inoculation. Collected urediniospores were desiccated, then stored temporarily in a refrigerator (4°C) or placed in long term storage in a cryofreezer at -80°C).

Phenotypic Evaluation. Virulence phenotypes were evaluated on the set of nine internationally accepted differentials (Table 2.3) (Gulya and Masirevic, 1996). To evaluate virulence phenotype, each isolate was inoculated onto three plants of each differential and repeated. Differentials were planted in 4 x 9 cell packs (T & O Plastics, St. Paul, MN) using Sunshine mix as a growth medium. Plants were grown in a greenhouse with diurnal temperatures of 24°C and 20°C 16 hour photoperiod. After 14 days, fresh urediniospores were inoculated on the first set of true leaves using methods described above. Infection type was evaluated 13-15 days post-inoculation according to a modified 0-5 scale from Yang et al., (1986): 0 = immune, ; = flecks, 1 = pustules smaller than 0.2 mm, 2 = pustules 0.2-0.4 mm, 3 = pustules 0.4-0.6 mm, 4

= 0.6-0.8, and 5 = pustules larger than 0.8mm. Infection types 3 or greater were considered to be a susceptible reaction.

Table 2.3. Sunflower lines used as rust differentials, resistance gene(s) or alleles, and scoring values.

	Differential	**Resistance Gene/Alleles**	**Scoring Value[a]**
Set 1	7350		1
	MC90	R_1	2
	MC29	$R_2 + R_{10}$	4
Set 2	P386	R_{4e}	1
	HA-R1	R_{4a}	2
	HA-R2	R_5	4
Set 3	HA-R3	R_{4b}	1
	HA-R4	R_{4c}	2
	HA-R5	R_{4d}	4

[a] Scoring value is the numerical value associated with virulence on a specific differential. The additive score for each set is the digit in the three digit race name.

Results

2011 Collections. Seventeen races were detected from the 129 single pustule isolates tested. Races 300 and 304 comprised 69.7% of the tested isolates (Figure 2.2). The least virulent race detected was race 300, which confers virulence to only two of the nine differentials (including the susceptible). The most virulent race detected was 776, which is virulent on eight of the nine differentials. Race 776 was detected only once in 2011, originating from a north-central confectionary field in North Dakota. Multiple races were identified from approximately 77% of the locations sampled. For example, three races were characterized from three isolates at 10 locations in North Dakota, and at one location in Nebraska. Few differences existed between race and sunflower host type. Race 736 was the only race identified from a wild population that

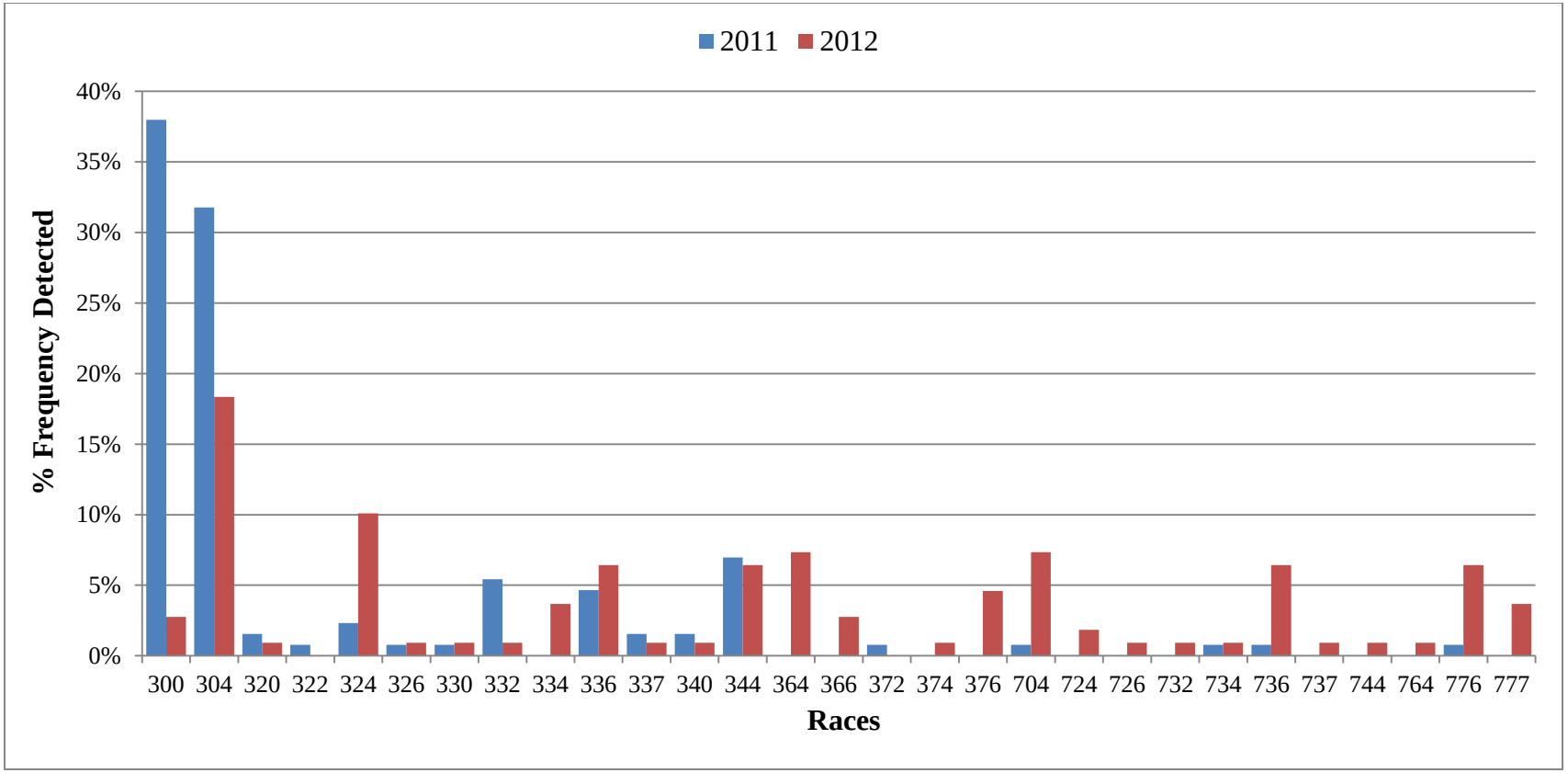

Figure 2.2. Frequency of *Puccinia helianthi* races detected in 2011 and 2012.

was not identified from cultivated sunflowers, whereas race 776 was only detected on a confection hybrid. Numerous races were detected from oil hybrids that were not represented in either confections or wilds.

Resistance gene R_{4b} found in the differential line HA-R3, conferred resistance to 98.4% of the isolates evaluated in 2011 (Figure 2.3). Resistance genes R_2 and R_{10} found in differential line MC29 and R_5 found in HA-R2 conferred resistance to 96.9% and 89.9% of the isolates, respectively. The resistance genes R_1 did not confer any resistance to any of the isolates tested, and resistance gene R_{4d} conferred resistance to only 48.8% of the isolates.

2012 Collections. A total of 27 races were detected from 109 single pustule isolates evaluated in 2012. The most common race detected was 304, which was found in five out of the seven states sampled and comprised over 18% of the total collection (Figure 2.2). Races 324, 364 and 704 were detected 10.1%, 7.3%, and 7.3% of the time respectively. The least virulent race detected was 300, the same race least detected in 2011. The most virulent race detected was 777, which is virulent on all nine differentials. Race 777 was detected at four locations in Nebraska, North Dakota and Manitoba, Canada.

The resistance gene R_{4b}, found in differential line HA-R3, conferred resistance to 94.5% of the isolates tested (Figure 2.3). Resistance genes R_2, R_{10}, and R_5 found in differential lines MC29 and HA-R2, conferred resistance to 68.3% and 65.1% of the tested isolates, respectively. Resistance genes $R4_d$ and $R4_a$ found in lines HA-R5 and HA-R1, conferred resistance to only 7.3% and 50.5% of the tested isolates, respectively. At locations where multiple single-pustules were collected, multiple races were recovered at 81% of them. In one location, six different races were detected from six different isolates all originating from the same location (ND12_18).

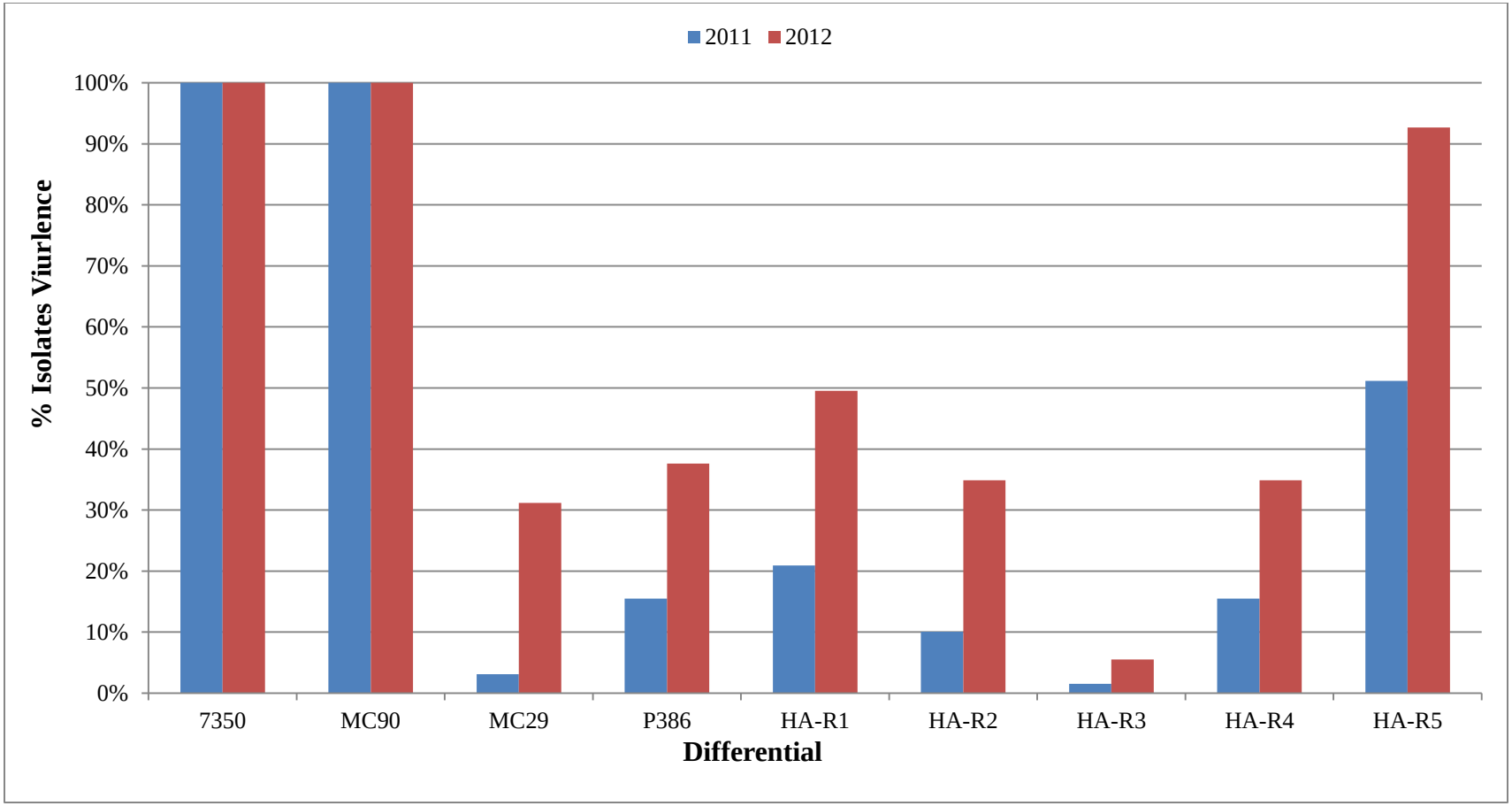

Figure 2.3. Percent virulence on the nine internationally accepted sunflower rust differentials from all *Puccinia helianthi* isolates evaluated in 2011 and 2012.

Virulence Phenotypes across Year, Host, and Geography. A total of 29 races were detected collectively in 2011 and 2012 (Figure 2.2). Two rust races detected in 2011 were not detected in 2012, while 12 races detected in 2012 were not detected in 2011 (Figure 2.2). Race 304 was the most common race identified in both years, followed by race 300 in 2011 and race 324 in 2012. Race 300 was only found 2.8% of the time in 2012 and race 324 was only found 2.3% of the time in 2011.

All isolates used this study were virulent on differentials 7350 and CM90 (Figure 2.2), and limited variation of virulence among states and hosts was found. Virulence to all differentials was found in pathogen collections from only North Dakota and California, while isolates from Iowa conferred virulence to only five differentials (Figure 2.4). Detection frequency of virulence on differentials was similar among host type (Figure 2.4). HA-R3 was most commonly resistant to *P. helianthi* isolates, regardless of geography or host (Figures 2.3, 2.4, 2.5)

Discussion

To the best of our knowledge, this is the first race survey done with single pustule isolates on *P. helianthi* in the US. Many different races were detected throughout the study, and differences between year and among host type and geography were examined. The geographic scope of this study was sunflower production areas in the United States, but the focus was in North Dakota.

Frequency of detection of some races in the population varied greatly between years. For example, race 300 was detected approximately 38% of the time in 2011, but was only apparent in approximately 3% of the tested isolates in 2012. Similarly, race 776 was detected six times in 2012 compared to being detected once in 2011. Many reasons for these contrasts may exist.

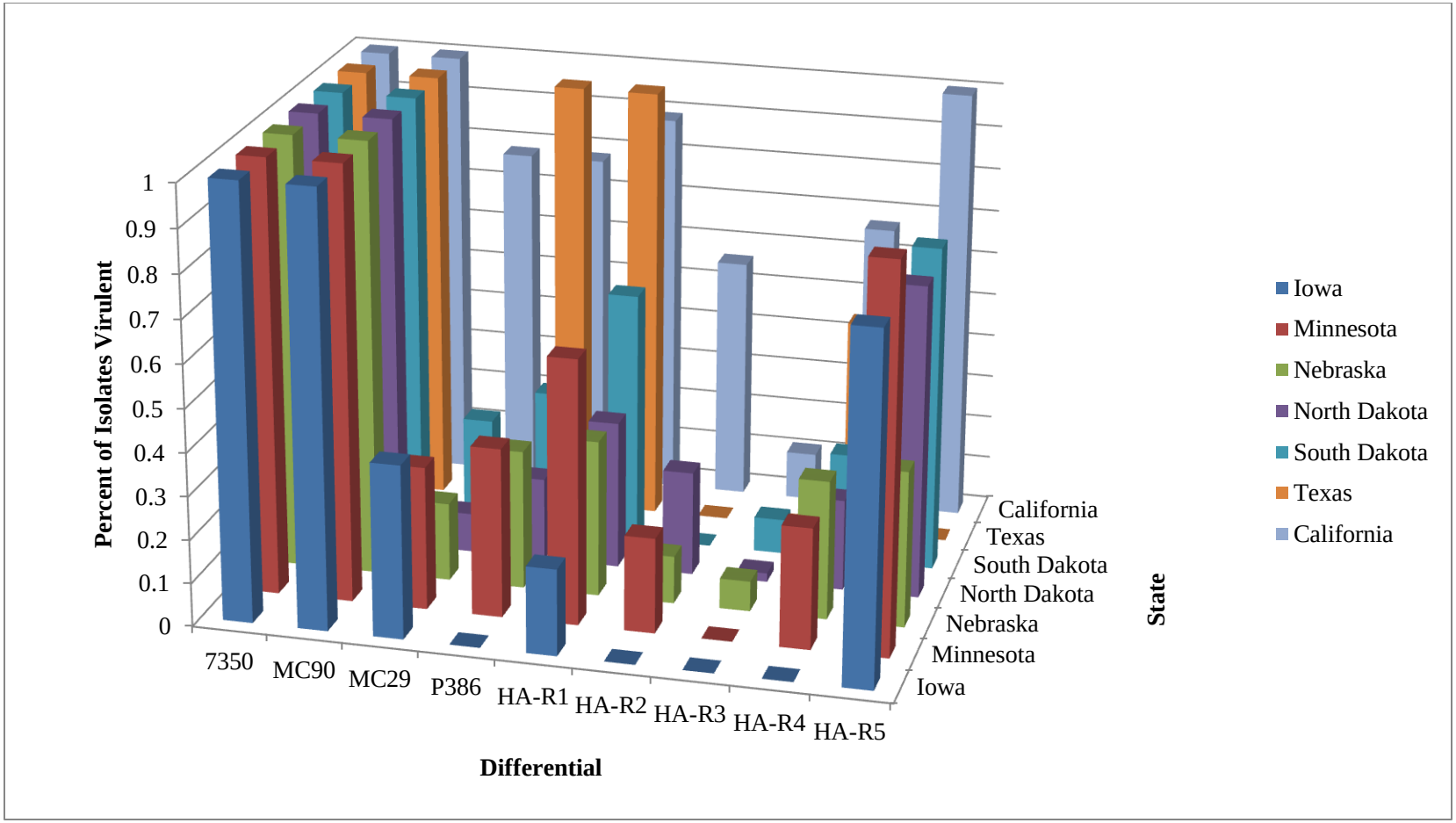

Figure 2.4. Percent of isolates virulent on each of the nine internationally accepted differentials, by state.

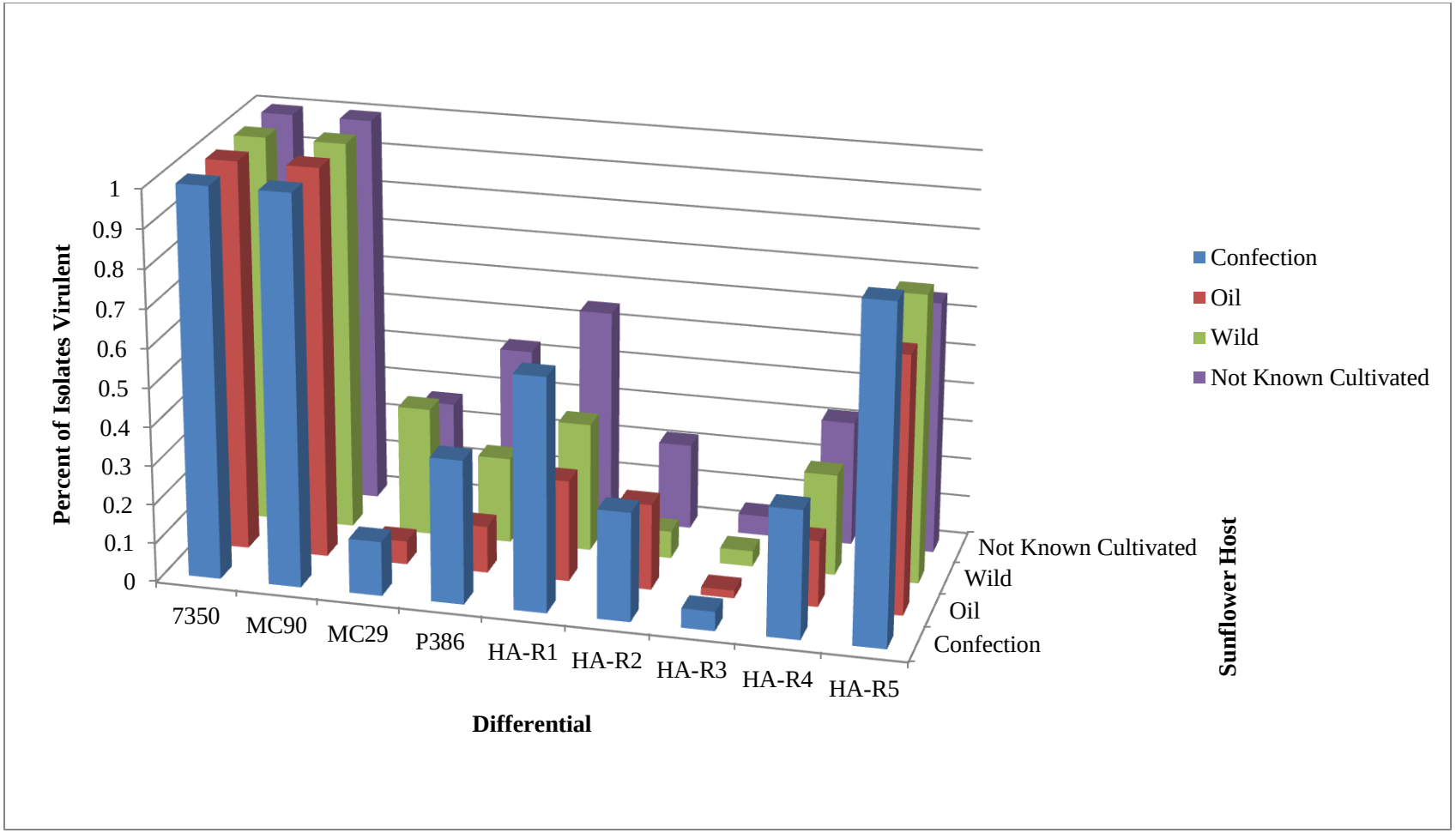

Figure 2.5. Percent of isolates virulent on each of the nine internationally accepted differentials, by host.

First, the sampling areas between 2011 and 2012 were different, and the expansion into new geographic areas could be a reason for differences in detection frequency. In 2011, a majority of the isolates collected were from North Dakota and to a lesser extent from Nebraska and South Dakota. In 2012, the sampling area covered the states of California, Iowa, Minnesota, Nebraska, North Dakota, South Dakota, and Texas. Secondly, it is possible that differences in virulence phenotypes of the *P. helianthi* population might differ at time of collection and level of disease severity in the growing season. In 2012, North Dakota surveys were conducted in July and in September and rust was limited in both incidence and severity at both times. In 2011, a North Dakota survey was conducted at the end of July only, and incidence and severity were much higher than in 2012. It is possible that a very high level of infection may have followed a high level of sexual recombination, and more races were detected.

Although a limited number of isolates were collected from most states, isolates from California generally appeared to be more virulent than isolates collected other states. Most cultivated fields in California are used primarily for seed production. With the diverse amount of host genetics, selection pressure to many resistance genes may be high and facilitate generation of more virulent races. Surveys could provide a better understand of virulence using intense sampling methods from California seed production fields (Jochua et al., 2007). No virulence differences were found for isolates collected from different hosts. This implies that the *P. helianthi* population may be consistent between wilds and cultivated sunflowers.

Numerous isolates were characterized from North Dakota in 2011 and 2012. Even though 21 races were detected collectively, only eight races (304, 324, 326, 336, 344, 704, 736, and 776) were found in both years. Race 300 was detected from 35 North Dakota isolates in 2011, yet it wasn't detected in 2012. One explanation for the differences in races could be to sampling

location. The 2011 North Dakota survey covered the central and north central portions of the state, whereas the 2012 North Dakota survey focused on the west and southwest portion of the state. Also, no location sampled in 2011 was sampled again in 2012, due to crop rotation and the destruction of wild populations. To test the recombination ability of the pathogen, a single location could be sampled for consecutive years to obtain both phenotype and genotype data of the *P. helianthi* population present.

Previous sunflower rust race surveys indicated that the most bulk virulence phenotypes coded to races 336 and 337. In this study, races 336 and 337 were found in low frequency. However, the identification of multiple races being reported in the same field supports the idea that virulence phenotypes from bulk collections may confer virulence to more differentials than individual collections. Notably, when six different single-pustule isolates were derived from the bulk sample ND12_18, six different races were detected. This indicates that phenotypic richness of *P. helianthi* can be high from within a field, and is consistent with other autoecious and macrocyclic rusts such as dry bean rust (Jochua et al., 2007).

Five genetic loci are represented in the differential set. However, multiple alleles of the R4 gene are present in the lines P386, HA-R1, HA-R3, HA-R4, and HA-R5. The results of the survey indicated that a majority of the *P. helianthi* isolates were avirulent on HA-R3 (R_{4b}) and is overwhelmingly more effective than the other R_4 alleles. Additionally, compared to the other differentials, the genes in MC29 (R_2 + R_{10}) and HA-R2 (R_5), conferred resistance to 17.2% and 22.8% of the isolates, respectively. From a breeding perspective, these findings are important. The genes in these lines would be able to be stacked for gene pyramiding because four different genetic loci are present.

Avenues for expansion for *P. helianthi* are numerous. To our knowledge, no study has been completed using the phenotypic and genotypic data exclusively from North America. Also, due to the sunflower plant's architecture, studies on rust location in the canopy could be done to evaluate the pathogen diversity during the growing season. Newer molecular methods exist that can be used for characterization of genetic pathogen diversity (Brueggeman et al., 2013). Evaluations of *P. helianthi* virulence from growing regions in other parts of the world could provide critical information to sunflower breeders in the US and abroad.

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CHAPTER THREE: EVALUATION AND IDENTIFICATION OF SUNFLOWER RUST RESISTANCE (*PUCCINIA HELIANTHI*) IN THE CORE-SET OF *HELIANTHUS ANNUUS* GERMPLASM

Introduction

Sunflower rust, caused by *Puccinia helianthi* Schwein., is an economically important disease in North Dakota and other sunflower producing states in the United States. Yield losses associated with this disease can approach 80% in a given year (Markell et al., 2009). Recently, an increase in incidence and severity has been documented in North Dakota, which leads the U.S. annually in planted acreage of the crop (Kandel, 2012). This may be due in part to the lack of resistance in the majority of commercial hybrids produced (Friskop et al., 2011a). Also, frequent sexual recombination can facilitate an earlier onset of the disease and increase the likelihood of race changes (Markell et al., 2009). As a result of increased incidence and severity and a genetically variable pathogen, additional sources of rust resistance are needed to help prevent yield loss (Hulke et al., 2010). With limited rust resistance available in commercially available hybrids, new sources of rust resistance may provide an important management tool for years to come (Friskop et al., 2011a).

Two types of sunflower are commercially grown, and differ primarily in use. Oilseed sunflowers account for approximately 80% of the acreage in North Dakota and are primarily used for cooking oil (NASS, 2012). Confectionary sunflowers are used for snacks and baking products. Confectionary hybrids tend to be more susceptible to rust than oil hybrids. This could be a consequence of breeding efforts, which for confection germplasm have focused primarily on agronomic and market traits such as seed size and quality. Also, there is limited data elucidating the impact rust has on quality characteristics (Lilleboe, 2012).

Screening sunflower germplasm for rust resistance is a way to potentially identify novel sources of resistance. In rust pathosystems, resistance has been categorized into vertical and horizontal depending on the number of genes needed to confer resistance; race specific or non-race specific, depending on the spectrum of races to which they are effective; and seedling (all stage) or adult plant, depending on the stage of the host in which resistance is effective (Kolmer, 1996; Mmbaga et al., 1996). Major gene (vertical) resistance is a commonly used resistance against rust pathogens. However, when only one gene is incorporated, the pathogen can quickly overcome this type of resistance. Minor gene (horizontal) resistance is comprised of multiple minor genes for resistance and is often considered to be more durable (Agrios, 2005; Kolmer and Liu, 2001; and Singh et al., 2011). Most of the rust resistance genes that have been categorized in sunflower are major genes (Qi et al., 2011; Sendall et al., 2006).

Germplasm screening has been conducted as a way to identify novel sources of resistance for other important sunflower pathogens, including downy mildew (*Plasmopara halstedii*) and sclerotinia head rot (*Sclerotinia sclerotiorum*) (Block et al., 2012; Gulya and Hulke, 2010; Hulke et al., 2010). Similarly, rust resistant germplasm has been derived from plant introduction (PI) lines. Notably, rust resistance detected in PI 650362 and PI 432512 have been incorporated into USDA germplasm HAR 6 and HAR 8, respectively (Miller and Gulya, 2001). Additionally, in other rust pathosystems, such as soybean rust (*Phakopsora pachyrhizi*), wheat stem rust (*Puccinia graminis* f.sp. *tritici*), and dry bean rust (*Uromyces appendiculatus*), novel sources of resistance have been identified by screening accessions lines (Acevedo et al., 2013; Newcomb et al., 2013; Rouse et al., 2011; Twizeyimana et al., 2008).

The USDA-North Central Regional Plant Introduction Station at Ames, IA, houses the national *Helianthus* germplasm collection, which as of 2012, consists of over 1800 cultivated

sunflower accessions and over 2200 wild accessions of wild *Helianthus* species (Marek et al., 2012). The cultivated accessions originate from 54 counties, with the largest percentage of collections coming from United States and Russia. The collection consists of old land races, open-pollinated varieties, lines at varying stages of inbreeding, and some fixed homozygous inbred lines. Due to the large collection of accessions, a statistically representative core set was created (Brothers and Miller, 1999). The statistical core subset was created using 20 variables, including country of origin, morphological characteristics, disease resistance, and insect resistance. The resultant core-subset is comprised of 112 accessions representing 39 countries (Brothers and Miller, 1999). To our knowledge, the core-set of *Helianthus annuus* germplasm has not been screened for rust resistance. The objective of this study is to evaluate the core subset of *H. annuus* germplasm for rust resistance.

Materials and Methods

Plant Material. Seed for the 112 PI core subset were obtained from the USDA-North Central Regional Plant Introduction Station in Ames, IA. Additionally, the internationally accepted set of nine sunflower rust differentials (Gulya and Masirevic, 1996) and nine USDA lines (RHA 397, HA-R6, HA-R8, RHA 464, Rf ANN-1742, PH3, PH4, PH5, and TX16) previously reported to be rust resistant were obtained (Qi et al., 2011) (Table 3.1).

Pathogen Material. Sunflower lines were screened individually to five *P. helianthi* isolates obtained from North Dakota in 2011 and 2012 coding to races 300, 304, 336, 337, and 777. *Puccinia helianthi* races 300 and 304 were selected because they were the most common races identified in a 2011 and 2012 survey (Chapter 2). Races 336 and 337 were selected because they were the most commonly detected virulence phenotpyes from bulk field collection during surveys conducted in 2007 and 2008 (Gulya and Markell, 2009). Race 777 was selected because

Table 3.1. Predominant greenhouse infection types and field severity on *Helianthus annuus* accessions and USDA lines, to multiple races of *P. helianthi*.

Sunflower Line	300[a]	304	336	337	777	Fargo[b]	Langdon
PI 162454	4	4	4	4	5	12.6	14.6
PI 170412	4	5	5	4	5	22.4	4.1
PI 170419	4	4	4	4	4	16.7	4.6
PI 171655	4	4	4	5	4	13.2	6.0
PI 175723	4	5	4	4	4	12.1	3.7
PI 184048	3	4	5	4	4	16.2	4.4
PI 195573	5	4	4	4	5	14.5	3.1
PI 213175	4	4	4	4	4	13.0	5.1
PI 221441	4	5	5	4	5	21.0	5.6
PI 221693	4	5	4	4	4	17.9	16.1
PI 232904	4	4	4	4	5	18.6	6.4
PI 243074	4	4	4	4	5	26.1	6.0
PI 251901	5	5	4	5	4	7.9	3.6
PI 251990	4	5	4	4	4	16.0	4.9
PI 256334	4	4	4	4	5	20.5	2.2
PI263178	4	4	4	4	4	17.5	2.0
PI 265099	4	5	4	4	4	10.7	6.5
PI 265499	4	4	4	4	4	19.6	6.1
PI 287230	4	4	4	4	4	10.0	5.6
PI 289626	4	3	4	4	4	14.2	6.8
PI 291404	4	5	4	4	4	19.4	9.1
PI 296289	4	5	4	4	4	12.7	5.1
PI 307831	4	4	4	4	4	13.6	5.6
PI 307934	4	4	4	4	4	7.5	4.9
PI 307942	4	4	4	4	4	11.6	3.2
PI 323281	4	4	4	4	5	18.3	3.6
PI 331176	0;	0;	1	;	4	2.6	0.6
PI 340784	4	4	4	5	4	8.6	2.9
PI 340790	4	5	4	4	4	12.3	4.3
PI 343798	4	4	4	5	4	9.2	3.8
PI 343809	4	4	4	4	4	19.9	4.3
PI 369358	0;	0;	4	0;	4	3.8	1.2
PI 369359	0;	4	3	;	4	3.6	3.5
PI 369360	4	4	4	4	4	6.9	2.1
PI 371936	5	4	4	4	5	9.5	3.7
PI 372173	4	4	4	4	4	10.5	4.8
PI 372258	3	4	4	4	4	9.6	4.9
PI 372259	4	4	3	4	4	10.9	2.6
PI 377528	4	4	5	4	4	13.3	3.3

Table 3.1. Predominant greenhouse infection types and field severity on *Helianthus annuus* accessions and USDA lines, to multiple races of *P. helianthi* (continued).

Sunflower Line	300	304	336	337	777	Fargo	Langdon
PI 377530	3	4	4	4	4	11.5	2.2
PI 378894	4	4	4	4	4	13.6	3.6
PI 378895	0;	4	4	4	;	4.1	0.9
PI 380576	5	4	4	4	4	17.0	5.2
PI 386096	4	4	4	4	5	15.8	9.0
PI 386230	4	4	4	5	4	20.7	6.7
PI 408726	0;	4	4	4	;	5.4	2.3
PI 424926	4	4	4	4	5	13.5	3.0
PI 430539	4	4	4	4	4	6.7	2.4
PI 430541	5	4	4	4	4	7.0	2.7
PI 431516	5	4	4	4	4	14.1	8.2
PI 431529	5	4	4	5	4	14.4	2.6
PI 431538	2	0;	;	;	4	0.2	1.0
PI 431542	4	4	4	4	5	1.9	0.5
PI 431558	4	3	4	H[c]	4	8.9	5.1
PI 432504	4	4	4	4	5	13.9	4.4
PI 432512	;	0;	;	0;	;	0.7	0.2
PI 432519	4	4	4	4	4	12.3	5.9
PI 433377	4	4	4	4	4	11.8	2.6
PI 480472	4	4	4	4	4	11.1	3.3
PI 483077	4	4	4	4	4	10.9	7.6
PI 487194	4	4	4	4	4	9.4	2.4
PI 490281	4	4	4	4	5	15.3	4.5
PI 490324	4	4	4	4	4	13.3	4.6
PI 496263	4	5	4	5	5	21.8	7.7
PI 496265	4	4	4	4	4	14.7	7.6
PI 497247	4	4	4	5	4	11.7	3.4
PI 497250	4	4	4	4	4	3.0	2.6
PI 497937	4	4	4	4	5	12.2	5.6
PI 497939	4	4	4	4	5	13.9	3.7
PI 500688	4	5	4	4	4	15.4	5.7
PI 505839	4	4	4	4	4	6.7	2.4
PI 507899	4	4	4	4	4	15.6	3.4
PI 507901	5	4	4	4	4	10.5	2.3
PI 531339	3	5	4	4	4	11.1	3.7

Table 3.1. Predominant greenhouse infection types and field severity on *Helianthus annuus* accessions and USDA lines, to multiple races of *P. helianthi* (continued).

Sunflower Line	300	304	336	337	777	Fargo	Langdon
PI 531345	4	4	4	4	4	9.2	3.0
PI 531350	4	4	4	5	4	14.0	4.3
PI 531351	4	4	5	4	4	12.4	2.1
PI 535890	5	4	4	4	4	18.4	3.9
PI 535894	4	4	4	4	4	13.0	6.1
PI 600705	4	5	4	4	5	18.9	10.0
PI 600717	4	4	4	4	4	17.1	8.3
PI 600721	4	4	4	5	4	11.8	5.4
PI 650337	4	4	4	4	4	13.9	2.1
PI 650343	4	4	4	4	4	13.8	3.1
PI 650344	4	4	4	4	4	10.8	4.2
PI 650350	4	5	4	4	5	13.6	2.5
PI 650362	0;	;	0;	;	0;	0.0	0.0
PI 650370	4	4	4	5	4	11.0	4.0
PI 650391	4	5	4	5	4	20.9	2.6
PI 650406	4	4	4	4	4	9.1	1.4
PI 650407	4	5	4	4	4	18.5	3.7
PI 650413	4	5	4	5	4	9.1	4.2
PI 650415	4	4	4	4	4	11.1	6.1
PI 650420	4	4	4	4	4	17.1	6.8
PI 650438	4	4	4	4	5	20.3	18.3
PI 650467	4	4	4	4	4	16.8	3.5
PI 650472	5	4	4	4	4	10.7	4.8
PI 650497	4	5	4	4	5	8.9	3.9
PI 650530	5	5	4	4	4	11.5	2.8
PI 650534	4	5	4	4	4	3.8	7.4
PI 650558	4	5	4	4	4	14.6	8.7
PI 650649	4	4	4	4	4	15.6	8.3
PI 650650	5	4	4	4	4	12.7	5.9
PI 650655	4	4	4	4	4	18.8	5.4
PI 650657	5	4	4	4	4	17.5	9.4
PI 650727	4	4	5	4	4	11.6	3.7
PI 650731	4	4	4	4	4	13.3	1.7
PI 650735	4	4	4	4	4	13.1	3.0
PI 650741	5	4	4	5	4	6.5	2.4

Table 3.1. Predominant greenhouse infection types and field severity on *Helianthus annuus* accessions and USDA lines, to multiple races of *P. helianthi* (continued).

Sunflower Line	300	304	336	337	777	Fargo	Langdon
PI 650781	4	5	4	5	4	16.5	4.5
PI 650788	4	4	4	4	5	21.2	6.6
PI 664140	4	5	4	4	4	15.2	4.0
HAR 6	0;	0;	0;	…[d]	…	0.0	0.0
HAR 8	0;	0;	0;	…	…	0.9	0.6
RHA 397	0;	0;	0;	…	…	0.0	0.2
RHA 464	0;	;	;	…	…	0.0	0.0
PH3	0;	0;	0;	;	;	0.1	0.0
PH4	0;	0;	;	;	;	1.1	0.4
PH5	0;	0;	0;	;	;	1.5	0.5
TX16	0;	0;	0;	…	0;	0.0	0.0
RH-ANN-1742	0;	0;	0;	…	…	0.0	0.0

[a]Predominant IT: 0 = immune; = flecks, 1 = pustules smaller than 0.2 mm, 2 = pustules 0.2-0.4 mm, 3 = pustules 0.4-0.6 mm, 4 = 0.6-0.8, and 5 = pustules larger than 0.8
[b]Year-end Severity: mean leaf area severity according to diagrams (Gulya et al., 1990 and Friskop et al., 2011b)
[c]H = accession was heterogeneous for resistance and susceptibility based on IT
[d]… = no seed available

it is virulent on all nine differentials, and was detected multiple times in 2012. All isolates were derived from single pustules and increased on a susceptible hybrid ('Jaguar' – Seeds 2000).

For isolate increase and inoculation of sunflower lines, the susceptible confection hybrid 'Jaguar' (Seeds 2000) was planted in 7.62 cm cone-tainers filled with potting soil (Sunshine mix, SunGro Horticulture Distribution Inc., Bellevue, WA). When plants were 14 days old, isolates were inoculated individually on the plants. Inoculations were performed by suspending uredioniospores in a light petroleum based oil (Soltrol 170; ConocoPhillips Inc., Houston) at approximately 275,000 spores/ml. The urediniospore suspension was sprayed onto the first true leaves of sunflower. Inoculated plants were allowed to dry for 30-40 minutes and placed into misting chambers for 18-20 hours at 22 ± 2°C with a photoperiod of 16 hours. Light was supplemented by a 400 watt halogen bulb (Phillips, Royal Philips of the Netherlands) using the P. L light systems model PL2000 HPS Super (P. L. Light Systems, Beamsville, ON, Canada).

Urediniospores were collected 12-14 days after inoculation. Inoculation timing was scheduled to provide fresh urediniospores for subsequent experiments.

Greenhouse Screening. Greenhouse evaluations were done using a complete randomized design with two replications and repeated twice. Seeds were planted in a 10.16 cm by 22.86 cm cell packs (4 cells by 9 cells (T & O Plastics, St. Paul, MN)) filled with Sunshine Mix. Two sunflower seeds were planted in three cells per rep for accession lines, USDA lines, differentials, and the susceptible check. Three cells per tray were planted with a susceptible check. Plants were grown in a greenhouse at 22 ± 2°C diurnal temperature regime and a 16 hour photoperiod. Light was supplemented by a 400 watt halogen bulb (Phillips, Royal Philips of the Netherlands) using the P. L light systems model PL2000 HPS Super (P. L. Light Systems, Beamsville, ON, Canada).

Sunflower lines were inoculated with fresh urediniospores 13-15 days after planting (as above). Infection types were recorded 13-15 days post inoculation according to a modified 0-5 scale from Yang et al (1986): 0 = immune, ; = flecks, 1 = pustules smaller than 0.2 mm, 2 = pustules 0.2-0.4 mm, 3 = pustules 0.4-0.6 mm, 4 = 0.6-0.8, and 5 = pustules larger than 0.8 The letters N and C were used to denote necrotic or chlorotic reactions. Heterogeneous infection types on the same plant were denoted by the most frequent infection type followed by "/", and subsequent reactions were recorded. Heterogeneous infection types occurring on different plants were separated by ",". The infection type was recorded for three to six plants for each line for each replicate.

Additional Greenhouse Screening. Plant Introduction (PI) lines that had resistant infection types in greenhouse studies (Table 3.2), and/or low severity in field trials, were screened using additional races. Nine accessions were resistant to at least one race in the

Table 3.2. Origin, seed type, days to flower, and plant height of nine accessions that were resistant to at least one race in the greenhouse, or had low severity in the field.

PI #	Origin	Seed Type	Days to Flower	Plant Height (cm)
331176	Argentina	Striped	71	200
369358	United States	Striped	82	240
369359	United States	Striped	76	Not Available
378895	Argentina	Striped	80	260
408726	France	Striped	61	180
431538	Serbia	Striped	73	125
431542	Serbia	Striped	73	165
432512	United States	Striped	85	285
650362	France	Black	62	125

greenhouse or had a low year end mean severity at each location. Therefore, PI 331176, PI 369358, PI 369359, PI 378895, PI 408726, PI 431538, PI 431542, PI 432512, and PI 650362 were screened to single pustule *P. helianthi* isolates coding to races 324, 332, 344, 364, and 732, using techniques described above. Specific races were chosen to provide more information on the possible resistance present in accession lines. The experiment was completed twice using two replicates in a completely randomized design.

Field Screening. To evaluate rust reactions in the field, all lines were planted at three locations in 2012; Fargo, ND, Langdon, ND, and Staples, MN. Both Fargo and Landon were under dry land field conditions and Staples was under irrigation. Planting dates for the locations were 6 June 2012 at Fargo, 17 May 2012 at Langdon, and 18 May 2012 at Staples. Single row plots 5.48 meters long and spaced 0.76 meters were established at Fargo and Staples. In Langdon, double row plots 3.05 meters long with row spacing of 0.76 meters were planted. Seed spacing at all locations was 17.78 cm. The experiments at all locations were designed in a random complete block with three replications. At all locations, the susceptible confection

hybrid ‘Jaguar’ (Seeds2000) was planted every third row to help facilitate disease pressure by serving as ‘spreader rows’.

Sunflower rust epidemics were initiated by inoculating spreader rows with a mixture of isolates coding to races 300, 304, 336 and 337. Race 777 was not used in the field studies due to its high virulence and low frequency in the growing regions. Urediniospores were suspended in Soltrol 170 at approximately 275,000 spores/ml and inoculated onto spreader rows using a modified leaf blower. Inoculation dates were July 3, July 12, and July 16 for Langdon, Staples, and Fargo, respectively. Growth stage at time of inoculation varied from R1 to R4 depending on maturity differences among and within the sunflower lines. Rust evaluations were conducted two to three times at each location. Two to eleven plants, depending on stand establishment, were rated for disease severity for each line for each replicate. Severity was evaluated for four leaves on the middle portion of the plant with the aid of disease severity diagrams (Gulya et al., 1990; Shtienberg, 1995; Friskop et al., 2011b).

Statistical Analysis. Deviation from normality of field screening severity data was tested using the Shapiro-Wilk test in the univariate procedure within the SAS 9.2 (SAS Institute, Cary, NC) program. Due to significance from the Shapiro-Wilk test, data was transformed [square root (field severity)] to normalize data.

Results

Greenhouse Screening. The number of accessions with predominantly resistant infection types (0, ;, 1, and 2) was 8, 5, 4, 6, and 4 when inoculated with races 300, 304, 336, 337, and 777 respectively (Table 3.1, Figure 3.1). Eight PI lines had a predominant resistant infection type to at least one race, while five PI lines had infection types resistant to three or more races. PI 432512 and PI 650362 had resistant infection types across all five races. Based on infection

types, PI 408726 had an equal distribution of resistant and susceptible plants across all five races. Additionally, all nine USDA lines had infection types classified as resistant for races tested (Table 3.1).

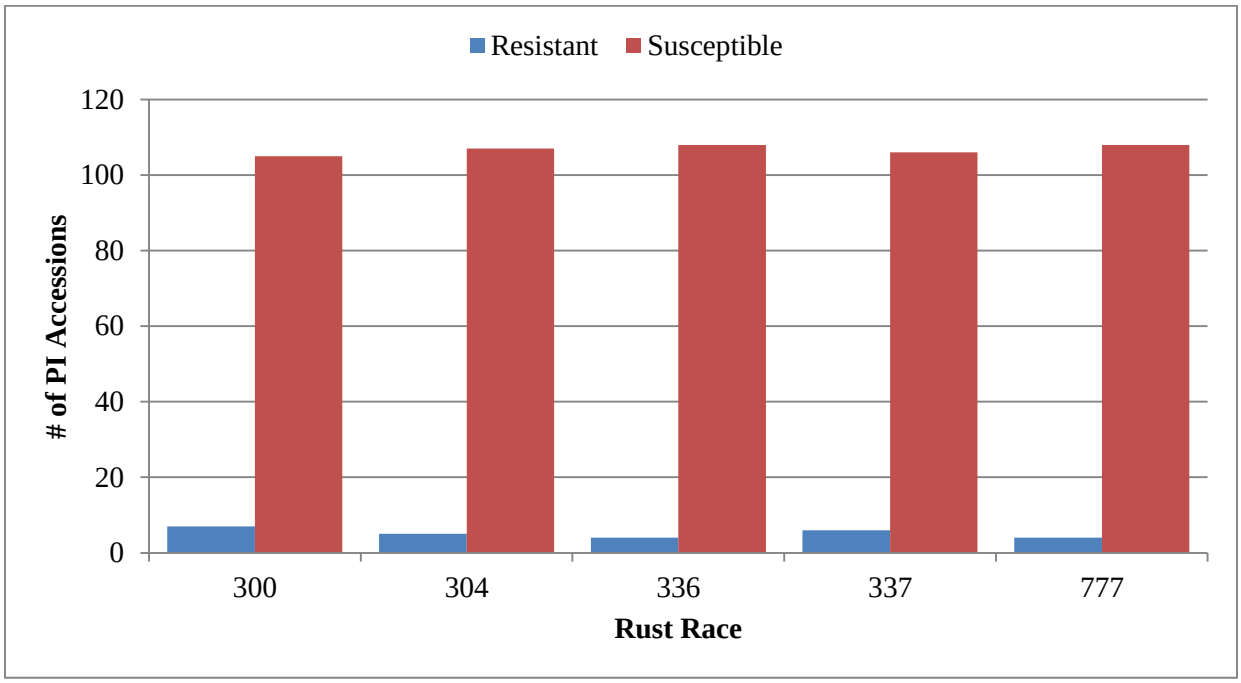

Figure 3.1. Number of accessions with a resistant (IT=0-2) and susceptible (IT=3-5) infection types to *Puccinia helianthi* races 300, 304, 336, 337, and 777.

Additional Greenhouse Screening. *P. helianthi* isolates coding to races 324, 332, 344, 364, and 732 were used in this supplementary screening. No line screened had a resistance response consistent with that of a known differential (Table 3.3). PI 650362 was resistant to all additional races tested. PI 432512, which was previously resistance across the races tested, was found to be susceptible to rust races 324 and 732. Interestingly, PI 431542, which was susceptible to all of the previously tested races (including 300 and 304), conferred resistance to race 332.

Field Screening. Mean severity on the susceptible check was 9.8%, 3.3%, and 0.01% at Fargo, Langdon, and Staples, respectively (data not shown). Due to the low disease pressure at

Table 3.3. Virulence and avirulence comparison of sunflower rust differentials and selected PI accessions to ten races of *Puccinia helianthi*.

	Differentials									PI Lines								
Rust Races	7350	CM90	CM29	P386	HAR1	HAR2	HAR3	HAR4	HAR5	331176	369358	369359	378895	408726	431538	431542	432512	650362
300	V[a]	V	A[b]	A	A	A	A	A	A	A	A	A	A	A	A	V	A	A
304	V	V	A	A	A	A	A	A	V	A	A	V	V	V	A	V	A	A
324	V	V	A	A	V	A	A	A	V	A	V	V	A	V	A	V	V	A
332	V	V	A	V	V	A	A	V	A	V	A	V	V	V	A	A	A	A
336	V	V	A	V	V	A	A	V	V	A	V	V	V	V	A	V	A	A
337	V	V	A	V	V	A	V	V	V	A	A	A	V	V	A	V	A	A
344	V	V	A	A	A	V	A	A	V	A	V	V	A	H[c]	V	V	A	A
364	V	V	A	A	V	V	A	A	V	A	A	V	V	H	A	V	A	A
732	V	V	V	V	V	A	A	V	A	A	V	V	A	H	A	V	V	A
777	V	V	V	V	V	V	V	V	V	V	V	V	A	A	V	V	A	A

[a]V = virulence
[b]A = avirulence
[c]H= heterogeneous reaction

Staples, only data from Fargo and Langdon is presented (Table 1.1). A majority of the accessions had rust severity greater than 1% at Fargo and Langdon (Figure 3.2; Figure 3.3). Three and six accessions had rust severities between 0-1% at Fargo and Langdon, respectively. Three accessions; PI 431538, PI 432512, and PI 65036) had low rust severities at both Fargo and Langdon. Statistical analysis showed that severity data was not normally distributed, thus a mean separation test was not performed.

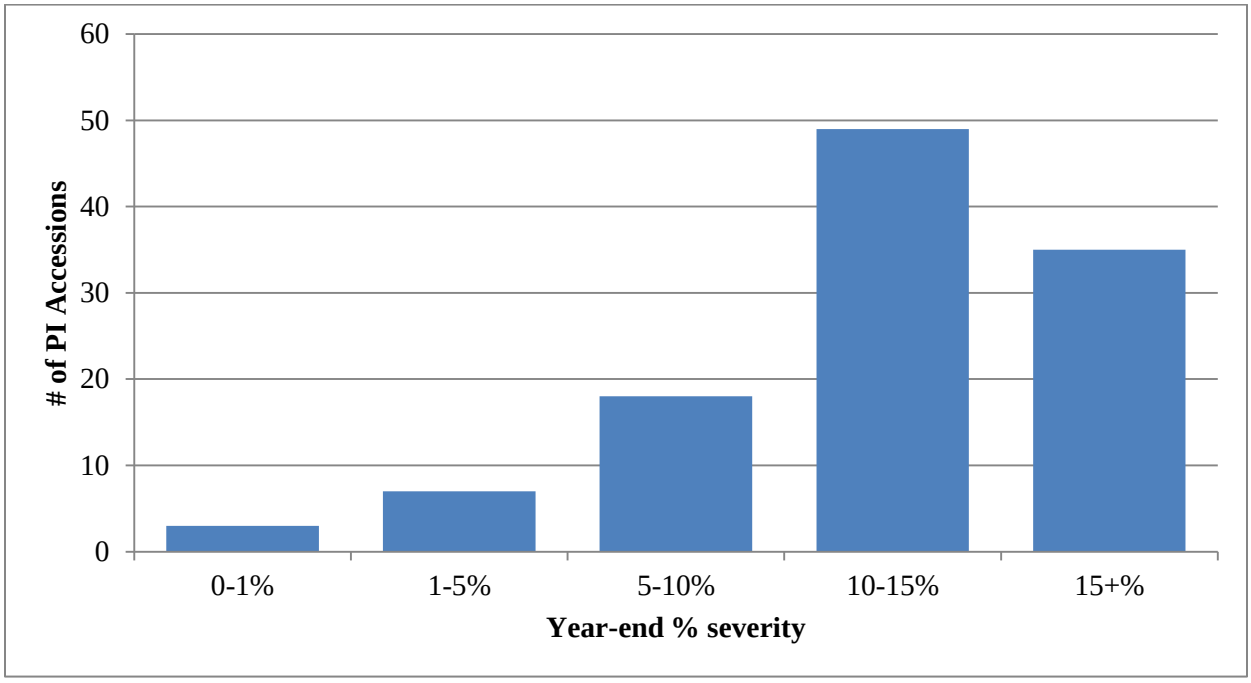

Figure 3.2. Number of accessions grouped by mean foliar rust severity from Fargo, ND 2012.

Discussion

Several accessions in this study were identified as sources of resistance. PI 650362 was resistant across the races tested in the greenhouse and had low year-end severities in the field. PI 432512 was resistant to most races tested in the greenhouse and also had low year-end severities. These results agree with previous reports demonstrating resistance in these lines (Miller and Gulya, 2001; Qi et al., 2011). None of the aforementioned lines had a resistance response

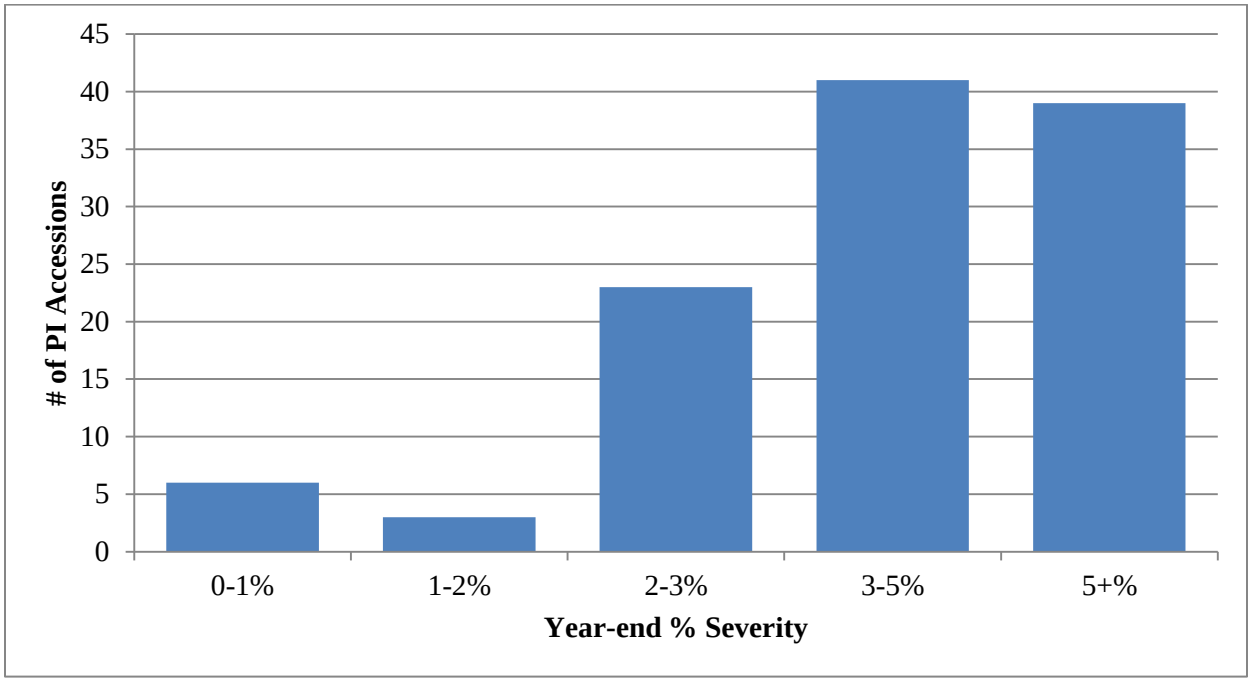

Figure 3.3. Number of accessions grouped by mean foliar rust severity from Langdon, ND 2012.

consistent with any differential, suggesting that the gene, or gene combinations, may not be represented in the differential set. Additionally, several other accessions appear to be segregating for resistance and further assessments may be beneficial.

A large majority of the accessions were susceptible in both greenhouse and field experiments. PI 432512 and PI 650362 had the lowest year-end severities at both locations and were resistant to all five races tested in the greenhouse. Additionally, PI 331176, PI 431538, and PI 369358 greenhouse results were consistent with field severity results. PI 408726 had varying levels of rust severity in the field, which corresponds with results in the greenhouse. Only two USDA germplasm lines had rust severity greater than 1% at Fargo, while most had trace levels of rust. At Langdon, all USDA germplasm had rust severities <1%.

Adult plant resistance, where resistance is effective in adult plants but the seedling is susceptible, has been utilized for management of wheat rusts (Jin et al., 2007; Wamishe and

Milus, 2004). Adult plant resistance has not been documented to *P. helianthi*, but could be very useful in sunflower production regions because rust does not often appear in grower fields until reproductive growth stage. The reactions of PI 431542 are consistent with a line carrying an adult plant resistant gene(s). In the greenhouse experiment, the first true leaves of the plant were inoculated, while in the field experiment, inoculation was completed at approximately growth stage R1. PI 431542 had susceptible infection types to the five races tested in the greenhouse and to four out of the five additional races tested, but low year-end rust severity in the field. This was the only line in this study with seedling susceptibility and adult plant resistance. In addition to potentially being a source of adult plant resistance, when PI431542 was screened to five additional races in the greenhouse, it was resistant to race 332 but to no other races. This suggests that an additional resistance gene may be present in the line, which is not present in the differential set. Genetic studies (Olivera et al. 2013) to elucidate the resistance gene(s) present in PI431542 are needed.

Plants of some accessions had both resistant and susceptible infection types in greenhouse evaluations. PI 408726 collectively had a 1:1 ratio of resistance and susceptibility (data not presented). This line may be heterozygous for a resistance gene. Field data suggest that PI 378895 and PI 331176 may also be heterozygous for resistance gene(s), and could be evaluated further as a potentially novel source of resistance.

This work identifies accession lines that contain a source of resistance to many races of rust, including one accession that appears to be a source of adult plant resistance. The PI lines are a public resource and can be obtained for incorporation into breeding programs. Incorporation of rust resistant into commercial hybrids may provide an important management option for growers in the future.

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APPENDIX: RACE INFORMATION FOR *PUCCINA HELIANTHI* ISOLATES

Puccinia helianthi isolate designation, sampling location, host-type, and race detected in 2011 and 2012.

Isolate	State/Country	Host	Race
NE11_01 C	Nebraska	Not Known Cultivated	300
NE11_01 F	Nebraska	Not Known Cultivated	300
NE11_03 A	Nebraska	Not Known Cultivated	304
NE11_04 B	Nebraska	Not Known Cultivated	300
NE11_04 D	Nebraska	Not Known Cultivated	372
NE11_04 F	Nebraska	Not Known Cultivated	300
NE11_05 D	Nebraska	Not Known Cultivated	300
NE11_05 E	Nebraska	Not Known Cultivated	300
NE11_06 A	Nebraska	Not Known Cultivated	336
NE11_06 B	Nebraska	Not Known Cultivated	336
NE11_07 A	Nebraska	Not Known Cultivated	330
NE11_07 E	Nebraska	Not Known Cultivated	304
NE11_07 F	Nebraska	Not Known Cultivated	300
NE11_08 B	Nebraska	Not Known Cultivated	300
NE11_08 E	Nebraska	Not Known Cultivated	300
NE11_09 B	Nebraska	Not Known Cultivated	332
NE11_09 D	Nebraska	Not Known Cultivated	332
NE11_10 B	Nebraska	Not Known Cultivated	300
NE11_10 D	Nebraska	Not Known Cultivated	300
NE11_10 E	Nebraska	Not Known Cultivated	300
ND11_01 D	North Dakota	Oil	704
ND11_01 E	North Dakota	Oil	300
ND11_01 F	North Dakota	Oil	320
ND11_02 A	North Dakota	Not Known Cultivated	344
ND11_02 B	North Dakota	Not Known Cultivated	304
ND11_03 B	North Dakota	Wild	304
ND11_03 H	North Dakota	Wild	304
ND11_04 D	North Dakota	Wild	340
ND11_04 F	North Dakota	Wild	324
ND11_05 C	North Dakota	Confection	776
ND11_05 D	North Dakota	Confection	304
ND11_05 H	North Dakota	Confection	304
ND11_06 C	North Dakota	Oil	304
ND11_06 E	North Dakota	Oil	304
ND11_06 F	North Dakota	Oil	304
ND11_07 A	North Dakota	Oil	304

Isolate	State/Country	Host	Race
ND11_07 B	North Dakota	Oil	300
ND11_07 H	North Dakota	Oil	300
ND11_08 D	North Dakota	Confection	332
ND11_08 I	North Dakota	Confection	304
ND11_08 N	North Dakota	Confection	304
ND11_09 E	North Dakota	Oil	300
ND11_09 G	North Dakota	Oil	304
ND11_09 H	North Dakota	Oil	336
ND11_10 D	North Dakota	Oil	304
ND11_10 G	North Dakota	Oil	322
ND11_10 H	North Dakota	Oil	300
ND11_11 A	North Dakota	Oil	300
ND11_11 E	North Dakota	Oil	324
ND11_11 F	North Dakota	Oil	326
ND11_12 E	North Dakota	Confection	304
ND11_12 F	North Dakota	Confection	300
ND11_12 I	North Dakota	Confection	304
ND11_13 A	North Dakota	Oil	304
ND11_13 B	North Dakota	Oil	734
ND11_13 D	North Dakota	Oil	304
ND11 _14 E	North Dakota	Oil	300
ND11_14 C	North Dakota	Oil	300
ND11_14 H	North Dakota	Oil	340
ND11_15 A	North Dakota	Oil	300
ND11_15 D	North Dakota	Oil	300
ND11_15 F	North Dakota	Oil	300
ND11_16 B	North Dakota	Oil	300
ND11_16 C	North Dakota	Oil	324
ND11_16 D	North Dakota	Oil	300
ND11_17 B	North Dakota	Oil	304
ND11_17 D	North Dakota	Oil	300
ND11_17 F	North Dakota	Oil	337
ND11_18 A	North Dakota	Wild	300
ND11_18 C	North Dakota	Wild	736
ND11_18 E	North Dakota	Wild	304
ND11_19 H	North Dakota	Confection	320
ND11_19 I	North Dakota	Confection	344
ND11_19 J	North Dakota	Confection	332
ND11_20 A	North Dakota	Wild	304
ND11_20 D	North Dakota	Wild	332

Isolate	State/Country	Host	Race
ND11_20 E	North Dakota	Wild	336
ND11_21 B	North Dakota	Oil	300
ND11_21 C	North Dakota	Oil	344
ND11_21 G	North Dakota	Oil	304
ND11_22 D	North Dakota	Oil	304
ND11_22 E	North Dakota	Oil	304
ND11_22 F	North Dakota	Oil	300
ND11_23 C	North Dakota	Oil	304
ND11_23 D	North Dakota	Oil	300
ND11_23 F	North Dakota	Oil	300
ND11_24 D	North Dakota	Oil	332
ND11_24 I	North Dakota	Oil	304
ND11_24 J	North Dakota	Oil	332
ND11_25 D	North Dakota	Oil	304
ND11_25 F	North Dakota	Oil	304
ND11_25 J	North Dakota	Oil	336
ND11_26 B	North Dakota	Oil	300
ND11_26 E	North Dakota	Oil	300
ND11_26 G	North Dakota	Oil	300
ND11_27 A	North Dakota	Oil	304
ND11_27 G	North Dakota	Oil	300
ND11_27 J	North Dakota	Oil	300
ND11_28 B	North Dakota	Oil	344
ND11_28 C	North Dakota	Oil	300
ND11_28 G	North Dakota	Oil	304
ND11_29 A	North Dakota	Oil	300
ND11_29 B	North Dakota	Oil	337
ND11_29 D	North Dakota	Oil	304
ND11_30 B	North Dakota	Oil	304
ND11_30 C	North Dakota	Oil	300
ND11_30 F	North Dakota	Oil	300
ND11_31 G	North Dakota	Oil	300
ND11_31 J	North Dakota	Oil	304
ND11_32 D	North Dakota	Oil	304
ND11_32 G	North Dakota	Oil	344
ND11_32 H	North Dakota	Oil	344
ND11_33 D	North Dakota	Oil	304
ND11_33 E	North Dakota	Oil	300
ND11_34 A	North Dakota	Oil	304
ND11_34 B	North Dakota	Oil	344

Isolate	State/Country	Host	Race
ND11_34 E	North Dakota	Oil	304
ND11_35 C	North Dakota	Oil	344
ND11_35 D	North Dakota	Oil	300
ND11_35 G	North Dakota	Oil	344
ND11_36 A	North Dakota	Oil	304
ND11_36 E	North Dakota	Oil	300
ND11_36 G	North Dakota	Oil	300
ND11_37 A	North Dakota	Wild	300
ND11_37 C	North Dakota	Wild	336
ND11_37 H	North Dakota	Wild	304
SD11_01 A	South Dakota	Confection	300
SD11_01 B	South Dakota	Confection	304
SD11_01 C	South Dakota	Confection	300
CA12_04 A	California	Not Known Cultivated	704
CA12_05 A	California	Not Known Cultivated	776
CA12_05 B	California	Not Known Cultivated	776
CA12_08 A	California	Not Known Cultivated	736
CA12_09 B	California	Not Known Cultivated	376
CA12_09 C	California	Not Known Cultivated	724
CA12_10 B	California	Not Known Cultivated	776
CA12_11 B	California	Not Known Cultivated	337
CA12_12 B	California	Not Known Cultivated	776
CAN12_01 C	Canada	Not Known Cultivated	304
CAN12_03 A	Canada	Confection	777
CAN12_06 A	Canada	Confection	324
CAN12_06 C	Canada	Confection	324
IA12_01 B	Iowa	Wild	320
IA12_02 A	Iowa	Wild	304
IA12_02 B	Iowa	Wild	304
IA12_03 A	Iowa	Wild	704
IA12_03 B	Iowa	Wild	704
MN12_03 C	Minnesota	Wild	300
MN12_06 A	Minnesota	Wild	704
MN12_06 B	Minnesota	Wild	726
MN12_06 C	Minnesota	Wild	704
MN12_07 A	Minnesota	Confection	304
MN12_07 B	Minnesota	Confection	324
MN12_07 C	Minnesota	Confection	324
MN12_08 A	Minnesota	Confection	336
MN12_08 B	Minnesota	Confection	336

Isolate	State/Country	Host	Race
MN12_08 C	Minnesota	Confection	336
MN12_09 A	Minnesota	Not Known Cultivated	334
MN12_09 B	Minnesota	Not Known Cultivated	334
MN12_11 A	Minnesota	Not Known Cultivated	340
MN12_11 B	Minnesota	Not Known Cultivated	374
MN12_12 A	Minnesota	Not Known Cultivated	744
MN12_14 B	Minnesota	Not Known Cultivated	736
MN12_14 C	Minnesota	Not Known Cultivated	364
MN12_16 A	Minnesota	Not Known Cultivated	704
NE12_01 A	Nebraska	Wild	704
NE12_01 B	Nebraska	Wild	732
NE12_02 A	Nebraska	Wild	777
NE12_03 A	Nebraska	Not Known Cultivated	304
NE12_05 A	Nebraska	Not Known Cultivated	300
NE12_05 B	Nebraska	Not Known Cultivated	304
NE12_06 B	Nebraska	Not Known Cultivated	777
NE12_07 B	Nebraska	Not Known Cultivated	724
ND12_01 B	North Dakota	Oil	304
ND12_02 A	North Dakota	Not Known Cultivated	344
ND12_02 E	North Dakota	Not Known Cultivated	334
ND12_03 A	North Dakota	Not Known Cultivated	324
ND12_05 B	North Dakota	Oil	366
ND12_06 A	North Dakota	Confection	777
ND12_06 B	North Dakota	Confection	364
ND12_06 C	North Dakota	Confection	736
ND12_06 D	North Dakota	Confection	364
ND12_07 A	North Dakota	Confection	776
ND12_07 B	North Dakota	Confection	334
ND12_07 C	North Dakota	Confection	376
ND12_10 C	North Dakota	Confection	364
ND12_10 D	North Dakota	Confection	324
ND12_11 A	North Dakota	Confection	304
ND12_12 A	North Dakota	Oil	324
ND12_12 B	North Dakota	Oil	364
ND12_12 C	North Dakota	Oil	344
ND12_12 E	North Dakota	Oil	704
ND12_14 A	North Dakota	Oil	336
ND12_14 D	North Dakota	Oil	304
ND12_14 G	North Dakota	Oil	326
ND12_15 C	North Dakota	Confection	344

Isolate	State/Country	Host	Race
ND12_15 E	North Dakota	Confection	304
ND12_15 G	North Dakota	Confection	336
ND12_16 B	North Dakota	Oil	736
ND12_16 C	North Dakota	Oil	364
ND12_17 A	North Dakota	Oil	764
ND12_17 D	North Dakota	Oil	344
ND12_17 E	North Dakota	Oil	376
ND12_18 A	North Dakota	Confection	344
ND12_18 B	North Dakota	Confection	324
ND12_18 D	North Dakota	Confection	304
ND12_18 E	North Dakota	Confection	736
ND12_18 F	North Dakota	Confection	336
ND12_18 H	North Dakota	Confection	376
ND12_19 A	North Dakota	Oil	304
ND12_19 B	North Dakota	Oil	776
ND12_19 C	North Dakota	Oil	344
ND12_19 F	North Dakota	Oil	304
ND12_20 B	North Dakota	Oil	364
ND12_20 C	North Dakota	Oil	364
ND12_20 E	North Dakota	Oil	376
ND12_20 F	North Dakota	Oil	304
ND12_20 G	North Dakota	Oil	366
ND12_20 H	North Dakota	Oil	304
ND12_21 A	North Dakota	Oil	366
ND12_21 C	North Dakota	Oil	776
ND12_21 D	North Dakota	Oil	304
ND12_21 G	North Dakota	Oil	304
ND12_21 H	North Dakota	Oil	344
ND12_23 C	North Dakota	Not Known Cultivated	736
ND12_25 B	North Dakota	Wild	304
ND12_26 A	North Dakota	Wild	304
SD12_01 B	South Dakota	Not Known Cultivated	734
SD12_02 A	South Dakota	Not Known Cultivated	336
SD12_02 B	South Dakota	Not Known Cultivated	300
SD12_04 B	South Dakota	Not Known Cultivated	324
SD12_05 A	South Dakota	Not Known Cultivated	737
SD12_05 B	South Dakota	Not Known Cultivated	324
SD12_06 B	South Dakota	Not Known Cultivated	304
SD12_07 A	South Dakota	Not Known Cultivated	324
SD12_08 A	South Dakota	Not Known Cultivated	736

Isolate	State/Country	Host	Race
TX12_01 A	Texas	Not Known Cultivated	332
TX12_01 B	Texas	Not Known Cultivated	330