DEVELOPMENT OF MANAGEMENT TOOLS FOR SUNFLOWER DOWNY MILDEW

(*PLASMOPARA HALSTEDII*) AND RUST (*PUCCINIA HELIANTHI*)

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Ryan Michael Humann

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Plant Pathology

March 2016

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Development of Management Tools for Sunflower Downy Mildew
(*Plasmopara halstedii*) and Rust (*Puccinia helianthi*)

By

Ryan Michael Humann

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

SUPERVISORY COMMITTEE:

Dr. Samuel Markell
Co-Chair

Dr. Thomas Gulya
Co-Chair

Dr. Maricelis Acevedo

Keith D. Johnson

Dr. Hans Kandel

Approved:

March 29, 2016	Dr. Jack Rasmussen
Date	Department Chair

ABSTRACT

Downy mildew (*Plasmopara halstedii*) and rust (*Puccinia helianthi*) are two economically important diseases of sunflower (*Helianthus annuus*) in North Dakota. Both diseases are capable of causing significant reductions in yield and quality. Effective disease management tools for both diseases are limited. Genetic resistance to both pathogens is frequently overcome by new pathogen races and only one efficacious fungicide is currently available to manage downy mildew. In order to identify additional management tools for downy mildew and rust, three research studies were done. The objective of the first study was to evaluate the efficacy of a novel fungicide, oxathiapiprolin, for the management of sunflower downy mildew. Seventeen inoculated field trials were conducted from 2011-2015 to test the efficacy of oxathiapiprolin. Results indicate that oxathiapiprolin significantly and consistently reduced downy mildew incidence and determined the optimal effective rate, which ranged from 9.37 – 18.75 µg active ingredient per seed. The second and third objectives focused on identifying accessions with novel sources of genetic resistance to *P. halstedii* and *P. helianthi* isolates collected in North Dakota. In the past, a disproportionate amount of resistance genes have been identified in wild *Helianthus* germplasm originating from Texas. For both studies, 182 wild *H. annuus* and 33 wild *H. argophyllus* accessions originating from Texas were obtained from the USDA North Central Regional Plant Introduction Station and screened to both pathogens in a greenhouse environment. Results from these individual studies identified numerous accessions with high levels of resistance to *P. halstedii* and *P. helianthi*, some accessions had high levels of resistance to both. Overall, results from these three studies will provide information and tools that will be useful for the long-term management of both diseases.

ACKNOWLEDGEMENTS

I would like to say a special thanks to all those who have helped me in countless ways over the last four years. First, I would like to thank my major advisor, Dr. Sam Markell, for his endless support, guidance, and trust. Simply believing in me made all the difference; the confidence you have instilled in me has allowed me to reach potentials that I never thought were possible. I hope to pay it forward someday. I would also like to extend a sincere thanks to the rest of my graduate committee members: Dr. Tom Gulya, Dr. Maricelis Acevedo, Keith Johnson, and Dr. Hans Kandel. Your valuable expertise and input have helped me grow in my professional and personal life.

Also, thanks to the National Sunflower Association, DuPont Crop Protection, the North Dakota Agricultural Experiment Station, and the North Dakota Extension Service for providing financial support for my research projects.

Additionally, I would like to extend a thanks

To Dr. Andrew Friskop: for introducing me to the wonderful world of plant pathology and always making time for me; who would’ve thought it would’ve turned into this.

To Elizabeth Crane: for always giving me that extra push when I needed it; I’ll miss all of the miles spent traveling together and countless thought-provoking conversations.

To Jessica Halvorson: for all of your clever suggestions and willingness to help; so many great memories working together.

To Jim Jordahl and Scott Meyer: for showing me the ropes of plant pathology and always keeping me on my toes.

To Austin Streifel, Christian Steffen, Brandt Berghuis, Sam Haugen, Casey Schuh, Kelby Opsahl, Michelle Gilley, and Dr. Febina Mathew: for all of your assistance in the field, greenhouse, and lab which collectively helped make this all possible.

To the NDSU Plant Pathology department: for making my graduate school experience better than I ever thought possible, especially Dr. Jack Rasmussen, Darla Bakko, Aimee Thapa, and Barbara Nilles.

Last and most importantly, I would like to thank all of my loving and supportive family; for without them, none of this would have been possible. Thanks

To my Dad: for the countless hours spent together on the farm, from which I developed my passion for agriculture and learned that good things come to those who work hard.

To my Mom: for the endless love and encouragement that always helped me weather the storms.

Thanks Dad and Mom, for always loving and believing in me in ways that only a parent can.

To my brother, Derick and sister-in-law, Kendra: for all the laughs and advice; I look up to you in more ways than you'll ever know.

To my sister, Megan: for your kindness and willingness to listen; your smile and loving support lit fires that always kept me going.

To my grandparents: for all of the phone calls and cards. Thanks for all the wonderful memories.

This dissertation would have meant nothing if I didn't have you all to share it with.

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS iv

LIST OF TABLES x

LIST OF FIGURES xii

LIST OF APPENDIX TABLES xiii

LITERATURE REVIEW 1

Sunflower 1

History 1

Sunflower production in the United States. 3

Sunflower uses. 3

Sunflower taxonomy and genetics. 4

Physical appearance and growth stages. 5

Plasmopara halstedii (downy mildew) 6

Taxonomy and host range. 6

Biology and life cycle. 6

Sunflower downy mildew. 7

Symptoms and signs. 7

Epidemiology. 8

Disease management. 9

Puccinia helianthi (rust) 10

Taxonomy and host range. 10

Biology and life cycle. 11

Sunflower rust. 11

Symptoms and signs. 12

Epidemiology. 12

Disease management. 13

Literature cited 15

CHAPTER 1. EVALUATION OF OXATHIAPIPROLIN FOR THE MANAGEMENT OF SUNFLOWER DOWNY MILDEW 18

Introduction 18

Materials and Methods 20

Locations and experimental design. 20

Pathogen and inoculation. 22

Fungicide efficacy. 23

Data collection. 24

Statistical analysis. 25

Results 25

Fungicide efficacy. 25

Discussion 36

Literature Cited 40

CHAPTER 2. IDENTIFYING *HELIANTHUS* ACCESSIONS RESISTANT TO *PLASMOPARA HALSTEDII* 43

Introduction 43

Materials and Methods 45

Host material. 45

Pathogen material and inoculation. 46

Initial screening. 47

Advanced screening. 48

Initial screening analysis. 49

Advanced screening analysis. 50

Results ... 50

Initial screening. ... 50

Advanced screening. ... 52

Discussion ... 58

Literature Cited ... 63

CHAPTER 3. IDENTIFYING *HELIANTHUS* ACCESSIONS RESISTANT TO *PUCCINIA HELIANTHI* ... 67

Introduction ... 67

Materials and Methods ... 70

Host material. ... 70

Pathogen material and inoculation. ... 71

Initial screening. ... 72

Advanced screening. ... 73

Additional accessions of interest. ... 74

Initial screening analysis. ... 74

Advanced and additional accessions of interest screening analysis. ... 75

Results ... 76

Initial screening. ... 76

Advanced screening. ... 79

Additional accessions of interest. ... 83

Discussion ... 87

Literature Cited ... 92

APPENDIX A. SUMMARY OF STATISTICAL ANALYSES FOR THE 2011-2014 SUNFLOWER DOWNY MILDEW FUNGICIDE SEED TREATMENT EFFICACY TRIALS ... 97

APPENDIX B. SUMMARY OF RESULTS OF SCREENING *HELIANTHUS* GERMPLASM TO *PLASMOPARA HALSTEDII* ... 103

APPENDIX C. SUMMARY OF RESULTS OF SCREENING *HELIANTHUS* GERMPLASM TO *PUCCINIA HELIANTHI* 109

LIST OF TABLES

Table | Page

1.1. Agronomic and sunflower downy mildew trial information for all experimental locations. 21

1.2. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2011. 26

1.3. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Fargo, ND in 2011. 27

1.4. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Thompson, ND in 2011. 28

1.5. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trials in Carrington and Thompson, ND in 2012. 29

1.6. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2013. 30

1.7. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial 1 in Fargo, ND in 2013. 31

1.8. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial 2 in Fargo, ND in 2013. 32

1.9. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Thompson, ND in 2013. 33

1.10. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2014. 34

1.11. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Fargo, ND in 2014. 35

1.12. Treatment list for sunflower downy mildew seed treatment efficacy trial in 2015. 36

2.1. Total plants and percent resistance for the above-ground evaluation of 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates. 53

2.2. Total asymptomatic roots, percent resistance, and median root color for the root evaluation of 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates. 55

3.1. Susceptible, mixed, and resistant plants and percent resistance for 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates. 80

3.2. Susceptible, mixed, and resistant plants and percent resistance for 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions of interest to a bulk mixture of six *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*.. 84

LIST OF FIGURES

Figure Page

2.1. Percent of resistant and susceptible plants for each of the 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714. 51

2.2. Percent of resistant and susceptible plants for each of the 33 wild *Helianthus argophyllus* accessions in the initial screening to *Plasmopara halstedii* race 714.......... 52

2.3. Percent of root color values (1, 2, 3, and 4) for the root evaluation of each of the 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates.. 57

3.1. Percent of resistant, mixed, and susceptible plants for each of the 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336..... 77

3.2. Percent of resistant, mixed, and susceptible plants for each of the 33 wild *Helianthus argophyllus* accessions in the initial screening to *Puccinia helianthi* race 336.. 78

3.3. Percent of infection types (0, ;, 1, 2, 3, 4, and 5) for each of the 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates...................................... 81

3.4. Percent of plants showing symptoms of necrosis or chlorosis for 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates...................................... 82

3.5. Percent of infection types (0, ;, 1, 2, 3, 4, and 5) for each of the 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions of interest to a bulk mixture of six *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*.. 85

3.6. Percent of plants showing symptoms of necrosis or chlorosis for 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions to a bulk mixture of *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*... 86

LIST OF APPENDIX TABLES

Table Page

A.1. Analysis of variance for the sunflower downy mildew incidence rating 31 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial. 97

A.2. Analysis of variance for the sunflower downy mildew incidence rating 46 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial. 97

A.3. Analysis of variance for the sunflower downy mildew incidence rating 60 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial. 97

A.4. Analysis of variance for the sunflower downy mildew incidence rating 26 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial. 97

A.5. Analysis of variance for the sunflower downy mildew incidence rating 37 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial. 97

A.6. Analysis of variance for the sunflower downy mildew incidence rating 51 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial. 98

A.7. Analysis of variance for the sunflower downy mildew incidence rating 65 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial. 98

A.8. Analysis of variance for the sunflower downy mildew incidence rating 36 days after planting across ten fungicide seed treatments for the 2011 Thompson, ND field trial. 98

A.9. Analysis of variance for the sunflower downy mildew incidence rating 54 days after planting across ten fungicide seed treatments for the 2011 Thompson, ND field trial. 98

A.10. Analysis of variance for the sunflower downy mildew incidence rating 37 days after planting across nine fungicide seed treatments for the 2012 Carrington, ND field trial. 98

A.11. Analysis of variance for the sunflower downy mildew incidence rating 48 days after planting across nine fungicide seed treatments for the 2012 Carrington, ND field trial. 98

A.12. Analysis of variance for the sunflower downy mildew incidence rating 45 days after planting across nine fungicide seed treatments for the 2012 Thompson, ND field trial. 99

A.13. Analysis of variance for the sunflower downy mildew incidence rating 52 days after planting across nine fungicide seed treatments for the 2012 Thompson, ND field trial. 99

A.14. Analysis of variance for the sunflower downy mildew incidence rating 34 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial. 99

A.15. Analysis of variance for the sunflower downy mildew incidence rating 48 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial. 99

A.16. Analysis of variance for the sunflower downy mildew incidence rating 59 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial. 99

A.17. Analysis of variance for the sunflower downy mildew incidence rating 23 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1. 99

A.18. Analysis of variance for the sunflower downy mildew incidence rating 38 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1. 100

A.19. Analysis of variance for the sunflower downy mildew incidence rating 52 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1. 100

A.20. Analysis of variance for the sunflower downy mildew incidence rating 28 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2. 100

A.21. Analysis of variance for the sunflower downy mildew incidence rating 40 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2. 100

A.22. Analysis of variance for the sunflower downy mildew incidence rating 61 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2. 100

A.23. Analysis of variance for the sunflower downy mildew incidence rating 27 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial. 100

A.24. Analysis of variance for the sunflower downy mildew incidence rating 35 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial. ... 101

A.25. Analysis of variance for the sunflower downy mildew incidence rating 46 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial. ... 101

A.26. Analysis of variance for the sunflower downy mildew incidence rating 23 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial. ... 101

A.27. Analysis of variance for the sunflower downy mildew incidence rating 39 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial. ... 101

A.28. Analysis of variance for the sunflower downy mildew incidence rating 57 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial. ... 101

A.29. Analysis of variance for the sunflower downy mildew incidence rating 24 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial. ... 101

A.30. Analysis of variance for the sunflower downy mildew incidence rating 35 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial. ... 102

A.31. Analysis of variance for the sunflower downy mildew incidence rating 49 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial. ... 102

B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714. ... 103

B.2. Susceptible and resistant plants and percent resistance for 33 wild *Helianthus argophyllus* accessions in the initial screening to *Plasmopara halstedii* race 714. ... 108

C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336. ... 109

C.2. Susceptible, mixed, and resistant plants and percent resistance for 33 wild *Helianthus argophyllus* accessions in the initial screening to *Puccinia helianthi* race 336. ... 114

LITERATURE REVIEW

Sunflower

History. Sunflower (*Helianthus annuus* L.) is native to North America, where it was cultivated by Native Americans as early as 3000 BCE. Many believe it was even domesticated in North America prior to maize being introduced from the south. Initially, sunflower was grown for food purposes, where it was found to be a good source of fat that supplemented diets which were restricted to lean meats. Later, Native Americans discovered a wide array of non-food uses for the crop which included medicinal remedies and use in religious ceremonies. Also, pigments were extracted from ray flowers which could be used for dyes (Putt 1997).

Around 1500 CE, it was believed that sunflower was brought from North America and introduced into Europe in a number of countries, including Spain, France, and Britain. In Europe, it gained popularity not as a food crop, but as an exotic ornamental flowering plant. The crop was not widely used as a food crop in Europe until the 1700s, especially the larger seeded types which were preferred and selected for during this time. Eventually the sunflower made its way east across Europe and was introduced into Russia. The Russians were the first to widely utilize sunflower for its vegetable-like oil, which quickly gained popularity. By the late 1800s, sunflowers for oil production were grown on more than 120,000 hectares (ha) with over 80 mills operating to crush seeds and extract the oil. Breeding programs were developed in the early 1900s which improved head size, seed quality, and oil content (Putt 1997). Additionally, disease resistance was improved by crossing *H. annuus* with its wild relatives. By the 1930s, sunflower was the most important oilseed crop throughout all of Europe, where it was heavily relied on by the Germans during World War II. In the 1950s, sunflower slowly spread to many other

temperate areas of the world, including the South American countries of Argentina and Uruguay. Eventually, this production was expanded to Australia (Harveson 2016; Putt 1997).

Even though the center of origin for sunflower is North America, it did not emerge as a cultivated food crop until the 1800s. Prior to this in the late 1700s, numerous Christian religious groups (e.g. Mennonites) moved from present day Poland and Germany to Russia (Putt 1997). The Russian leader of the time, Catherine the Great, promised these people free land, self-government, and religious freedom. Nearly 100 years later, these promises were revoked by Alexander II. Consequently, many of these groups decided to emigrate from Russia to North America, especially south central Canada and the north central United States (U.S.), where they could continue to freely practice their Christian religion. During this period of immigration by the so called "Germans from Russia", the sunflower was reintroduced to North America after the crop had been widely grown in Russia. Initially, sunflower was grown in present day Manitoba and Saskatchewan, after which it slowly moved into the U.S. in the present-day states of North Dakota, South Dakota, and Nebraska (Harveson 2016; Putt 1997).

In North America during the early 1900s, sunflower varieties, many originating from Russia, were primarily planted for use as a silage and forage crop fed to animals. Over time, the sunflower production area continued to grow in Canada, where efforts were being put into developing some of the first North American sunflower breeding programs. In addition to this, local Canadian cooperatives in Manitoba built facilities dedicated solely for processing the increasing number of sunflowers. During the 1940s, sunflower production spread into the northern U.S., where the longer growing season proved to be more advantageous for sunflower production than in the southern provinces of Canada (Putt 1997).

Sunflower production in the United States. Widespread production of sunflowers in the U.S. did not begin until the 1960s, which was facilitated by the discovery of cytoplasmic male sterility (CMS). The CMS gene, which was derived from a wild annual sunflower, allowed breeders to begin the production of sunflower as a hybrid crop on a large scale (Putt 1997). Sunflower hybrid production was arguably one of the greatest advances in sunflower, which created vigorous plants that were higher yielding, had improved seed quality, and better disease resistance (Harveson 2016). Consequently, the sunflower hectarage rapidly increased from 1,000 ha in 1970 to more than 2.2 million ha in 1979, which was the maximum hectares planted in a single year for the U.S. (Harveson 2016; USDA-NASS 2016). From 1979-1986, the annual planted hectarage decreased to approximately 800,000 ha, where it has consistently remained for the past 30 years. In 2015, 750,000 ha were planted in the U.S. with almost 80% of those hectares raised in the three North Central states of North Dakota, South Dakota, and Minnesota. Other states with significant sunflower production in the U.S. include Nebraska, Colorado, Kansas, and Texas. Additionally, sunflowers are grown in California where the climate can support year-round sunflower production, which is ideal for breeding programs. North Dakota typically leads all states in the total number of hectares planted. In 2015, the U.S. sunflower crop averaged a yield of 1,821 kg ha^{-1} which produced 1.3 billion kg valued at 559 million U.S. dollars (USDA-NASS).

Sunflower uses. Currently in the U.S., two market types of sunflowers are produced, which include oilseed types and non-oilseed (confectionary) types. Oilseed types have seeds with higher oil content. These can be crushed, have the oil extracted, and used for cooking oil. Non-oilseed types are typically larger seeded and may be roasted with or without the shell for human food or may be sold as bird food. Other relatively minor uses of sunflower include types grown

for ornamental purposes (Berglund 2007). In 2015, approximately 80% of the U.S. hectarage was planted to oilseed types, while non-oilseed types accounted for the remaining 20% (USDA-NASS 2016).

Sunflower taxonomy and genetics. Cultivated sunflower *(H. annuus*) is a member of the Asteraceae, or Compositae family, which is the largest family of flowering plants. Approximately 10% of all flowering plants are in this family. Since the family is so large, sunflower is further categorized in the Asteraceae subfamily Asteroideae, tribe Heliantheae, and subtribe Helianthinae. *Helianthus* is the genus of the sunflower, which is derived from the two Greek words, *helios* (sun) and *anthus* (flower), which was appropriately named since sunflower is well known for possessing the heliotropism trait (Seiler 2016). The *Helianthus* genus is comprised of 52 species; of these, 14 species are annual (e.g. *H. annuus*, *H. argophyllus*, *H. petiolaris*, *H. praecox*, etc.) and 38 are perennial (e.g. *H. giganteus*, *H. tuberosis*, *H. occidentalis*, and *H. augustifolius*) (Heiser et al. 1969; Schilling 2006). The genus of *Helianthus* has a haploid chromosome number of n = 17. The ploidy levels of the 52 species include diploid (2n = 2x = 34), tetraploid (2n = 4x = 68), and hexaploid (2n = 6x = 102). All 14 of the annual *Helianthus* species are diploid; the perennials can be divided into four categories of diploid, tetraploid, hexaploid, and mixiploid (Berglund 2007; Seiler 2016).

The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) National Plant Germplasm System (NPGS) sunflower collection is one of the largest collections of *Helianthus* germplasm and is housed at the USDA-ARS North Central Regional Plant Introduction Station in Ames, Iowa. A large number of genetically diverse sunflower germplasm is stored for research purposes and for crop improvements. In total, the NPGS sunflower collection is composed of 4,032 accessions of cultivated *H. annuus*, wild *H. annuus*,

other wild annual *Helianthus* species, and perennial *Helianthus* species. These sources of genetically diverse germplasm are extremely valuable to the sunflower industry (Marek et al. 2012). The most significant contributions from sunflower germplasm include the CMS gene, herbicide tolerance genes, salt tolerance genes, and sources of major and minor resistance genes to a large number of economically important diseases of sunflower including rust, downy mildew, *Phomopsis* stem canker, and *Sclerotinia* diseases (Marek et al. 2012; Seiler 2016).

Physical appearance and growth stages. Cultivated sunflowers are much different in appearance to their wild relatives. A cultivated sunflower plant has a single head (capitulum) that sits on a single stem, while many wild, non-domesticated types have branched stems from which multiple smaller heads are produced. Characteristics that make up the general ideotype of a mature, hybrid sunflower include: a large central taproot that can reach depths of 150-270 cm with a lateral root system spanning 60-150 cm in the top 60 cm of soil; a single stem that is 1-10 cm thick and 80-180 cm in height; 23-26 simple leaves (ranging in sizes and shapes) attached to the stem by a petiole; and a head that is 8-35 cm wide. Nut-like fruits called achenes are produced within the head and are made up of the kernel (seed) and the surrounding pericarp (hull). The achene dimensions of oilseed and non-oilseed types can range from 6-9 mm in diameter and 6-25 mm in length (Seiler 2016).

The growth cycle of a sunflower is broken down into three stages. Vegetative emergence (VE) occurs when the seedling has emerged and first true leaves are less than 4 cm in length. Vegetative stages (V-number of leaves) are determined by counting the number of true leaves that are a minimum of 4 cm in length. Reproductive stages (R1-R9) start when the terminal bud is in the form of a floral head and no longer looks like a cluster of leaves (R1) and end when bracts turn yellow and brown at physiological maturity (R9). The days it takes for a sunflower to

reach maturity from planting will vary greatly depending on the cultivar. Typically, a range of 75-150 growing days are needed for maturity. Sunflowers flower independently of day-length and require an average of 2,300-2,400 growing degree days (GDD) (Berglund 2007).

***Plasmopara halstedii* (downy mildew)**

Taxonomy and host range. The fungal-like organism, *Plasmopara halstedii*, was first described in 1876 by American mycologist, B.D. Halsted on *Eupatorium purpureum* L. (red hemp), a plant from the Family Compositae. In 1883, this pathogen was re-described by W.G. Farlow on the basis of Halsted's sample. The fungus was originally called *Peronospora halstedii* Far. In 1888, Berlese and de Toni assigned the fungus to the genus *Plasmopara* and then assigned the specific name *Plasmopara halstedii* (Farl.) Berl. and de Toni which is the present pathogen name (Novotelnova 1966; Sackston 1981; Viranyi and Spring 2011).

Plasmopara halstedii is an obligate organism which belongs to the Kingdom Straminipila, Phylum Oomycota, Order Peronosporales, and Family Peronosporaceae. *Plasmopara halstedii* is pathogenic and capable of causing downy mildew diseases on 80 species within 35 genera belonging to the subfamilies Asteroidae and Cichorioidae of the Family Compositae (Gulya et al. 1997).

Biology and life cycle. *Plasmopara halstedii* produces oospores, a diploid sexual resting structure that has a distinct thick wall around the perimeter of the spore. Oospores form in the tissue underneath the epidermis and are more likely to form in root tissue than in the above ground tissue. It has been hypothesized that oospores must be exposed to several months of freezing before the spore will germinate (Gulya et al. 1997). Oospores germinate and give rise to zoosporangia which are capable of releasing numerous motile, haploid, asexual zoospores that bear two flagella. Primary infection of the host plant occurs when the zoospores contact and

attach to the root, where an appressorium is formed, penetrates the root tissue, and nutrients are extracted from the plant cells via haustoria. In response to cool temperatures and high humidity, asexual sporulation can occur on the underside of leaves of systemically infected plants. Each zoosporangium can release more than a dozen zoospores. Secondary infection by these zoospores can occur, but infection will typically only result in local lesions, which are not of economic importance. Eventually, male antheridia and female oogonia structures form on the tips of hyphae and merge to form oospores, the sexual stage of the fungus (Gulya et al. 1997).

Sunflower downy mildew. Sunflower downy mildew is caused by the pathogen *Plasmopara halstedii* and is an economically important monocyclic disease in sunflower producing regions. The obligate Oomycete pathogen is indigenous to North America where it had been co-evolving with its sunflower host, *Helianthus annuus*, for thousands of years (Gulya et al. 1997). Yield losses in North Dakota from downy mildew can be substantial, but will vary depending on the percentage of infected plants, distribution within the field, and the weather conditions 3-15 days after planting. Healthy sunflower plants adjacent to infected ones can often compensate with larger heads, which offsets yield loss totals. When entire areas are infected, in low spots of the field for example, yield losses can be substantial (Bradley et al. 2007, Friskop et al. 2009; Gulya et al. 2013).

Symptoms and signs. Symptoms are broadly classified as either systemic or localized infections depending on time of infection. When seedlings are exposed to high inoculum concentrations, pre- or post-emergence damping off is often observed (Gulya et al. 1997). When seedling death does not occur, typical symptoms of systemic infection include stunting of the plant due to shortened internodes and puckered leaves with chlorosis bordering the main veins of the leaves. If the systemically infected plant reaches maturity, the heads will be shrunken and

horizontal with no viable seeds. Symptoms of systemic downy mildew can be mistaken or confused with that of herbicide damage caused by plant growth regulators such as 2,4-D. The telltale difference between downy mildew and herbicide damage is to look for signs of white mycelial growth and zoosporangia production on the underside of the leaves. Local lesions, which are non-systemic infections resulting from secondary zoospores, can occur over a much longer period of time than systemic root infections. The local lesions are usually angular in shape and turn chlorotic and eventually necrotic, and produce limited sporulation in humid environments (Friskop et al. 2009; Gulya et al. 1997).

Epidemiology. Sunflower seedlings are susceptible to systemic infection for approximately 12 days after radicle emergence. A combination of inoculum presence and a conducive environment is essential for systemic infection. Large outbreaks of disease occur when sunflower seeds are planted into an area where high concentrations of soil-borne inoculum exist, including; oospores increased from previously infected crops, windborne zoosporangia from neighboring fields, or water runoff containing zoosporangia or oospores (Gulya et al. 1997). Cool, optimum temperatures ranging from 16-20°C and water-saturated soils soon after planting favor systemic infection by providing a conducive environment for the motile zoospores to reach the sunflower seedling roots (Sackston 1981). The percentage of infected plants is dependent upon the amount of rain that is received 3-15 days after the seeds are planted (Gulya et al. 1997). Warm soil temperatures in excess of 26°C and dry conditions are conducive for fast seedling emergence, which greatly reduces the chance of systemic infection (Sackston 1981). Dry and warm soils may negatively affect the ability of the oospore to germinate, and may inhibit the zoospore mobility. Low-lying areas in the field where water can collect can have a

high incidence level of infected plants usually leaving bare spots in the field (Gulya et al. 1997, Gulya et al. 2013; Sackston 1981).

Disease management. Downy mildew can be managed with both fungicide seed treatments and genetic resistance (Gulya et al. 1997). While both methods have been used to effectively manage the pathogen, the genetically variable pathogen has been able to overcome one fungicide mode of action and several resistance genes (Gulya et al. 1999; Gulya and McMullen 2012). Consequently, identification of efficacious fungicides and new sources of resistance are critical for long-term management of downy mildew.

Historically, two phenylamide (PA) fungicides (FRAC 4), metalaxyl (Allegiance FL, Bayer Crop Science, Research Triangle Park, NC) and mefenoxam (Apron XL, Syngenta Crop Protection, Greensboro, NC) were widely used in North America to manage downy mildew. However, in 1999, it was reported that pathogen isolates had developed resistance to both fungicides and was found to be widespread in North Dakota by the 2000's (Gulya et al. 1999). As a result, the chemistry is ineffective wherever insensitive pathogen isolates are present. During the 2000s, two quinone outside inhibitors (QoI) (FRAC 11), azoxystrobin (Dynasty, Syngenta Crop Protection, Greensboro, NC) and fenamidone (Idol, Bayer Crop Science, Research Triangle Park, NC), were registered and labeled for downy mildew management in sunflowers. Today, fenamidone is no longer registered for use in sunflowers, which leaves azoxystrobin as the only labeled fungicide for suppression of downy mildew. Neither QoI compounds are as effective as the two PA chemistries prior to development of resistance (Gulya et al. 2013). Additionally, FRAC 11 compounds are at a high risk for fungicide resistance development, which means there is a need for continued research to find other efficacious fungicides that may provide suppression (Friskop et al. 2014; Gulya et al. 2013).

Downy mildew has been managed in the past with single dominant resistance genes, named *Pl* genes. As many as 20 major resistance genes (Pl_{1-18}, Pl_{21}, and Pl_{arg}) to *P. halstedii* have been identified, at least nine of which have been derived from wild annual *Helianthus* germplasm (Qi et al. 2016; Qi et al. 2015; Vear et al. 2008). However, many of these genes have been overcome by the pathogen in some areas. Since the early 2000s, the Pl_6 and Pl_7 genes were being used to effectively manage the disease in North America until resistant isolates were detected in 2009. By 2011, 31% of isolates screened were virulent on these genes (Gulya and McMullen 2012). As of 2015, seven races (304, 314, 704, 714, 734, 774, and 707) have been identified that confer virulence on Pl_6 and Pl_7 genes (Gilley et al. 2015). Two genes (Pl_{arg} and Pl_{15}) that are effective against these new races are known, but none are believed to be incorporated into commercial hybrids. Given the pathogen's history of overcoming resistance genes, identification of new sources of resistance is critical for long-term management of downy mildew (Gulya and McMullen 2012, Gulya et al. 2013).

***Puccinia helianthi* (rust)**

Taxonomy and host range. The causal pathogen of sunflower rust was first described by Schweinitz in 1882. The material was collected from *Helianthus mollis* in Pennsylvania and was initially named *Aecidium helianthi-mollis*. Before Schweinitz named the pathogen, it was observed causing sunflower rust in several countries, including; Canada, Germany, Austria, Italy, Serbia, Romania, Sweden, and Russia. The pathogen's name was changed to *Puccinia helianthi-mollis*, and eventually the suffix *mollis* was dropped to form the present name *Puccinia helianthi* Schwein. (Bailey 1923). Interestingly, the neotype for *P. helianthi*, designated by Parmalee in 1907, is housed at the Arthur Herbarium at Purdue University in West Lafayette, IN (Sam Markell, *personal communication*).

Puccinia helianthi is an obligate organism belonging to the Kingdom Fungi, Phylum Basidiomycota, Class Pucciniomycetes, Order Uredinales, and Family Pucciniaceae (Cummins and Hiratsuka 2003). *Puccinia helianthi* is pathogenic and capable of causing rust on all annual and perennial *Helianthus* species, and, additionally, on *Heliopsis helianththoides* and *Iva xanthiifolia*. *Puccinia helianthi* is known to exist everywhere in the world where cultivated sunflowers are grown (Gulya et al. 1997).

Biology and life cycle. *Puccinia helianthi* is an autoecious and macrocyclic rust. The five spore stages that all occur on one host include pycnia, aeciospores, urediniospores, teliospores, and basidiospores. Telia are the overwintering structures and are the only true diploid stage occurring within the lifecycle. Each of the two-celled teliospores germinate to form four haploid basidiospores via meiosis. Basidiospores are spread by wind and rain to infect new tissue upon contact. The basidiospore penetrates the epidermis and produces monokaryotic mycelium composed of either the (+) or (-) mating types. The mycelium near the upper side of the leaf develops into pycnia that have male gametes and receptive female hyphae. At this stage, the fungus cross fertilizes and the new mycelium forms aecia (aecial cups) on the underside of the leaf. Binucleate aeciospores have the capability of surviving long periods and are also able to be disseminated over long distances. The aeciospore lands and penetrates the leaf surface through the stomata and produces dikaryotic mycelium and single-celled dikaryotic urediniospores on both the upper and lower surfaces of the leaf. Urediniospores will continue to re-infect tissue throughout the growing season until environmental conditions that are favorable for telia development occur (Gulya et al. 1997).

Sunflower rust. Sunflower rust, caused by *Puccinia helianthi*, is a polycyclic disease that is economically important in all areas of North America where sunflower is cultivated (Gulya et

al. 1997). Rust is known to overwinter as telia on sunflower in the major growing regions of the U.S. (Markell et al. 2009). This overwintering enables urediniospores to develop early in the growing season which can repeatedly infect healthy tissue of the leaves, stem, and head while conditions are conducive. Rust can reduce photosynthetic processes that can cause reductions in yield, oil, seed size, and test weight in either wet or dry environments (Gulya et al. 1997). Yield reductions of up to 80% have been documented when urediniospores are observed in the early vegetative growth stages (Markell et al. 2009).

Symptoms and signs. *Puccinia helianthi* produces small (0.1-1 mm), pustules on the upper and lower surface of the host leaves. Pustules may even be found on bracts, petioles, and stems. When pustules are found on the stem, they are usually linear in shape, unlike the round pustules on the leaf surfaces. Four of the five spore stages can be visually observed without a microscope. The earliest stage visible is the pycnial stage. Pycnia are small (0.1-1 mm), yellow-orange in color, and found on the upper side of the cotyledons and lower leaves. Aecia will appear in clusters of orange cups on the underside of the leaf immediately opposite the pycnia. Typically 10-14 days after aecia release aeciospores, uredinia will be observed on both sides of the leaves. Uredinia pustules are often surrounded by a chlorotic halo. Uredinia pustules contain dusty, cinnamon-brown urediniospores which can be easily rubbed off the leaf tissue. Uredinia will convert to black telial structures that cannot be rubbed off. The pathogen will overwinter in the telial form until the following spring when they will produce the microscopic, haploid basidiospores (Friskop et al. 2011a, Gulya et al. 1997).

Epidemiology. Sunflower rust can occur anytime during the growing season with the environment and inoculum sources having a major impact on disease onset. If the disease occurs early in the sunflower growing season, it is likely a result of the pathogen overwintering as telia

on the previous year's sunflower residue. If the disease is not observed mid-season or later, it is likely that urediniospores were blown from adjacent fields or from states south of North Dakota on the *Puccinia* pathway (Friskop et al. 2011a).

Favorable conditions for infection include free moisture or dew coinciding with temperatures ranging from 13-30°C. Because the disease is favored by free moisture, it is more common to observe uredinia pustules in leaf depressions or along veins where moisture will persist for prolonged periods of time. When the temperatures become unfavorable for disease development, the repeating uredinial cycle slows until conditions become favorable again. As the season progresses and temperatures become colder and the host ages, the uredinia are triggered to change into the overwintering telial stage. Once telia begin to form, the disease cycle is complete for the remainder of the season (Friskop et al. 2011a).

<u>Disease management.</u> Recommended management tools for sunflower rust include foliar fungicides or genetic resistance (Gulya et al. 1997). Other cultural methods used include crop rotations and controlling wild sunflower populations (Friskop et al. 2011a).

Foliar fungicides are critical to manage rust epidemics. If fungicides are applied in a timely manner, they can help limit new infections and slow the disease progression. Current fungicides registered for use on sunflower rust in North Dakota include the demethylation inhibitor (DMI) tebuconazole (Tebuzol 3.6F, United Phosphorus, Inc., King of Prussia, PA) (FRAC 3); the QoIs pyraclostrobin (Headline, BASF, Research Triangle Park, NC) and azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC) (FRAC 11), and Priaxor (BASF, Research Triangle Park, NC) which includes the succinate dehydrogenase inhibitor (SDHI) fluxapyroxad (FRAC 7) plus pyraclostrobin (Friskop et al. 2014). Recent fungicide

evaluations have demonstrated that available fungicides can effectively manage rust when application is made at an appropriate time (Friskop et al. 2015a).

Timing of a fungicide application is very important to mitigate rust epidemics. If sunflower rust is found early in the season, multiple applications during the growing season may be necessary to prevent economic loss. Fungicide applications should be made when severity on the upper four leaves reaches approximately 1% prior to or at bloom (R5). Fungicide applications made at R6 or later have been shown to have little or no effect on yield (Friskop et al. 2015a).

Sunflower rust can also be managed with resistance genes. However, less than 10% of commercial hybrids have been found to be resistant in North Dakota (Friskop et al. 2011b). Furthermore, shifts in pathogen virulence can occur quickly and overcome resistance. The most virulent race possible (777), as determined by the universal *P. helianthi* differential set, has been isolated and identified in North Dakota (Friskop et al. 2015b). To the best of our knowledge, no hybrids with resistance to race 777 are available in North Dakota.

Crop rotation may help break up the rust cycle which will reduce occurrence of the sexual cycle and limit the frequency of race changes. Controlling wild sunflower populations will reduce reservoir populations and delay the onset of infection. Similarly, controlling wild sunflowers will help delay the onset of early disease development and also help limit the amount of sexual recombination that is occurring in the area. Limiting sexual recombination events will limit development of new races capable of overcoming available resistance (Friskop et al. 2011a).

Literature cited

Bailey, D. L. 1923. Sunflower Rust. University of Minnesota Tech. Bull. 16.

Berglund, D. 2007. Introduction. Pages 1-5 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Bradley, C., Markell, S., and Gulya, T. 2007. Diseases of sunflower. Pages 54-77 in: Sunflower Production Guide. D. R., Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Cummins, G. B. and Hiratsuka, Y. 2003. Illustrated genera of rust fungi. APS Press, St. Paul, MN. 225 pp.

Friskop, A. J., Gulya, T. J., Halley, S. A., Schatz, B. G., Schaefer, J. P., Jordahl, J. G, Meyer, S. M., Misek, K. W., Hendrickson, P., and Markell, S. G. 2015a. Effect of fungicide and timing of application on management of sunflower rust. Plant Dis. 99:1210-1215.

Friskop, A. J., Gulya, T. J., Harveson, R. M., Humann, R. M., Acevedo, M., and Markell, S. G. 2015b. Phenotypic diversity of *Puccinia helianthi* (sunflower rust) in the United States from 2011 and 2012. Plant Dis. 99:1604-1609.

Friskop, A., Markell, S., and Gulya, T. 2009. Downy mildew of sunflower. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1402. Fargo, ND.

Friskop, A., Markell, S., Gulya, T., Halley, S., Schatz, B., Schaefer, J., Wunsch, M., Meyer, S., Kandel, H., Acevedo, M., Venette, J., and Venette R. 2011a. Sunflower rust. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1557. Fargo, ND.

Friskop, A., Markell, S. G., Khan, M., Gudmestad, N., and Secor, G. 2014. 2015 North Dakota field crop plant disease management guide. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-622. Fargo, ND.

Friskop, A. J., Schatz, B., Mathew, F., Hendrickson, P., Gulya, T., and Markell, S. 2011b. Sunflower hybrid susceptibility and the impact on yield to sunflower rust in North Dakota. Phytopathology 101:S2.2.

Gilley, M. A., Markell, S. G., Gulya, T. J., and Misar, C. G. 2015. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers in 2014. Proc. 37th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1245/gilley.downy.mildew.poster_2015.pdf

Gulya, T., Draper, M., Harbour, J., Holen, C., Knodel, J., Lamey, A., and Mason, P. 1999. Metalaxyl resistance in sunflower downy mildew in North America. Proc. 21st Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/510/1999_gulya_metalaxyl.pdf

Gulya, T., Kandel, H., McMullen, M., Knodel, J., Berglund, D., Mathew, F., Lamey, H. A., Nowatzki, J., and Markell, S. 2013. Prevalence and incidence of sunflower downy mildew in North Dakota between 2001 and 2011. Online. Plant Health Progress doi:10.1094/PHP-2013-0522-01-RS.

Gulya, T. J. and McMullen, M. P. 2012. New virulent races of downy mildew: What's new in 2011. Proc. 34th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. www.sunflowernsa.com/.../new-virulent-races-of-downy-mildew---gulya.pdf

Gulya, T., Rashid, K. Y., and Masirevic, S. M. 1997. Sunflower diseases. Pages 263-379 in: Sunflower Technology and Production. A. A. Schneiter ed. American Society of Agronomy, Madison, WI.

Harveson, R. M. 2016. History of sunflower use and production. Pages 1-4 in: Compendium of Sunflower Diseases and Pests. R. M. Harveson, S. G. Markell, Block, C. C., and T. J. Gulya eds. APS Press, St. Paul, MN. In press.

Heiser, C. B., Smith, D. M., Clevenger, S. B., and Martin, W. C. 1969. The North American sunflowers (*Helianthus*). Mem. Torrey Bot. Club 22:1-213.

Marek, L. F., Block, C. C., and Gardner, C. C. A. 2012. 2012 Update: New sunflower genetic resources in the U.S. national sunflower collection and potential use for crop improvement. Proc. 18th Intl. Sunflower Conf. International Sunflower Association. Mar del Plata, Argentina. Online Publication.

Markell, S., Gulya, T., McKay, K., Hutter, M., Hollingsworth, C., Ulstad, V., Koch, R., and Knudsvig, A. 2009. Widespread occurrence of the aecial stage of sunflower rust caused by *Puccinia helianthi* in North Dakota and Minnesota in 2008. Plant Dis. 93:6.

Novotelnova, N. S. 1966. Downy mildew of sunflower. U.S. Dept. Commerce, Natl. Tech. Info. Serv., Springfield, VA.

Putt, E. D. 1997. Early History of Sunflower. Pages 1-20 in: Sunflower Technology and Production. A.A. Schneiter, ed. American Society of Agronomy, Madison, WI.

Qi, L. L., Foley, M. E., Cai, X. W., and Gulya, T. J. 2016. Genetics and mapping of a novel downy mildew resistance gene, Pl_{18}, introgressed from wild *Helianthus argophyllus* into cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. doi 10.1007/s00122-015-2662-2.

Qi, L. L., Long, Y. M., Jan, C. C., Ma, G. J., and Gulya, T. J. 2015. *Pl_{17}* is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (Helianthus annuus L.). Theor. Appl. Genet. 128:757–767.

Sackston, W. E. 1981. Downy mildew of sunflower. Pages 545-576 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London, England.

Schilling, E. E. 2006. *Helianthus*. Pages 141-169 in: Flora of North America North of Mexico. Vol. 21. Flora of North America Editorial Committee, eds. Oxford University Press, New York, NY.

Seiler, G. J. 2016. Botany of the sunflower plant. Pages 4-11 in: Compendium of Sunflower Diseases and Pests. R. M. Harveson, S. G. Markell, Block, C. C., and T. J. Gulya eds. APS Press, St. Paul, MN. In press.

USDA, National Agricultural Statistics Service. 2016. Sunflower quick statistics. Available at: http://quickstats.nass.usda.gov/results/C714A9DF-EFBF-3E42-902C-DE1316523C76?pivot=short_desc

Vear, F., Serieys, H., Petit, A., Serre, F., Boudon, J. P., Roche, S., Walser, P., and Tourvieille de Labrouhe, D. 2008. Origins of major genes for downy mildew resistance in sunflower. Proc. 17th Int. Sunflower Conf. International Sunflower Association, Cordoba, Spain. Online Publication. http://isasunflower.org/fileadmin/documents/aaProceedings/17thISC_CordobaVol1/p125vear.pdf

Viranyi, F. and Spring, O. 2011. Advances in sunflower downy mildew research. Eur. J. Plant Pathol. 129:207-220.

CHAPTER 1. EVALUATION OF OXATHIAPIPROLIN FOR THE MANAGEMENT OF SUNFLOWER DOWNY MILDEW

Introduction

Downy mildew of sunflower (*Helianthus annuus* L.) is caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni and is an economically important disease in the northern sunflower producing regions of the United States (U.S.). The pathogen is an obligate Oomycete that is capable of overwintering as oospores in soils for up to 10 years. Disease development is favored by saturated soils and cool soil temperatures (15-20°C) that facilitate oospore germination shortly after sunflower is planted. Systemic infections occur when motile zoospores infect seedling roots. Most systemically infected plants damp-off prior to emergence, but those that survive grow up stunted and eventually die or go on to produce sterile heads. Secondary infection can occur when windblown zoosporangia from infected plants land on sunflower leaf tissue causing acute, angular lesions. This type of foliar infection does not lead to systemic infection and is not economically important (Friskop et al. 2009; Gulya et al. 2013; Gulya et al. 1997; Sackston 1981).

Downy mildew is a frequent problem in North Dakota (ND), which tends to have cooler temperatures following planting than other states. Results from disease surveys show that prevalence of downy mildew in fields surveyed has ranged from 2-70% and incidence within fields can be as high as 7% since the early 2000's (Gulya et al. 2013). Yield losses are most frequently observed when large water-saturated areas of the field (i.e. low spots) create conditions conducive for systemic infection resulting in significant stand losses in concentrated areas of the field. In ND, it is not uncommon for areas of fields many hectares in size to have nearly 100% yield loss (Gulya et al. 2013).

Due to the high yield loss potential and frequent occurrence of downy mildew, management of the disease is very important. However, management of downy mildew is challenging. Management practices such as crop rotation or altering planting dates are ineffective due to the ability of the oospore to survive many years in the soil until conditions are favorable for germination and infection (Gulya et al. 1997). Genetic resistance can be an effective tool to manage disease and numerous resistance genes have been released and incorporated into many commercial sunflower hybrids over the years, but frequent changes in pathogen races have allowed many of these resistance genes to be overcome (Gulya et al. 2011; Gulya and McMullen 2012). Since systemic infection can only occur during a short time after planting (3-15 days), seed treatments can be a very effective management tool (Gulya et al. 2013; Gulya et al. 1997).

Throughout the 1980s and 1990s, two phenylamide (FRAC 4) fungicides, metalaxyl (Allegiance FL, Bayer Crop Science, Research Triangle Park, NC) and mefenoxam (Apron XL, Syngenta Crop Protection, Greensboro, NC) were used to effectively manage *P. halstedii*. Both of these compounds had excellent efficacy and were commonly used in the U.S. However, in the 1990's, *P. halstedii* isolates had evolved insensitivity to the phenylamide fungicides. By 1999, 91% of isolates collected and screened in ND were insensitive to the phenylamides (Gulya et al. 1999). It was not until the 2000s, when two quinone outside inhibitors (QoIs) (FRAC 11), azoxystrobin (Dynasty, Syngenta Crop Protection, Greensboro, NC) and fenamidone (Idol, Bayer Crop Science, Research Triangle Park, NC) were labeled for downy mildew in sunflower. Today, fenamidone is no longer labeled for use in sunflower production systems, leaving azoxystrobin as the only labeled fungicide currently on the market. However, azoxystrobin is not as effective as metalaxyl and mefenoxam were before insensitivity developed, and is only labeled for suppression. Additionally, QoIs such as azoxystrobin target a single site of action and

are therefore considered high risk for fungicide resistance development (Friskop et al. 2014; Gulya et al. 2013). In order to better manage downy mildew with seed treatments, and to prolong the life of new resistance genes and available fungicides, it is necessary to find other fungicides with efficacy on *P. halstedii*. The objective of this study was to evaluate the efficacy of a novel fungicide compound, oxathiapiprolin (Lumisena, DuPont Crop Protection, Wilmington, DE), for the management of downy mildew on sunflower.

Materials and Methods

Locations and experimental design. Between 2011 and 2015, 17 trials were conducted at three locations in ND: the Fargo Agricultural Experiment Station in Fargo, ND (FAR), the DuPont Agricultural Research Farm in Thompson, ND (THOM), and the North Dakota State University (NDSU) Carrington Research Extension Center in Carrington, ND (CREC). All trials were conducted in a randomized complete block design with four to six replicates. All locations were dry-land, however FAR and CREC had temporary drip irrigation systems in place during artificial inoculations to provide supplemental water to facilitate infection. Single row plots were planted in all locations with the exception of CREC 2011 and 2012, where two row plots were established. A cone type planter (Almaco, Nevada, IA) was used for planting in the FAR location and vacuum type planters (Monosem, Edwardsville, KS) were used in the THOM and CREC locations. Row width was 76.2 cm at all locations. Seed spacing, plot length, planting date, inoculation date, and incidence assessments varied by location and years (Table 1.1). Recommended agronomic practices for sunflower production were followed for all trials (Berglund 2007).

Table 1.1. Agronomic and sunflower downy mildew trial information for all experimental locations.

							Days after planting						
								Incidence evaluation					
Location	Planting date	Row width (cm)	Rows plot^{-1}	Plot width (cm)	Plot length (m)	Seed spacing (cm)	Artificial inoculation	1	2	3	4	Number of treatments	Measurable disease pressure
Carrington	13 May 11	76.2	2	152.4	5.8	6.8	10	31	46	60	-	10	Yes
Fargo	18 May 11	76.2	1	76.2	7.6	15.2	7	26	37	51	65	10	Yes
Thompson	16 May 11	76.2	1	76.2	7.6	15.2	7	36	54	-	-	10	Yes
Carrington	15 May 12	76.2	2	152.4	5.8	7.6	10	37	48	-	-	9	Yes
Fargo 1	17 May 12	76.2	1	76.2	7.6	15.2	12	-	-	-	-	9	No
Fargo 2	5 July 12	76.2	1	76.2	7.6	15.2	5	-	-	-	-	9	No
Thompson	14 May 12	76.2	1	76.2	7.6	15.2	7	45	52	-	-	9	Yes
Carrington	24 May 13	76.2	1	76.2	5.8	7.6	7	34	48	59	-	8	Yes
Fargo 1	28 May 13	76.2	1	76.2	7.6	15.2	7	23	38	52	-	8	Yes
Fargo 2	25 July 13	76.2	1	76.2	7.6	15.2	7	28	40	61	-	8	Yes
Thompson	16 May 13	76.2	1	76.2	7.6	15.2	7	27	35	46	-	8	Yes
Carrington	15 May 14	76.2	1	76.2	6.4	13.3	8	23	39	57	-	5	Yes
Fargo	9 June 14	76.2	1	76.2	7.6	15.2	7	24	35	49	-	5	Yes
Thompson	27 May 14	76.2	1	76.2	7.6	13.4	3	-	-	-	-	5	No
Carrington	12 May 15	76.2	1	76.2	4.5	13.3	9	-	-	-	-	8	No
Fargo	5 June 15	76.2	1	76.2	7.6	15.2	5	-	-	-	-	8	No
Thompson	22 May 15	76.2	1	76.2	7.6	15.2	5	-	-	-	-	8	No

Pathogen and inoculation. In order to facilitate infection, all plots were inoculated with pathogen zoosporangia. *Plasmopara halstedii* isolates selected for this study were originally collected from ND and virulence-phenotyped on an internationally recognized set of differentials to determine their race nomenclature; namely, 714 and 734. (Tourvieille de Labrouhe et al. 2000). For all trials in all years, a bulk mixture of isolates of races commonly found in ND and virulent on the susceptible hybrid '63N82' (Pioneer, Johnston, IA) were increased to ensure that the pathogen was virulent. Additionally, all isolates included were insensitive to metalaxyl and mefenoxam.

A source of fresh inoculum with high viability was used to inoculate each field trial. In order to accomplish this, inoculum increases were timed to coincide with the planting of field trials. Inoculum was increased by germinating the susceptible hybrids Mycogen '270' and '8D310' (Dow AgroSciences, Indianapolis, IN) in a germination chamber at 21°C for three days or until radicles grew to a length of 3 cm. *Plasmopara halstedii* zoosporangia were suspended in a 10 mM $CaCl_2$ solution at a concentration of approximately 4.0×10^4 zoosporangia ml^{-1}. Germinated seedlings were soaked in this zoosporangia suspension in a 15°C dark room for 3-4 h and then hand planted into a mixture of sand and perlite (3:2 ratio). The seedlings were grown in a greenhouse at 20-22°C under a 14 h diurnal photoperiod. After 10-11 days, the infected seedlings were moved into chambers in a 16-20°C dark room at 100% relative humidity and allowed to sporulate for 16-24 h (Gulya 1996). Cotyledons covered with zoosporangia were allowed to dry for four hours and harvested into plastic bags and stored at 4°C until they were used for the field inoculation (up to 10 days).

Field trials were inoculated when sunflower radicles reached a length of 1-3 cm, which was approximately 5-12 days after planting the trial depending on temperature and moisture after

planting. Immediately before inoculation, zoosporangia on the harvested seedlings were washed off by shaking them vigorously in distilled water. The solution was then poured through a strainer to remove the plant debris, leaving only the zoosporangia suspended in distilled water. The inoculum concentrations for trials with measurable disease pressure ranged from 5.2×10^5 – 6.9×10^7 zoosporangia per linear meter, with a combined average across all locations of 1.5×10^7 zoosporangia per linear meter.

Two equally effective techniques were used for artificially inoculating the field trials. Technique one introduced the spores to the plots via a drip tape system. Drip tape (T-tape 510-08-340, John Deere Water, San Marcos, CA) was placed over every plot row (76.2 cm intervals) and connected to a header hose (3.8 cm diameter) at the front of the trial. A chemical injection system was used to inject the spore solution into the header hose of the drip tape system. The second technique introduced the zoosporangia suspension via a 7.6 liter watering can, where 7.6 liters of the spore solution was poured overhead onto each furrow within the trial. Technique one was used for the five CREC 2011 – 2015 and three FAR 2011 – 2012 trials, while technique two was used for the four FAR 2013 – 2015 and five THOM 2011 – 2015 trials.

Fungicide efficacy. The number and combination of fungicide treatments in trials evaluated varied among years, but remained consistent across locations within the same year. Fungicides evaluated included mefenoxam (MEF) (Apron XL, Syngenta Crop Protection, Greensboro, NC) a phenylamide fungicide (PA, FRAC 4), azoxystrobin (AZO) (Dynasty, Syngenta Crop Protection, Greensboro, NC) a quinone outside inhibitor fungicide (QoI, FRAC 11), fenamidone (FEN) (Idol, Bayer Crop Science, Research Triangle Park, NC) a QoI (FRAC 11), fludioxonil (FLU) (Maxim, Syngenta Crop Protection, Greensboro, NC) a phenylpyrrole fungicide (PP, FRAC 12), acibenzolar-S-methyl (ACI) (BION, Syngenta Crop Protection,

Greensboro, NC) a benzo-thiadiazole fungicide (BTH, FRAC P1), and oxathiapiprolin (OXA) (Lumisena, DuPont Crop Protection, Wilmington, DE) a piperidinyl thiazole isoxazoline fungicide (FRAC U15). Additionally, AZO and OXA were combined with FLU and MEF (Maxim XL, Syngenta Crop Protection, Greensboro, NC) to form combination treatments, each with three different modes of action (Friskop et al. 2014). Inoculated, non-treated controls (NTC) and non-inoculated, non-treated controls (NI NTC) were included in all trials. All treatments at all locations in all years had a neonicotinoid insecticide, thiamethoxam (THI) (Cruiser, Syngenta Crop Protection, Greensboro, NC) applied at a rate of 250 µg active ingredient (ai) seed^{-1} to protect from insect pests (Knodel et al. 2014). All combinations of fungicides and insecticides were applied as a seed treatment by Incotec Coating and Seed Technology (Salinas, CA) and supplied by DuPont Crop Protection (Wilmington, DE).

Data collection. Disease evaluations were conducted visually by examining all plants within a treatment plot for signs and symptoms consistent with systemic infection by *P. halstedii*, including chlorosis bordering veins or on entire leaf, masses of white zoosporangia on undersides of chlorotic leaves, and stunting. Percent incidence was calculated as: incidence (%) = (number of systemically infected plants / total number of plants) x 100. Additionally, stand counts (plants hectare^{-1}) and phytotoxicity was assessed while rating incidence. Phytotoxicity was determined by visually comparing the overall appearance of sunflowers with fungicide seed treatments to the NTC. Phytotoxicity was recorded on a 0-10 scale, where 0 was equal to no phytotoxic symptoms present and 10 was equal to severe necrosis, bronzing, distorted or misshapen plant parts, and any other potential phytotoxic reactions. Two to four assessments were conducted when sunflowers were in early vegetative stages (VE-V4), mid-vegetative stages (V8-V12), late

vegetative stages and/or early reproductive stages (V14-R1); depending on time of symptom onset and development (Berglund 2007). No yield data was collected for any of the trials.

Statistical analysis. Data were analyzed at each location separately due to differences in disease development at each location. SAS version 9.4 (SAS Institute, Cary, NC) was used to run a general linear model (GLM) procedure to conduct an analysis of variance (ANOVA). When analyses were significant at the 95% level of confidence, Fisher's protected least significant differences (LSD) at $\alpha = 0.05$ were used to separate treatment means.

Results

Fungicide efficacy. In 2011, three trials were conducted; at FAR, THOM, and CREC (Tables 1.2, 1.3, and 1.4). A total of 10 treatments were evaluated including; five OXA treatments (at rates of 0.45, 1.8, 7.25, 29.0, and 116.0 µg ai seed^{-1}), MEF (29.0 µg ai seed^{-1}), AZO (50.0 µg ai seed^{-1}), FEN (189.0 µg ai seed^{-1}), NI NTC and NTC. The three trials all had measurable disease pressure. The incidence values of the NTC at the first rating for FAR, THOM, and CREC were 79.9%, 36.1%, and 65.7%, respectively. According to the ANOVAs, fungicide treatments had a significant effect on incidence for all ratings at all locations (P<0.0001). All treatments had significantly less incidence than the NTC, except MEF. For all trials, the four highest rates of OXA (at 1.8, 7.25, 29.0, and 116.0 µg ai seed^{-1}) had significantly lower incidence than the NTC and MEF. The three highest rates of OXA (at 7.25, 29.0, and 116.0 µg ai seed^{-1}) had the same incidence values, and all had statistically lower incidence values when compared to the AZO standard. Additionally, the three highest OXA rates had lower incidence levels than FEN at the CREC and THOM locations, but the same incidence at the FAR location. Overall, the three highest rates of OXA consistently had the lowest incidence levels for all three trials. No phytotoxicity was observed in any of the treatments containing fungicide.

Table 1.2. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2011.

Treatments		Carrington 2011					
		31 DAP[w]		46 DAP		60 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	0.45	30.9	b[z]	15.3	b	29.1	bc
OXA	1.8	10.4	c	13.7	bc	18.3	cde
OXA	7.25	3.0	d	3.0	cd	10.8	de
OXA	29	2.9	d	4.2	cd	9.5	e
OXA	116	1.9	d	1.1	d	8.3	e
MEF	29	59.5	a	49.3	a	52.8	a
AZO	50	34.2	b	15.8	b	31.0	bc
FEN	188.9	10.5	c	12.1	bc	23.1	cd
NI NTC	-	0.9	d	5.4	bcd	7.6	e
NTC	-	65.7	a	44.0	a	39.0	b
LSD ($\alpha = 0.05$)		7.0		10.9		12.7	
P value		<0.0001		<0.0001		<0.0001	
CV		21.9		45.6		38.2	

[w]DAP = days after planting

[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[y]µg active ingredient seed^{-1}

[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 1.3. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Fargo, ND in 2011.

Treatments		Fargo 2011							
		26 DAP[w]		37 DAP		51 DAP		65 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)		Inc 4 (%)	
OXA	0.45	33.0	c[z]	58.2	b	38.5	b	15.0	b
OXA	1.8	11.2	d	19.8	c	15.9	c	5.6	b
OXA	7.25	4.6	d	11.1	cd	8.0	c	3.0	b
OXA	29	0.0	d	0.6	d	1.3	c	6.4	b
OXA	116	0.0	d	0.6	d	3.5	c	4.5	b
MEF	29	60.9	ab	74.1	ab	48.7	b	39.6	a
AZO	50	45.8	bc	64.0	b	41.6	b	15.6	b
FEN	188.9	3.4	d	9.1	cd	11.5	c	7.6	b
NI NTC	-	73.4	a	82.1	a	72.2	a	43.9	a
NTC	-	79.9	a	83.2	a	55.2	ab	59.9	a
LSD (α = 0.05)		21.7		17.4		22.2		21.9	
P value		<0.0001		<0.0001		<0.0001		<0.0001	
CV		47.9		29.7		51.7		74.9	

[w]DAP = days after planting

[x]All treatments had thiamethoxam applied at a rate of 250 μg active ingredient seed^{-1}; OXA = oxathiapiprolin; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[y]μg active ingredient seed^{-1}

[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table 1.4. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Thompson, ND in 2011.

Treatments		Thompson 2011			
		36 DAP[w]		54 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)	
OXA	0.45	27.6	ab[z]	30.9	ab
OXA	1.8	10.9	cd	11.8	c
OXA	7.25	1.7	de	2.5	d
OXA	29	0.8	e	0.8	d
OXA	116	0.0	e	0.9	d
MEF	29	35.3	a	38.2	a
AZO	50	24.7	b	25.4	b
FEN	188.9	19.8	bc	22.8	b
NI NTC	-	0.0	e	0.0	d
NTC	-	36.1	a	36.8	a
LSD (α = 0.05)		9.4		9.1	
P value		<0.0001		<0.0001	
CV		41.5		37.0	

[w]DAP = days after planting
[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.
[y]µg active ingredient seed^{-1}
[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

In 2012, four trials were conducted; at FAR (two trials), THOM, and CREC where a total of nine treatments were tested, two of which were treatments with a combination of three fungicides (Table 1.5). The treatments included four OXA treatments (at rates of 4.69, 9.37, 18.75, and 37.5 µg ai seed^{-1}), OXA + FLU + MEF (9.37 + 2.5 + 29.0 µg ai seed^{-1}), AZO + FLU + MEF (100.0 + 2.5 + 29.0 µg ai seed^{-1}), FEN (188.9 µg ai seed^{-1}), NI NTC, and NTC. Measurable disease pressure developed in both the THOM and CREC locations, but disease failed to develop in both FAR trials (data not presented). The disease pressure was higher at THOM than at the CREC with incidence values of the NTC at the first rating of 62.9% and 11.7%, respectively. According to the ANOVAs, fungicide treatments had a significant effect on

incidence for all ratings at both the THOM and CREC locations at the 95% level of confidence. For the THOM location, all treatments with a fungicide had statistically lower incidence than the NTC. All treatments containing OXA had statistically lower incidence than all other treatments at the first evaluation, and all other treatments but FEN at the second evaluation. Low disease pressure in the CREC location resulted in all seven treatments containing a fungicide performing equally and having statistically lower incidence than the NTC. No phytotoxicity was observed in any of the treatments containing fungicides.

Table 1.5. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trials in Carrington and Thompson, ND in 2012.

Treatments		Carrington 2012				Thompson 2012			
		37 DAP[w]		48 DAP		45 DAP		52 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 1 (%)		Inc 2 (%)	
OXA	4.69	3.9	b[z]	5.3	bc	18.7	de	16.2	de
OXA	9.37	2.7	b	2.7	bc	12.8	ef	13.4	ef
OXA	18.75	2.4	b	2.8	bc	19.9	d	18.4	cd
OXA	37.5	1.6	b	1.0	bc	8.4	fg	7.3	fg
OXA+FLU+MEF	9.37+2.5+29	1.4	b	1.4	bc	7.2	fg	4.5	fg
AZO+FLU+MEF	100+2.5+29	6.3	ab	6.5	ab	40.1	b	37.7	b
FEN	188.9	1.9	b	2.5	bc	31.1	c	25.3	c
NI NTC	-	0.0	b	0.0	c	1.7	g	1.3	g
NTC	-	11.7	a	11.8	a	62.9	a	54.9	a
LSD (α = 0.05)		6.3		5.8		7.1		7.7	
P value		0.0301		0.0112		<0.0001		<0.0001	
CV		122.4		106.5		21.6		27.0	

[w]DAP = days after planting
[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.
[y]µg active ingredient seed^{-1}
[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

In 2013, four trials were conducted; at FAR (two trials), THOM, and CREC (Tables 1.6, 1.7, 1.8, and 1.9). All of the same treatments from 2012 were used in the 2013 trials except the

4.69 μg ai seed^{-1} rate of OXA. All four trials had measurable disease pressure and were among the highest at all location x years in the study. The incidence values of the NTC at the first rating for FAR 1, FAR 2, THOM, and CREC were 91.5%, 45.0%, 58.0%, and 67.3%, respectively. According to the ANOVAs, all fungicide treatments had a significant effect on the incidence for all ratings at all four locations (P<0.0001). All treatments containing a fungicide had lower disease incidence compared to the NTC. All treatments containing OXA or FEN had incidence values that were statistically the same, but lower than the AZO + FLU + MEF treatment. Overall, all treatments performed consistently at each of the four locations. No phytotoxicity was observed in any of the treatments containing fungicides.

Table 1.6. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2013.

Treatments		Carrington 2013		
		34 DAP[w]	48 DAP	59 DAP
Fungicide[x]	Rate[y]	Inc 1 (%)	Inc 2 (%)	Inc 3 (%)
OXA	9.37	4.0 c[z]	3.8 c	3.1 c
OXA	18.75	1.9 c	2.4 c	2.2 c
OXA	37.5	0.0 c	0.0 c	0.0 c
OXA+FLU+MEF	9.37+2.5+29	0.8 c	0.6 c	0.5 c
AZO+FLU+MEF	100+2.5+29	20.0 b	23.3 b	18.7 b
FEN	188.9	1.3 c	1.3 c	0.7 c
NI NTC	-	67.7 a	67.3 a	62.2 a
NTC	-	67.3 a	68.5 a	65.0 a
LSD (α = 0.05)		5.3	7.0	6.7
P value		<0.0001	<0.0001	<0.0001
CV		22.2	28.5	30.0

[w]DAP = days after planting
[x]All treatments had thiamethoxam applied at a rate of 250 μg active ingredient seed^{-1}; OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.
[y]μg active ingredient seed^{-1}
[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table 1.7. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial 1 in Fargo, ND in 2013.

Treatments		Fargo 1 2013					
		23 DAP[w]		38 DAP		52 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	9.37	0.0	d[z]	0.4	c	2.0	c
OXA	18.75	0.0	d	0.0	c	0.4	c
OXA	37.5	0.4	d	0.0	c	0.8	c
OXA+FLU+MEF	9.37+2.5+29	0.0	d	0.0	c	1.2	c
AZO+FLU+MEF	100+2.5+29	38.9	c	54.3	b	51.4	b
FEN	188.9	0.4	d	1.2	c	3.1	c
NI NTC	-	60.9	b	60.9	b	63.7	b
NTC	-	91.5	a	87.3	a	87.6	a
LSD ($\alpha = 0.05$)		14.5		14.9		16.9	
P value		<0.0001		<0.0001		<0.0001	
CV		51.6		49.7		54.7	

[w]DAP = days after planting

[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[y]µg active ingredient seed^{-1}

[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

Table 1.8. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial 2 in Fargo, ND in 2013.

Treatments		Fargo 2 2013					
		28 DAP[w]		40 DAP		61 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	9.37	0.0	c[z]	0.0	b	0.0	c
OXA	18.75	0.0	c	1.1	b	1.1	c
OXA	37.5	2.1	c	2.2	b	0.0	c
OXA+FLU+MEF	9.37+2.5+29	0.8	c	0.8	b	0.0	c
AZO+FLU+MEF	100+2.5+29	28.4	b	32.2	a	27.8	b
FEN	188.9	1.8	c	5.3	b	6.1	c
NI NTC	-	1.8	c	1.9	b	0.0	c
NTC	-	45.0	a	38.7	a	41.2	a
LSD (α = 0.05)		7.2		8.9		10.9	
P value		<0.0001		<0.0001		<0.0001	
CV		49.0		59.1		77.5	

[w]DAP = days after planting

[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient $seed^{-1}$; OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[y]µg active ingredient $seed^{-1}$

[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table 1.9. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Thompson, ND in 2013.

Treatments		Thompson 2013					
		27 DAP[w]		35 DAP		46 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	9.37	0.7	c[z]	3.0	c	3.5	c
OXA	18.75	1.2	c	3.5	c	3.5	c
OXA	37.5	0.7	c	1.7	c	1.1	c
OXA+FLU+MEF	9.37+2.5+29	0.3	c	2.2	c	2.6	c
AZO+FLU+MEF	100+2.5+29	12.8	b	23.5	b	27.5	b
FEN	188.9	0.4	c	2.4	c	3.3	c
NI NTC	-	8.5	b	9.6	c	8.2	c
NTC	-	58.0	a	55.8	a	52.3	a
LSD ($\alpha = 0.05$)		7.3		8.4		9.5	
P value		<0.0001		<0.0001		<0.0001	
CV		60.3		56.6		63.6	

[w]DAP = days after planting
[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; FLU = fludioxonil; MEF = mefenoxam; AZO = azoxystrobin; FEN = fenamidone; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.
[y]µg active ingredient seed^{-1}
[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference ($\alpha = 0.05$).

In 2014, three trials were conducted; at FAR, THOM, and CREC (Tables 1.10 and 1.11). The number of treatments tested was reduced from eight to five and included two OXA treatments (9.37 and 18.75µg ai seed^{-1}), AZO + FLU + MEF (100.0 + 2.5 + 29.0), NI NTC, and NTC. Measurable disease pressure developed at both the FAR and CREC locations, but disease failed to develop in THOM (data not presented). The disease pressure was higher at CREC than at FAR with incidence values of the NTC at the first rating of 83.4% and 36.5%, respectively. According to the ANOVAs, fungicide treatments had a significant effect on incidence for all ratings at both the FAR and CREC locations at the 95% level of confidence. In FAR, all fungicide treatments had significantly lower incidence levels than the NTC. Both rates of OXA had lower incidence levels than the AZO + FLU + MEF combination treatment. In CREC, all

treatments with fungicides had lower incidence levels than the NTC. Additionally, both treatments of OXA had lower incidence levels than the AZO + FLU + MEF treatment. Overall incidence levels of OXA treatments were greater under the higher disease pressure in CREC when compared to the incidence under lower disease pressure in the FAR location. No phytotoxicity was observed in any of the treatments containing fungicides.

Table 1.10. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Carrington, ND in 2014.

Treatments		Carrington 2014					
		23 DAP[w]		39 DAP		57 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	9.37	1.8	c[z]	21.6	c	30.7	c
OXA	18.75	1.7	c	17.8	c	40.5	bc
AZO+FLU+MEF	100+2.5+29	21.9	b	62.9	b	59.6	ab
NI NTC	-	0.0	c	13.8	c	26.7	c
NTC	-	83.4	a	90.3	a	72.2	a
LSD (α = 0.05)		8.9		8.7		21.3	
P value		<0.0001		<0.0001		0.0009	
CV		34.0		17.4		38.5	

[w]DAP = days after planting
[x]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; AZO = azoxystrobin; FLU = fludioxonil; MEF = mefenoxam; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.
[y]µg active ingredient seed^{-1}
[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

Table 1.11. Sunflower downy mildew incidence (%) for fungicide seed treatment efficacy trial in Fargo, ND in 2014.

Treatments		Fargo 2014					
		24 DAP[w]		35 DAP		49 DAP	
Fungicide[x]	Rate[y]	Inc 1 (%)		Inc 2 (%)		Inc 3 (%)	
OXA	9.37	1.2	b[z]	1.9	c	1.8	c
OXA	18.75	1.4	b	1.4	c	1.4	c
AZO+FLU+MEF	100+2.5+29	9.7	b	22.1	b	24.7	b
NI NTC	-	26.8	a	34.0	ab	25.0	b
NTC	-	36.5	a	49.8	a	44.1	a
LSD (α = 0.05)		16.0		18.8		15.7	
P value		0.0004		<0.0001		<0.0001	
CV		88.1		71.7		67.4	

[w]DAP = days after planting

[x]All treatments had thiamethoxam applied at a rate of 250 μg active ingredient seed^{-1}; OXA = oxathiapiprolin; AZO = azoxystrobin; FLU = fludioxonil; MEF = mefenoxam; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[y]μg active ingredient seed^{-1}

[z]Columns labeled with the same letter are not statistically different based on Fisher's protected least significant difference (α = 0.05).

In 2015, field trials were planted at FAR, THOM, and CREC where a total of eight treatments were tested, three of which were combinations of two or three fungicides (Table 1.12). The treatments included two OXA treatments (at rates of 9.37 and 18.75 μg ai seed^{-1}), ACI (25.0 μg ai seed^{-1}), OXA + ACI (9.37 + 25.0 μg ai seed^{-1}), OXA + ACI (18.75 + 25.0 μg ai seed^{-1}), AZO + FLU + MEF (100 + 2.5 + 29.0 μg ai seed^{-1}), NI NTC, and NTC. No measurable disease pressure developed at any of the three locations (data not presented). No phytotoxicity was observed in any of the treatments containing fungicides.

Table 1.12. Treatment list for sunflower downy mildew seed treatment efficacy trial in 2015.

Treatments	
Fungicide[a]	Rate[b]
OXA	9.37
OXA	18.75
ACI	25
OXA + ACI	9.37+25
OXA + ACI	18.75+25
AZO+FLU+MEF	100+2.5+29
NI NTC	-
NTC	-

[a]All treatments had thiamethoxam applied at a rate of 250 µg active ingredient seed^{-1}; OXA = oxathiapiprolin; ACI = acibenzolar-S-methyl; AZO = azoxystrobin; FLU = fludioxonil; MEF = mefenoxam; NI NTC = non-inoculated, non-treated control; NTC = non-treated control.

[b]µg active ingredient seed^{-1}

Discussion

The results of this study demonstrate that the new fungicide compound, oxathiapiprolin, significantly and consistently reduced downy mildew incidence under a wide range of disease pressure. During the course of this study, the rate range of oxathiapiprolin was narrowed from 0.45 – 116.0 µg ai seed^{-1} to an optimal effective rate of 9.37 – 18.75 µg ai seed^{-1}. Within the optimum range, oxathiapiprolin is more effective than the only currently available fungicide, azoxystrobin. Oxathiapiprolin outperformed azoxystrobin in 10 of the 11 trials with measurable disease pressure. The only exception was at the CREC 2012 location, where oxathiapiprolin performed the same as the azoxystrobin treatment. This was likely an artifact of low disease pressure in the CREC 2012 location where the incidence value observed for the NTC was 11.7%, which was the lowest value for a NTC in all 11 trials. Additionally, sunflowers treated with oxathiapiprolin showed no symptoms of phytotoxicity in any of the 17 trial locations, even when applied at the highest rate of 116.0 µg ai seed^{-1}.

Oxathiapiprolin is the only compound within the piperidinyl thiazole isoxazoline class of fungicides, which was discovered and developed by DuPont. It targets organisms' oxysterol binding complex and has been classified within the U15 FRAC group (Friskop et al. 2014). Oxathiapiprolin's mode of action is novel and is therefore being evaluated in many other crops to determine its efficacy on other economically important pathogens. Preliminary data shows that oxathiapiprolin is most effective on Oomycetes. Examples of other Oomycete pathogens that oxathiapiprolin has efficacy on include *Phytophthora sojae* which causes root rot of soybeans (*Glycine max*), *Phytophthora infestans* which causes late blight of potato (*Solanum tuberosum*), and *Plasmopara viticola* which causes downy mildew of grapes (*Vitis vinifera*) (Keith D. Johnson, *personal communication*). Both sunflower downy mildew and soybean root rots caused by Oomycetes are managed with oxathiapiprolin applied as a seed treatment. This is different from potato late blight and grape downy mildew, which must be managed with a foliar application of oxathiapiprolin. These results reveal that oxathiapiprolin is an excellent candidate fungicide that may be useful for managing economically important disease of several other crops (Keith D. Johnson, *personal communication*).

In ND, downy mildew remains one of the most economically important sunflower diseases, even though nearly all sunflower growers use a fungicide seed treatment and many use genetic resistance in attempt to manage the disease. Surveys of sunflower downy mildew conducted in the Northern Great Plains in 2014 and 2015 have found that downy mildew is still occurring in over 50% of the fields in ND (Gilley et al. 2015; 2016). The results of our study help explain why high incidence and yield loss continue to occur despite widespread adoption of management tools. In our trials, when azoxystrobin was applied alone it was often overwhelmed by downy mildew in high disease pressure situations. Additionally, even when the highest rate of

100 µg ai seed^{-1} was applied, disease incidence was only moderately (although statistically) reduced when compared to the non-treated check. The insufficient management of currently available seed treatment products is an economic problem for sunflower growers, and because downy mildew is one of the easiest diseases to identify (even from a distance), most sunflower growers are aware that currently available seed treatments are insufficient. Consequently, it is likely sunflower growers will quickly adopt oxathiapiprolin into their management programs as soon as it is registered and made available to them.

The development of fungicide resistance by *P. halstedii* is a significant concern, and should be considered as oxathiapiprolin is made available to growers. The pathogen is highly variable and notorious for quickly overcoming both fungicides and resistance genes, especially when only one of these management strategies is solely relied on for control. Both metalaxyl and mefenoxam were overcome in the U.S. in 15 years. Azoxystrobin was labeled in 2003, was adopted very quickly, and is still currently being heavily relied on by many growers (Gulya et al. 2013). This is placing a heavy selection pressure on the *P. halstedii* population, which could facilitate development of insensitivity to the fungicide. Resistance to the single-site-of-action QoI fungicides like azoxystrobin have been well documented in other crops (Pasche et al. 2004; Wise et al. 2009; Wong and Wilcox 2000). The widespread usage of a fungicide in a high risk FRAC group as a solo product continue to put azoxystrobin at considerable risk for fungicide resistance development.

Oxathiapiprolin targets different sites of action than metalaxyl (FRAC 4), mefenoxam (FRAC 4), and azoxystrobin (FRAC 11), which means that oxathiapiprolin will be able to effectively manage *P. halstedii* populations that are resistant to metalaxyl and mefenoxam or which may become resistant to azoxystrobin. It also means that a viable resistance management

strategy may be to rotate or blend azoxystrobin and oxathiapiprolin. Rotation of FRAC groups is critical for fungicide longevity, and the presumed availability of two products (oxathiapiprolin and azoxystrobin) could conceivably extend both of their effective lifespans.

Interestingly, acibenzolar-S-methyl, which is a compound believed to induce resistance has also been tested in sunflowers and was found to have efficacy on downy mildew. In 2015, our study attempted to test this compound in combination with oxathiapiprolin, but disease did not develop at any of the three locations. However, research conducted in a 2012 field trial found that acibenzolar-S-methyl significantly lowered downy mildew incidence when compared to both the NTC and the treatment containing azoxystrobin (Humann et al. 2014). This is further supported by a study in 2015 that showed that when acibenzolar-S-methyl was applied alone, it resulted in significantly lower disease incidence compared to the NTC in three separate field trials (Humann et al. 2016). These results suggest that acibenzolar-S-methyl could be another compound of a different mode of action than could complement either oxathiapiprolin or azoxystrobin if applied in combination.

In order to keep oxathiapiprolin viable for many years to come, a few good management practices need to be followed. The first is to not rely solely on fungicides for management of downy mildew, this can be accomplished by pairing genetically resistant varieties with the fungicidal seed treatments. This practice will not only work to help prolong the life of the fungicide but will also help slow genetic resistance from being overcome. The second good management practice would be to apply oxathiapiprolin with another fungicide compound within a different FRAC group, such as azoxystrobin, or with a compound that works entirely different, such as acibenzolar-S-methyl.

The discovery, development and presumptive labeling of oxathiapiprolin presents one of the most meaningful disease management advances that sunflower growers have ever had in the Northern Great Plains. Oxathiapiprolin appears to be at least as effective as metalaxyl and mefenoxam were before the development of fungicide resistance by *P. halstedii*. The widespread usage of these products (while they were effective) helped reduce the incidence of downy mildew to the lowest levels observed in many decades. Possibly more important, to the best of our knowledge the simultaneous availability of fungicides as efficacious as azoxystrobin and oxathiapiprolin from two different FRAC groups to sunflower growers has not occurred before. With potential widespread adoption of oxathiapiprolin and a careful resistance management plan, it is possible that the use of oxathiapiprolin in the future may help mitigate the yield loss from downy mildew experienced by growers for many years to come.

Literature Cited

Berglund, D. 2007. Introduction. Pages 1-5 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Friskop, A., Markell, S., and Gulya, T. 2009. Downy mildew of sunflower. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1402. Fargo, ND.

Friskop, A., Markell, S. G., Khan, M., Gudmestad, N., and Secor, G. 2014. 2015 North Dakota field crop plant disease management guide. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-622. Fargo, ND.

Gilley, M. A., Markell, S. G., Gulya, T. J., and Misar, C. G. 2015. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers in 2014. Proc. 37th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1245/gilley.downy.mildew.poster_2015.pdf

Gilley, M., Markell, S., Gulya, T., and Misar, C. 2016. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers. Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1259/prevalence.virulence_gilley.et.al_ppt-2016.pdf

Gulya, T. J. 1996. Everything you should know about downy mildew testing but were afraid to ask. Proc. 18th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/639/1996_gulya_everythingdowny.pdf

Gulya, T., Draper, M., Harbour, J., Holen, C., Knodel, J., Lamey, A., and Mason, P. 1999. Metalaxyl resistance in sunflower downy mildew in North America. Proc. 21st Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/510/1999_gulya_metalaxyl.pdf

Gulya, T., Kandel, H., McMullen, M., Knodel, J., Berglund, D., Mathew, F., Lamey, H. A., Nowatzki, J., and Markell, S. 2013. Prevalence and incidence of sunflower downy mildew in North Dakota between 2001 and 2011. Online. Plant Health Progress doi:10.1094/PHP-2013-0522-01-RS.

Gulya, T. J., Markell, S. G., McMullen, M. V., Harveson, R. M., and Osborne, L. E. 2011. Emergence of new virulent races of Plasmopara halstedii inciting downy mildew on sunflower in the United States. Phytopathology 101:S2.3.

Gulya, T. J. and McMullen, M. P. 2012. New virulent races of downy mildew: What's new in 2011. Proc. 34th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. www.sunflowernsa.com/.../new-virulent-races-of-downy-mildew---gulya.pdf

Gulya, T., Rashid, K. Y., and Masirevic, S. M. 1997. Sunflower diseases. Pages 263-379 in: Sunflower Technology and Production. A. A. Schneiter ed. American Society of Agronomy, Madison, WI.

Humann, R., Belles, D., Gilley, M., Halvorson, J., and Markell, S. 2016. Evaluation of fungicide seed treatment efficacy against three *Plasmopara halstedii* (downy mildew) races in inoculated field trials. Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1295/3Humann-2016NSASyngentaposter.pdf

Humann, R., Wunsch, M., Gulya, T., Kraft, B., Schaefer, M., Friskop, A., and Markell, S. 2014. Evaluation of sunflower seed treatment efficacy for downy mildew (*Plasmopara halstedii*) in North Dakota, 2012. Plant Disease Management Reports 8:ST026.

Knodel, J. J., Beauzay, P., and Boetel, M. 2014. 2015 North Dakota field crop insect management guide. North Dakota State Univ. Coop. Ext. Serv., Publ. E-1143. Fargo, ND.

Pasche, J. S., Wharam, C. M., and Gudmestad, N. C. 2004. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Dis. 88:181-187.

Sackston, W. E. 1981. Downy mildew of sunflower. Pages 545-576 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London, England.

Tourvieille de Labrouhe, D., Gulya, T. J., Masirevic, S., Penaud, A., Rashid, K., and Viranyi, F. 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). Proc. 15th Int. Sunflower Conf. International Sunflower Association, Toulouse, France. Online Publication. http://isasunflower.org/fileadmin/documents/aaProceedings/15thISCToulouse2000/PosterWorkshopI-O/I-F57.pdf

Wise, K. A., Bradley, C. A., Pasche, J. S., and Gudmestad, N. C. 2009. Resistance of QoI fungicides in *Ascochyta rabiei* from chickpea in the Northern Great Plain. Plant Dis. 93:528-536.

Wong, F. P., and Wilcox, W. F. 2000. Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola*. Plant Dis. 84:275-281.

CHAPTER 2. IDENTIFYING *HELIANTHUS* ACCESSIONS RESISTANT TO *PLASMOPARA HALSTEDII*

Introduction

Sunflower (*Helianthus annuus* L.) is indigenous to North America and is believed to have been domesticated by Native Americans more than 2,800 years ago, making it one of the oldest food crops in the world (Putt 1997). Sunflower is an economically important crop in the United States (U.S.) where approximately 800,000 hectares are planted annually (Sandbakken and Kleingartner 2007; USDA-NASS 2016). Sunflower hectarage is dominated by two major market classes; oilseed and non-oilseed (confectionary) types. Oilseed types account for approximately 80% of the annual planted hectares and are harvested and crushed to make cooking oils. Non-oilseed types account for the rest of the market share and are sold for human consumption or as bird food (Berglund 2007; Gulya et al. 2013).

Diseases are the most important biological yield-limiting factor of sunflower (Kandel 2014). One of the most economically important diseases is downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni. *Plasmopara halstedii* is an obligate, fungal-like Oomycete, which is indigenous to North America and overwinters in soils as a thick-walled oospore (Gulya et al. 1997; Viranyi and Spring 2011). Systemic infection occurs when oospores germinate and form zoosporangia that release numerous motile zoospores, each capable of swimming through the soil and infecting sunflower radicles. Systemic infection is favored by cool (15-20°C) and wet conditions, and commonly occurs 3-15 days after planting. Consequently, low spots where water pools in fields are ideal for large patches of sunflowers to be infected. Systemically infected plants commonly damp off pre or post emergence, thus resulting in significant stand reductions that impact yields. Infected plants that do survive will

become severely stunted and dwarfed with chlorotic and puckered leaves. During conditions of high humidity and cool temperatures, masses of white zoosporangia will form on the undersides of the chlorotic leaf tissue. When systemically infected plants live to reproductive stages, plants yield little to no viable seeds, while competing with healthy plants for water and nutrients, causing additional yield loss. Secondary infection can occur when zoosporangia on leaf tissue are wind-dispersed to other leaves causing small, acute, angular lesions on foliar tissue rarely leading to systemic infection. Secondary infection is not economically important (Friskop et al. 2009; Gulya et al. 2013; Gulya et al. 1997).

Downy mildew is one of the most common sunflower diseases in North Dakota (ND), where approximately half of the U.S. sunflower hectares are planted. From 2001 to 2011, an average of 34% of fields had downy mildew with a high of 70% in 2011 and a low of 2% in 2006 (Gulya et al. 2013). In surveys conducted in ND and South Dakota in 2014 and 2015, 65% and 78% of fields had downy mildew, respectively (Gilley et al. 2015; 2016).

The most economical way for growers to manage downy mildew is by planting genetically resistant hybrids. Downy mildew resistance is race-specific (qualitative) and commonly controlled by single dominant genes denoted, *Pl*, for *Plasmopara* (Miller and Gulya 1991; Sackston 1981; 1992; Tan et al. 1992). Hybrids deployed with a single resistance gene increases the selection pressure on the pathogen and has facilitated shifts in pathogen virulence (race changes). Most races confer virulence to genes deployed decades ago, for example Pl_1 and Pl_2 (Fick and Zimmer 1974; Gilley et al. 2015; 2016). However, more recently deployed genes have also been overcome. The gene Pl_6 was widely deployed in the early 2000's and by 2009, virulence was detected in *P. halstedii* isolates in ND. By 2011, 31% of isolates screened were found to be virulent on Pl_6 (Gulya and McMullen 2012). Recent virulence surveys show that nine

virulence phenotypes (races) of *P. halstedii* exist in the Northern Great Plains, seven of which confer virulence on Pl_6 (Gilley et al. 2015). As a consequence, novel sources of resistance are frequently needed because widespread race changes quickly render currently deployed resistance genes ineffective.

Useful resistance genes to downy mildew have been identified from several wild *Helianthus* spp., including Pl_6 and Pl_{17} from *H. annuus* and Pl_8, Pl_{18}, and Pl_{arg} from *H. argophyllus* (Miller and Gulya 1991; Qi et al. 2016; Qi et al. 2015; Vear et al. 2008). In 2005, Gulya identified novel sources of resistance to commonly detected *P. halstedii* races by screening wild *Helianthus* germplasm housed at the United States Department of Agriculture (USDA) North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa (Gulya 2005). Interestingly, a disproportionate number of the resistant *H. annuus* and *H. argophyllus* accessions originated from Texas (TX). This is in agreement with earlier work done in the 1970s, where multiple sources of resistance in wild *Helianthus* germplasm originated from TX (Sackston 1981). The objective of this study was to evaluate the USDA-NCRPIS's collection of wild *Helianthus annuus* and *H. argophyllus* germplasm derived from TX for resistance to common and highly virulent races of *P. halstedii*.

Materials and Methods

Host material. Wild *Helianthus* germplasm was obtained from the USDA-NCRPIS. One hundred eighty-two accessions of wild *H. annuus* and 33 accessions of wild *H. argophyllus*, all derived from TX were obtained for this study. The nine internationally accepted differential lines used for downy mildew race identification were obtained from the USDA Agricultural Research Service (ARS) Sunflower Unit in Fargo, ND; the nine lines include 270 (Mycogen Seeds, Indianapolis, IN) (susceptible), RHA-265, RHA-274, DM-2, PM-17, 803-1, HA-R4, HA-R5, and

HA-335 (Tourvieille de Labrouhe et al. 2000). Mycogen 270 (no resistance genes) and HA-335 (contains Pl_6) were used as susceptible checks during the initial and advanced screening of the germplasm.

Pathogen material and inoculation. All *P. halstedii* isolates used in this study were obtained from the USDA-ARS Sunflower Unit. Each isolate was originally collected from a single plant in the field and virulence phenotyped on the nine internationally accepted differentials (Tourvieille de Labrouhe et al. 2000). For the initial screening, race 714 (isolate ND12_01) was selected because it conferred virulence on the Pl_6 resistance gene, which was widely used in commercial hybrids at the time of the study (Gulya and McMullen 2012). For the advanced screening, a mixture of isolates of different races was used, namely; races 100, 330, 700, 710, 714, and 773. This combination of isolates conferred an aggregate virulence phenotype of 777, which is virulent on all resistance genes harbored by the differentials.

For all experiments, fresh *P. halstedii* inoculum was used. To increase inoculum, seeds of a susceptible hybrid were surface sterilized in a 20% bleach solution for 10 min, thoroughly rinsed off with warm (35-40°C), distilled water and placed on moistened paper towels. Next, the trays of seed were stored in a germination chamber (Seedboro Equipment Company, Chicago, IL) at 24°C until radicle lengths reached approximately 1-3 cm (commonly 72 h), which is ideal for inoculation of cultivated seedlings. When seedlings were ready to be inoculated, viable *P. halstedii* zoosporangia were suspended in a 10 mM calcium and distilled water solution at a concentration of approximately 20,000-40,000 zoosporangia ml^{-1}. Seedlings were soaked in the inoculum solution in the dark at 18°C for 3-4 h, allowing the zoosporangia time to release motile zoospores and infect and encyst the root hairs of the seedling radicles. Inoculated seedlings were planted in 48 x 33 x 6 cm trays (Edge Manufacturing, Bluffton, IN) filled with a sand-perlite

mixture (3:2 v/v ratio). Plants were grown for 10-11 days in a greenhouse room at 20-22°C with a 14 h diurnal photoperiod. To induce sporulation on the foliar tissue, plants were misted with water and moved into a dark and high humidity (> 90%) environment at 17-20°C for 16-24 h. Before inoculum collection, plants were removed from the humidity chambers and allowed to air dry for at least four hours (Gulya 1996). Inoculum was collected by clipping all plant tissue covered with zoosporangia. For temporary storage of fresh inoculum, the spore-covered clipped plant tissue was kept in the dark at 3.5°C for up to 10 days, at which time they begin to lose viability. For long term storage, the spore-covered cotyledons were desiccated, transferred to cryotubes and stored at -80°C (Gulya et al. 1993; Viranyi 1985).

Initial screening. To break dormancy, 60 seeds from each wild *Helianthus* accession were surface sterilized with a 20% bleach solution, placed in a sterile, 100 x 15 mm petri dish (Falcon brand, Corning, Corning, NY) and soaked in 35-40 ml water-ethephon solution created by adding 4.3 ml of ethephon (Florel brand, Monterey Lawn and Garden, Fresno, CA) to one liter of water (Harada 1982; Marek et al. 2012). Seeds were stored in the dark at 3.5°C for 24 h. Seeds were then rinsed with distilled water and stored on moistened blotter paper in the dark at 3.5°C for 14 days. After this cold stratification period, seeds were moved into a 24°C seed germinator until radicle length reached 1-3 cm.

Seedlings with radicles within the targeted length were selected for the experiments and inoculated by soaking in a 10mM calcium-water solution with a concentration of 20,000-40,000 zoosporangia ml^{-1} as previously described. For all experiments, 48 x 33 x 6 cm trays were used. Within each tray, 12 furrows (30 cm in length) were created in rows and inoculated seedlings from 10 accessions and two susceptible checks (270 and HA-335) were sown, one genotype per row. The trial was arranged as a complete randomized design with two replicates for each

accession and the trial was repeated once. Fresh inoculum was used for each replicate. Plants were grown under greenhouse conditions previously described.

After 11 days, flats of inoculated accessions were placed into misting chambers for 24 h to induce sporulation, then removed and allowed to air dry for four hours. Disease assessments were made by visually evaluating the cotyledons of each plant for symptoms and signs consistent with systemic infection by *P. halstedii*, including; chlorosis, sporulation, and post-emergence damping off. Incidence of systemically infected and non-infected plants was recorded.

Advanced screening. Based on the results of the initial screening, the most resistant 12% of accessions from each species were selected for advanced screening. In total, 22 *H. annuus* and three *H. argophyllus* accessions were selected.

Dormancy of 60 seeds from each of the 25 accessions was broken according to methods previously described. Pre-germinated seedlings with radicles within the targeted length (1-3 cm) were inoculated with zoosporangium inoculum composed of six races in equal parts conferring in aggregate, a virulence phenotype of 777. Total concentration of the bulk inoculum mixture was 27,500 zoosporangia ml^{-1} in a 10 mM calcium-water solution as previously described. The design of this trial was the same as the initial trial, except the same batch of fresh inoculum was used throughout.

In order to determine if plants were truly immune from the infection, evaluation of plants for infection was done in two ways. First, a visual above-ground evaluation of each plant was done using the methods previously described. Second, microscopic examination of the roots of asymptomatic plants was done to ensure no infection had occurred and escaped visual detection of symptoms and signs (Gulya and Rama Raje Urs 1995). To do this, the complete root systems of asymptomatic plants are excised from the soil immediately after the above-ground rating.

Gravel and soil particles on roots were gently rinsed off by dipping roots in water and then placed on moistened blotter paper in 27 x 16 x 4 cm plastic boxes. The boxes were moved to a dark, high humidity environment at 17-20°C for a period of 16-24 h to induce sporulation. First, the entire root system of plants was visually evaluated for presence of zoosporangia using a dissecting microscope with a total magnification of 10X. If any signs of zoosporangia were observed singly or borne in grape-like clusters on zoosporangiophores, then the roots were considered to be colonized by *P. halstedii*. Second, an additional color rating was needed to differentiate the resistant plants of an accession from the susceptible checks, 270 and HA-335, which had damped off post emergence and whose hypocotyls and root systems turned necrotic and black in color. These susceptible checks were systemically infected by *P. halstedii*, but the root tissue was not healthy enough for the obligate *P. halstedii* to produce zoosporangia. The color of the roots of the asymptomatic plants and the susceptible checks roots was visually evaluated using a categorical scale where four colors exist and are: 1 = white, 2 = light brown, 3 = dark brown, and 4 = black. Roots with color types of 1 and 2 were considered healthy and capable of supporting zoosporangia production, while types of 3 and 4 were considered to be incapable of supporting zoosporangia production, and considered susceptible to downy mildew.

Initial screening analysis. Plants were categorized as susceptible (systemically infected) or resistant (non-systemically infected) based on the symptoms and signs visually observed on the leaf tissue. Percent resistance was calculated by taking the number of resistant plants in an accession and dividing it by the total number of plants evaluated for that accession, and then multiplying the quotient by a factor of 100 to convert it to a percentage. Additionally, the number of susceptible and resistant plants for an accession was converted to a percent of the total number of plants screened for that accession. For example, if 25 susceptible and 40 resistant plants were

identified for an accession, then the percent susceptible and resistant would be 38% and 62%, respectively.

Advanced screening analysis. Above-ground plant screening was conducted using the methods previously described. For the root screenings, roots with visible zoosporangia colonizing the tissue were categorized as susceptible, while roots with no visible zoosporangia were categorized as resistant. The percent resistance for the root screening was calculated for all accessions using the method previously described. The root color evaluation data was evaluated using a categorical scale (1-4), therefore the median value of the root color was calculated for both the accessions and checks. To find the median, the root color values (1-4) for each accession and both susceptible checks were arranged from least to greatest. Additionally, the frequency of the four root color categories for each accession was presented. This was done by converting an accession's overall total of each of the four color type categories to a percent of the total number of roots rated for that accession. For example, if 12 white, 13 light brown, three dark brown, and zero black roots were identified for an accession, than the accession would be composed of 43% white, 46% light brown, 11% dark brown, and 0% black roots.

Results

Initial screening. Germination of accessions and the number of seedlings with radicles within the appropriate length (1-3 cm) for inoculation was variable. Of the 182 *H. annuus* accessions, the range of seedlings inoculated for an accession was 20-94 with an average of 60 seedlings per accession (Table B.1). Of the combined susceptible checks 270 and HA-335, 96% and 97% of the plants screened were susceptible, respectively. The percent resistance among the accessions ranged from 27-97% (Fig. 2.1). Ninety-two accessions had greater than 70% resistance. Twenty-two accessions were selected and included in the advanced screening.

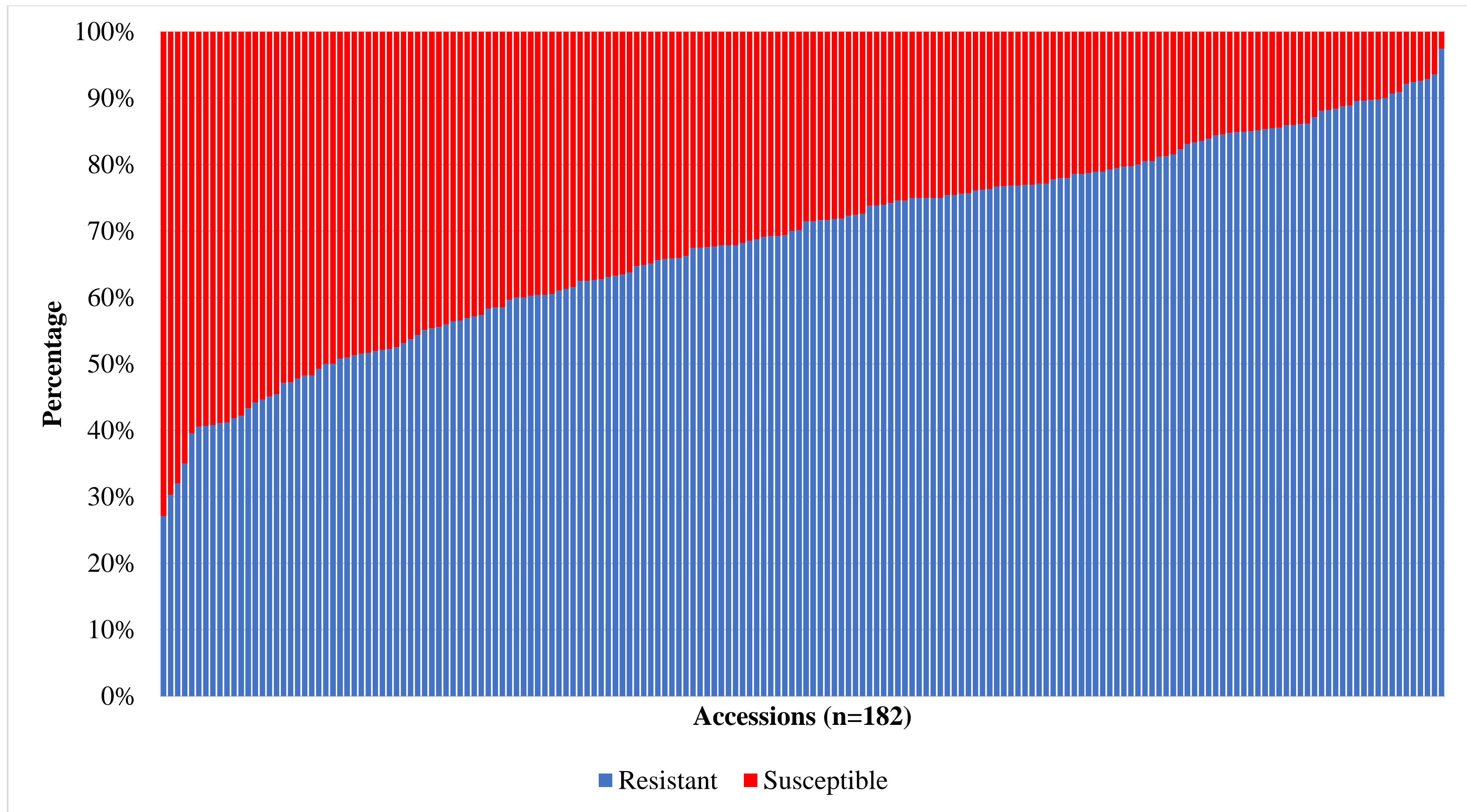

Figure 2.1. Percent of resistant and susceptible plants for each of the 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714.

Of the 33 *H. argophyllus* accessions, the range of seedlings inoculated for an accession was 26-86 with an average of 56 seedlings per accession (Table B.2). Of the combined susceptible checks 270 and HA-335, 99% and 100% of the plants screened were susceptible, respectively. The percent resistance among the accessions ranged from 46-97% (Fig. 2.2). Twenty-three accessions had greater than 70% resistance. Three accessions, all with greater than 95% resistance were selected and included in the advanced screening.

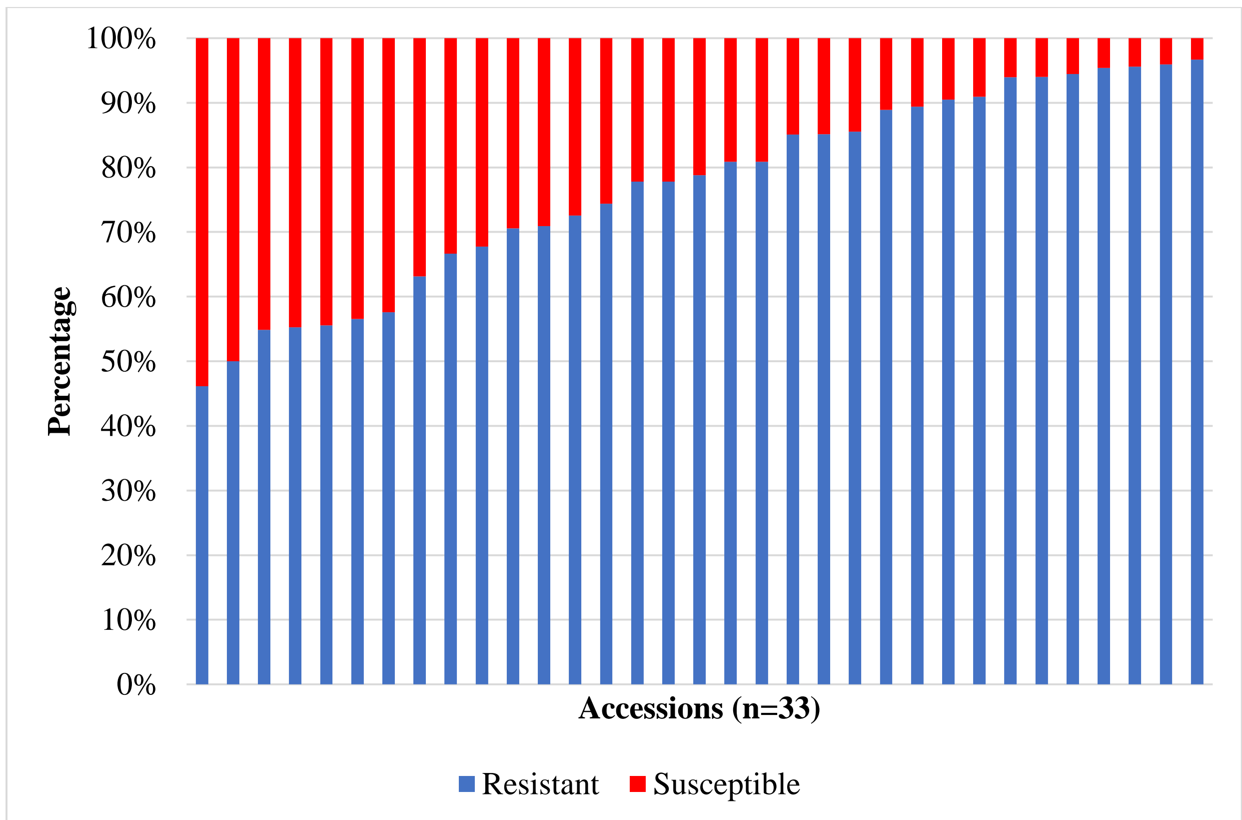

Figure 2.2. Percent of resistant and susceptible plants for each of the 33 wild *Helianthus argophyllus* accessions in the initial screening to *Plasmopara halstedii* race 714.

Advanced screening. For the above-ground evaluation of the 22 *H. annuus* and three *H. argophyllus* accessions, the range of seedlings inoculated for an accession was 12-80 with an average of 54 seedlings per accession (Table 2.1). Of the susceptible 270 and HA-335, 96% and 100% of the plants screened were susceptible, respectively. The percent resistance among the

accessions ranged from 55-93%. Thirteen accessions had greater than 75% resistance, which included all three *H. argophyllus*.

Table 2.1. Total plants and percent resistance for the above-ground evaluation of 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates.

			Above-ground evaluation	
Item No.	Accession No. (PI)	Species	Total plants	Resistance (%)
2	413161	*H. annuus*	45	80
27	435414	*H. annuus*	12	92
32	435419	*H. annuus*	25	76
33	435420	*H. annuus*	78	79
34	435421	*H. annuus*	63	89
44	435432	*H. annuus*	68	72
68	435482	*H. annuus*	12	83
71	435485	*H. annuus*	42	86
102	468445	*H. annuus*	76	71
105	468448	*H. annuus*	51	59
106	468449	*H. annuus*	73	73
113	468456	*H. annuus*	68	74
117	468460	*H. annuus*	72	81
126	468474	*H. annuus*	58	74
129	468477	*H. annuus*	47	60
142	468502	*H. annuus*	74	80
151	468511	*H. annuus*	48	73
165	468525	*H. annuus*	62	73
179	468543	*H. annuus*	51	55
183	494566	*H. annuus*	70	71
201	613746	*H. annuus*	28	89
207	649847	*H. annuus*	28	68
195	494578	*H. argophyllus*	56	93
196	494579	*H. argophyllus*	80	90
212	649863	*H. argophyllus*	67	78
Check	HA335	*H. annuus*	19	0
Check	Myc 270	*H. annuus*	228	4

Plants found to be asymptomatic in the above-ground rating had their root systems excised and visually evaluated for the presence or absence of zoosporangia on the roots. Due to difficulties during the excision process, not all resistant plants were successfully excised and

were therefore not included in the root evaluations. The total number of roots evaluated for an accession ranged from 8-40 with an average of 31 plants per accession (Table 2.2). The percent of plants resistant in the accessions ranged from 27-98%. Fifteen accessions had greater than 75% resistance, which included two of the three *H. argophyllus* accessions. None of the susceptible 270 or HA-335 plants' root systems were evaluated since the majority of the plants damped off post-emergence and any plants that were non-systemically infected were "escapes" that did not produce zoosporangia on the root tissue.

Table 2.2. Total asymptomatic roots, percent resistance, and median root color for the root evaluation of 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates.

			Root evaluation		
Item No.	Accession No. (PI)	Species	Total roots	Resistance (%)	Median root color
2	413161	*H. annuus*	33	85	2
27	435414	*H. annuus*	8	88	1
32	435419	*H. annuus*	19	95	2
33	435420	*H. annuus*	40	98	1
34	435421	*H. annuus*	40	68	1
44	435432	*H. annuus*	36	69	1
68	435482	*H. annuus*	11	27	2
71	435485	*H. annuus*	29	76	1
102	468445	*H. annuus*	40	70	2
105	468448	*H. annuus*	30	73	1
106	468449	*H. annuus*	37	54	2
113	468456	*H. annuus*	38	79	1
117	468460	*H. annuus*	38	82	1
126	468474	*H. annuus*	31	84	2
129	468477	*H. annuus*	24	88	2
142	468502	*H. annuus*	40	85	1
151	468511	*H. annuus*	28	75	1
165	468525	*H. annuus*	38	95	1
179	468543	*H. annuus*	28	71	2
183	494566	*H. annuus*	40	75	2
201	613746	*H. annuus*	16	94	1
207	649847	*H. annuus*	18	89	2
195	494578	*H. argophyllus*	40	95	2
196	494579	*H. argophyllus*	39	97	1
212	649863	*H. argophyllus*	40	73	2
Check	HA335	*H. annuus*	-	-	3
Check	Myc 270	*H. annuus*	-	-	4

In order to compare the susceptible checks 270 and HA-335 to the resistant accessions, the color of each root for all checks and accessions were evaluated with the categorical scale ranging from 1-4. Roots from all 25 accessions had median values ranging from 1-2, or white to light brown roots (Table 2.2). The 270 and HA-335 checks had median values of 4 (black) and 3 (dark brown), respectively. The white (1) and light brown (2) root colors combined, accounted for 89% of the total root colors observed for all 25 accessions (Fig. 2.3). Eighteen *H. annuus* and two *H. argophyllus* accessions were composed entirely of white (1) and light brown (2) root colors. The dark brown (3) and black (4) root colors combined, accounted for at least 80% of the root colors observed for all of the checks, Myc 270 and HA-335.

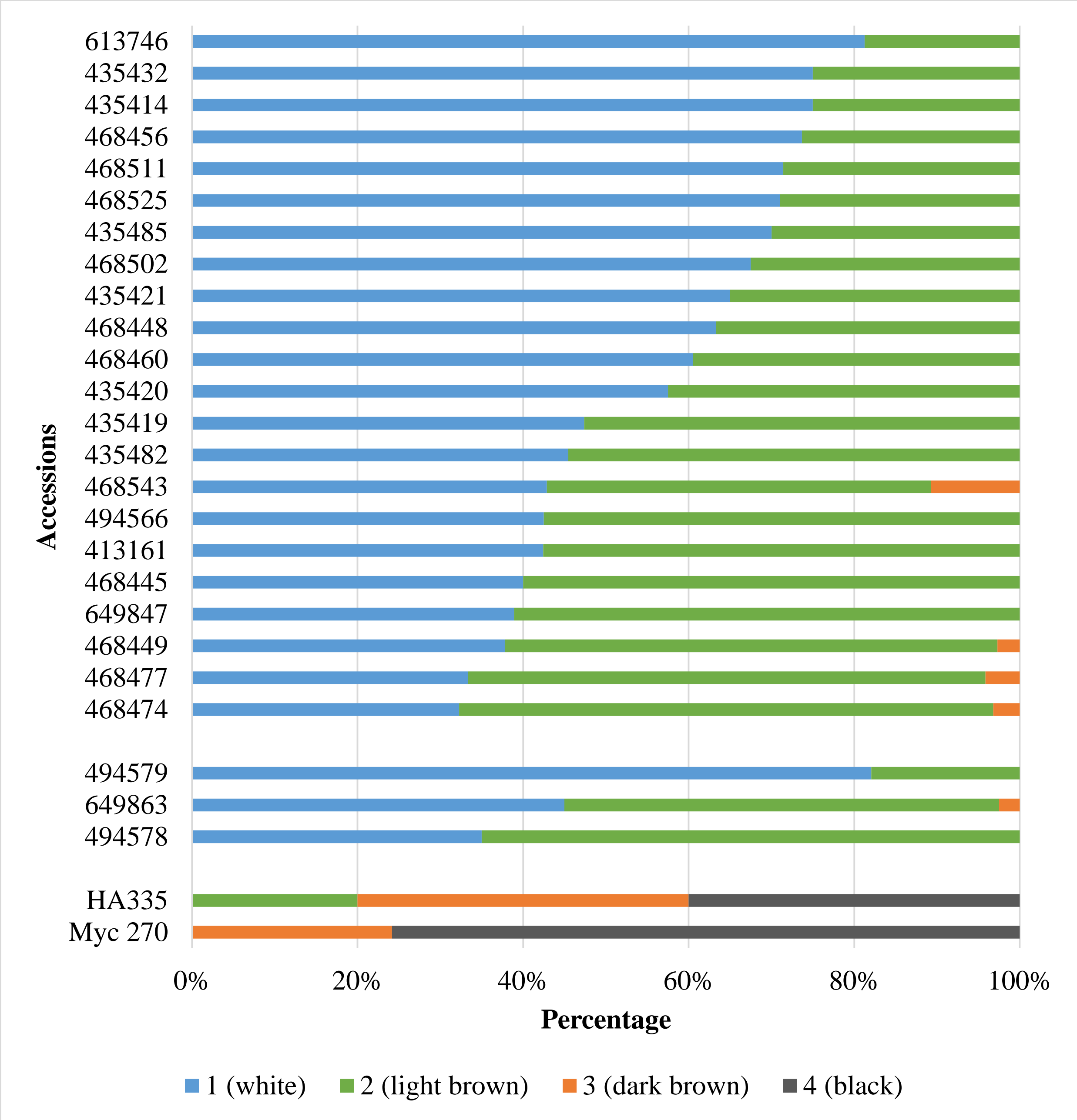

Figure 2.3. Percent of root color values (1, 2, 3, and 4) for the root evaluation of each of the 22 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Plasmopara halstedii* isolates.

Four *H. annuus* accessions (PI 413161 (2), PI 435414 (27), PI 468460 (117), and PI 613746 (201)) had greater than 80% resistance in the above-ground and root evaluations in the advanced screening. Two accessions (PI 494578 (195) and PI 494579 (196)), both *H. argophyllus*, had resistance levels greater than 80% in both the above-ground and root evaluations in the advanced screening.

Discussion

The results of this study determined that a large proportion of *H. annuus* and *H. argophyllus* accessions derived from TX were highly resistant to a single *P. halstedii* isolate virulent on Pl_6 and a mixture of isolates virulent on all genes represented in the nine international differentials. These results indicate that some of the more resistant accessions could be harboring novel sources of resistance, which is promising for the future management of sunflower downy mildew in the U.S.

One of the reasons high levels of resistance exists in wild populations is because the host has been naturally co-evolving with the *P. halstedii* pathogen for thousands of years without interruption. As a result of this, high levels of heterozygosity exists within the wild accessions. In order to account for these high levels of variability, a larger number of plants needed to be screened for each accession. Screening too few plants may skew the overall resistance or susceptibility that is detected within an accession, which could lead to misleading results. In our studies, an average of 60 and 56 plants were inoculated and evaluated for each accession in the initial and advanced screening, respectively. This was the maximum amount of plants we believed we could screen consistently, given the amount of seed that was available to us for each accession, and often low germination percentages and uneven radicle length development. The level of plants per accession evaluated for resistance in our study is much higher than in a similar

sunflower downy mildew study that screened 40 plants per accession (Gulya 2005). This is also true for other pathosystems; for example, a study (Sharma et al. 2012) evaluated 242 sorghum (*Sorghum bicolor*) accessions for resistance to *Colletotrichum sublineolum* (anthracnose), *Exserohilum turcicum* (leaf blight), and *Puccinia purpurea* (rust) and screened 30 plants per accession to each pathogen. In addition to the above-ground rating of plants for symptoms and signs consistent with systemic infection by *P. halstedii*, the roots of all resistant plants were rated for signs of the pathogen in the advanced screening. A past study conducted by Gulya and Rama Raje Urs (1995) concluded that even though a plant does not show signs of systemic infection on the above-ground tissue, there is still a chance that the pathogen was infecting and colonizing the root system. This root colonization could be indicating that the symptom development is delayed and that in reality, the plant may be susceptible. However, there is a chance that plants with colonized roots will never develop symptoms of systemic infection, but the pathogen may be able to grow and proliferate on the root tissue which could increase the amount of inoculum in the soil. When these two possibilities are considered, it seems risky to advance accessions that have a large percentage of resistant plants with roots colonized by the pathogen. In our study, the root evaluations provided valuable data by identifying accessions with greater than 90% of plants resistant during the above-ground and root evaluations. However, no correlation was identified between the above-ground evaluation and the root evaluation. Consequently, if a large proportion of an accession's plants are highly resistant during the above-ground rating, it does not necessarily mean that the roots of these resistant plants are not being colonized by the pathogen. Based on these results, the authors recommend the additional screening of the roots to help further narrow down which accessions may be truly resistant to infection and colonization by the pathogen.

To date, 20 downy mildew resistance genes have been identified, of these, at least nine downy mildew resistance genes including: Pl_1, Pl_2, Pl_6, Pl_7, Pl_8, Pl_{13}, Pl_{17}, Pl_{18}, and Pl_{arg} have been derived from annual wild *Helianthus* germplasm (Fick and Zimmer 1974; Hulke et al. 2010; Miller and Gulya 1988; Miller and Gulya 1991; Qi et al. 2016; Qi et al. 2015; Vear et al. 2008). As a result of this, much of the wild annual germplasm has been previously screened (some multiple times) for resistance to commonly detected *P. halstedii* isolates at the time of the studies. At least three of the mentioned downy mildew resistance genes have been derived from wild *Helianthus* accessions originally collected in TX, including Pl_6 (HA-335) derived from *H. annuus* 423 and Pl_8 (RHA-340) and Pl_{18} (HA-DM1) each derived from *H. argophyllus* PI 435629 and PI 494573, respectively (Miller and Gulya 1991; Qi et al. 2016; Vear et al. 2008). Since our study included the majority of the wild *H. annuus* and *H. argophyllus* germplasm from TX, there were several accessions from which resistance had been previously identified, from which genes had been characterized. This includes the two *H. argophyllus* accessions PI 435629 (94) and PI 494573 (190), where resistance genes Pl_8 and Pl_{18} were identified from, respectively. Both were screened in our initial study, and 67% (PI 435629 (94)) and 63% (PI 494573 (190)) of the plants screened were resistant, respectively. When compared to all other 215 accessions in our study, there were 131 accessions that had a greater percent of plants more resistant than both of these accessions. These results are promising and provide support that other sources of novel resistance exist within the TX collection we evaluated. Additionally, because all isolates used in our study were virulent on the Pl_6 gene, it can be concluded that none of the resistance we observed is being conferred by this gene. This is significant because the Pl_6 gene has been identified in multiple wild *Helianthus* accessions (especially *H. annuus*) derived from the southwestern U.S. (including TX) (Vear et al. 2008).

In a previous study (Gulya 2005), 286 wild *H. annuus* accessions were screened to *P. halstedii* isolates conferring virulence phenotypes of 300, 730, and 773. Even though the accessions included in this study were from all over the U.S., the overall results from Gulya's study found the greatest levels of resistance were identified in accessions derived from TX. Among the most resistant *H. annuus* accessions, were PI 413161 (2), PI 435414 (27), PI 435417 (30), PI 435424 (36), PI 435432 (44), and PI 435438 (50), all from TX. All plants of all six accessions were resistant to *P. halstedii* race 773, which was the most virulent race they were screened against. All six of these accessions were included in our initial screening, but only three were moved on to our advanced screening. PI 413161 (2), PI 435414 (27), and PI 435432 (44) had 80%, 92%, and 72% of all plants screened resistant, respectively. Both PI 413161 (2) and PI 435414 (27) showed high levels of resistance (>80% of plants resistant) in both the above-ground and root evaluations. Additionally, Gulya (2005) screened another 13 wild *H. argophyllus* accessions to a bulk mixture of *P. halstedii* isolates conferring in aggregate, a virulence phenotype of 777. Among the most resistant *H. argophyllus* accessions were PI 494576 (193), PI 494578 (195), PI 494579 (196), PI 494580 (197), and PI 494581 (198), all from TX. The percent of plants resistant in all five accessions ranged from 94-100%. All five accessions were included in our initial screening where resistance levels ranged from 86-97%. Two of these *H. argophyllus* accessions (PI 494578 (195), PI 494579 (196)) were moved onto our advanced screening to a bulk mixture of *P. halstedii* isolates conferring a race 777. It is important to point out that our combination of isolates were at least as virulent as the combination of isolates used in Gulya's study. Results from our advanced screening found that both *H. argophyllus* accessions, PI 494578 (195), PI 494579 (196), showed high levels of resistance (>90% of plants resistant) in both the above-ground and root evaluations. The results

from Gulya's study and our study provide strong evidence that these two *H. annuus* (PI 413161 (2) and PI 435414 (27)) and two *H. argophyllus* (PI 494578 (195), PI 494579 (196)) accessions could be harboring sources of novel resistance not already identified.

To the best of our knowledge, further studies to determine the source of resistance harbored in *H. annuus* accessions PI 435414 (27), PI 468460 (117), and PI 613746 (201) and *H. argophyllus* accession PI 494579 (196) have not been previously done. Both conferred high levels of resistance in both the initial and advanced screenings in this study and also in the study by Gulya (2005). It is prudent to mention that during the time our study was being conducted, a group at the USDA-ARS Sunflower Research Unit in Fargo, ND determined that resistance in the *H. argophyllus* accession PI 494578 (195) is being controlled by a single dominant gene (not Pl_6) that has yet to be characterized (Ma et al. 2016). Additionally, the *H. annuus* accession PI 413161 (2) is known to possess a single dominant resistant gene for downy mildew that is presumably not the Pl_6, Pl_7, or Pl_8 (Tan et al. 1992). In 1993, the gene from this accession was incorporated into a germplasm line, PLH4 and cooperatively released by the USDA-ARS and North Dakota Agricultural Experiment Station, both of Fargo, ND (Jan et al. 2004). To the authors' knowledge, the gene conferring resistance in PLH4 was never assigned an official name.

Overall, the results from this study are promising for the future management of sunflower downy mildew. There is a possibility that novel sources of resistance exist within the population of wild *Helianthus* screened in this study. Historically, resistance derived from wild *Helianthus* species has been controlled by a single dominant resistance gene. If new resistance genes are identified, it will be beneficial not to rely solely on a single resistance gene, which have been quickly overcome by the highly variable *P. halstedii* in the past. In order to preserve current and

future resistance genes, it is highly recommended that multiple effective sunflower downy mildew genes are pyramided into hybrids. In addition to the genetic resistance, fungicide seed treatments including azoxystrobin may be used to help suppress downy mildew (Friskop et al. 2014). Implementing an integrated management system utilizing multiple tools such as genetic resistance and a fungicide seed treatment will help prolong the life of all tools, which will contribute to the future long-term management of sunflower downy mildew.

Literature Cited

Berglund, D. 2007. Introduction. Pages 1-5 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Fick, G. N., and Zimmer, D. E. 1974. RHA271, RHA273, and RHA274 sunflower parental lines for producing downy mildew resistant hybrids. Online Publication. https://library.ndsu.edu/repository/bitstream/handle/10365/9694/farm_32_02_02.pdf?sequence=1

Friskop, A., Markell, S., and Gulya, T. 2009. Downy mildew of sunflower. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1402. Fargo, ND.

Friskop, A., Markell, S. G., Khan, M., Gudmestad, N., and Secor, G. 2014. 2015 North Dakota field crop plant disease management guide. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-622. Fargo, ND.

Gilley, M. A., Markell, S. G., Gulya, T. J., and Misar, C. G. 2015. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers in 2014. Proc. 37th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1245/gilley.downy.mildew.poster_2015.pdf

Gilley, M., Markell, S., Gulya, T., and Misar, C. 2016. Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers. Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1259/prevalence.virulence_gilley.et.al_ppt-2016.pdf

Gulya, T. J. 1996. Everything you should know about downy mildew testing but were afraid to ask. Proc. 18th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/639/1996_gulya_everythingdowny.pdf

Gulya, T. 2005. Evaluation of wild *Helianthus* species for resistance to downy mildew and Sclerotinia stalk rot. Proc. 27th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/265/Gulya_WildHelianthus_studies_05.pdf

Gulya, T., Kandel, H., McMullen, M., Knodel, J., Berglund, D., Mathew, F., Lamey, H. A., Nowatzki, J., and Markell, S. 2013. Prevalence and incidence of sunflower downy mildew in North Dakota between 2001 and 2011. Online. Plant Health Progress doi:10.1094/PHP-2013-0522-01-RS.

Gulya, T. J., Masirevic, S., and Thomas, C. E. 1993. Preservation of air-dried downy mildew sporangia in liquid nitrogen without cryoprotectants or controlled freezing. Mycol. Res. 97:240-244.

Gulya, T. J. and McMullen, M. P. 2012. New virulent races of downy mildew: What's new in 2011. Proc. 34th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. www.sunflowernsa.com/.../new-virulent-races-of-downy-mildew---gulya.pdf

Gulya, T. J. and Rama Raje Urs, N. V. 1995. A simple method to assess root response of downy mildew "resistant" sunflower lines. Proc. 17th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/682/1995_gulya_simplemethodassessroot.pdf

Gulya, T., Rashid, K. Y., and Masirevic, S. M. 1997. Sunflower diseases. Pages 263-379 in: Sunflower Technology and Production. A. A. Schneiter ed. American Society of Agronomy, Madison, WI.

Harada, W. S. 1982. The effects of ethephon on dormant seeds of cultivated sunflower (*Helianthus annuus* L.). Proc. 10th Int. Sunflower Conf., International Sunflower Association, Surfers Paradise, Australia. Online Publication. http://isasunflower.org/fileadmin/documents/Proceedings/10thISC1982/Agronomy/T1982AGR03.pdf

Hulke, B. S., Miller, J. F., Gulya, T. J., and Vick, B. A. 2010. Registration of the oilseed sunflower genetic stocks HA 458, HA 459, and HA 460 possessing genes for resistance to downy mildew. J. Plant Regist. 4:93-97.

Jan, C. C., Tan, A. S., and Gulya, T. J. 2004. Registration of four downy mildew resistant sunflower germplasms. Crop Sci. 44:1887.

Kandel, H. 2014. 2013 National Sunflower Crop Survey. Proc. 36th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1227/kandel_2013.crop.survey_paper_2014.pdf

Ma, G., Markell, S., and Qi, L. L. 2016. 2015 progress for development of super confection sunflower effectively resistant to downy mildew and rust. Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1279/super.confection_ma.etal_ppt-2016.pdf

Marek, L. F., Block, C. C., and Gardner, C. C. A. 2012. 2012 Update: New sunflower genetic resources in the U.S. national sunflower collection and potential use for crop improvement. Proc. 18th Intl. Sunflower Conf. International Sunflower Association. Mar del Plata, Argentina. Online Publication.

Miller, J. F. and Gulya, T. J. 1988. Registration of six downy mildew resistant sunflower germplasm lines. Crop Sci. 28:1040-1041.

Miller, J. F. and Gulya, T. J. 1991. Inheritance of resistance to race 4 of downy mildew derived from interspecific crosses in sunflower. Crop Sci. 31:40-43.

Putt, E. D. 1997. Early History of Sunflower. Pages 1-20 in: Sunflower Technology and Production. A.A. Schneiter, ed. American Society of Agronomy, Madison, WI.

Qi, L. L., Foley, M. E., Cai, X. W., and Gulya, T. J. 2016. Genetics and mapping of a novel downy mildew resistance gene, Pl_{18}, introgressed from wild *Helianthus argophyllus* into cultivated sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. doi 10.1007/s00122-015-2662-2.

Qi, L. L., Long, Y. M., Jan, C. C., Ma, G. J., and Gulya, T. J. 2015. Pl_{17} is a novel gene independent of known downy mildew resistance genes in the cultivated sunflower (Helianthus annuus L.). Theor. Appl. Genet. 128:757–767.

Sackston, W. E. 1981. Downy mildew of sunflower. Pages 545-576 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London, England.

Sackston, W. E. 1992. On a treadmill: Breeding sunflower for resistance to disease. Annual Rev. Phytopathol. 30:529-551.

Sandbakken, J. and Kleingartner, L. 2007. U.S. Production. Pages 6-8 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Sharma, R., Upadhyaya, H. D., Manjunatha, S. V., Rao, V. P., and Thakur, R. P. 2012. Resistance to foliar diseases in a mini-core collection of sorghum germplasm. Plant Dis. 96:1629-1633.

Tan, A. S., Jan, C. C., and Gulya, T. J. 1992. Inheritance of resistance to race 4 of sunflower downy mildew in wild sunflower accessions. Crop Sci. 32:949-952.

Tourvieille de Labrouhe, D., Gulya, T. J., Masirevic, S., Penaud, A., Rashid, K., and Viranyi, F. 2000. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). Proc. 15th Int. Sunflower Conf. International Sunflower Association, Toulouse, France. Online Publication. http://isasunflower.org/fileadmin/documents/aaProceedings/15thISCToulouse2000/PosterWorkshopI-O/I-F57.pdf

USDA, National Agricultural Statistics Service. 2016. Sunflower quick statistics. Available at: http://quickstats.nass.usda.gov/results/C714A9DF-EFBF-3E42-902C-DE1316523C76?pivot=short_desc

Vear, F., Serieys, H., Petit, A., Serre, F., Boudon, J. P., Roche, S., Walser, P., and Tourvieille de Labrouhe, D. 2008. Origins of major genes for downy mildew resistance in sunflower. Proc. 17th Int. Sunflower Conf. International Sunflower Association, Cordoba, Spain. Online Publication. http://isasunflower.org/fileadmin/documents/aaProceedings/17thISC_CordobaVol1/p125vear.pdf

Viranyi, F. 1985. A simple technique for long-term storage of *Plasmopara halstedii* sporangia at low temperature. T. Brit. Mycol. Soc. 85:529-531.

Viranyi, F. and Spring, O. 2011. Advances in sunflower downy mildew research. Eur. J. Plant Pathol. 129:207-220.

CHAPTER 3. IDENTIFYING *HELIANTHUS* ACCESSIONS RESISTANT TO *PUCCINIA HELIANTHI*

Introduction

Sunflower (*Helianthus annuus* L.) is an economically important crop in the United States (U.S.). Two market classes of sunflowers are produced in the U.S.; oilseeds used for cooking oils and non-oilseeds (confectionary types) used for human consumption and bird food markets (Berglund 1997). Over the past 20 years, the average hectarage of sunflowers planted in the U.S. has remained consistent at approximately 800,000 hectares (USDA-NASS 2016). Around 95% of those hectares are planted in the states of North Dakota (ND), South Dakota, Minnesota, Kansas, Colorado, Nebraska, and Texas (TX), with the majority concentrated in the Northern Great Plains (Sandbakken and Kleingartner 2007).

Sunflower is native to North America, where it was first domesticated by the Native Americans (Putt 1997). In North America alone, over 50 different species exist within the *Helianthus* genus, with the majority of these species growing exclusively as wild populations. Fourteen of these species are categorized as annuals while the remaining 38 are perennials (Heiser et al. 1969; Marek et al. 2012; Schilling 2006). Cultivated *H. annuus*, all other closely related wild annual species, and a few of the perennial species are diploid. This makes it possible for breeders to incorporate genes expressing desirable traits into cultivated sunflowers hybrids by making crosses between cultivated and wild annual species (Sackston 1992). Crossing wild and cultivated lines can facilitate the production of hybrids with favorable traits, including greater resistance to economically important sunflower diseases (Putt and Sackston 1957; Putt and Sackston 1963).

Sunflower rust, caused by *Puccinia helianthi* Schwein. is one of the most economically important diseases of sunflower that occurs in the U.S. and many other countries (Friskop et al. 2011; Gulya et al. 1997). *P. helianthi* is an autoecious, macrocyclic rust pathogen capable of completing its entire sexual life cycle on sunflower in one growing season (Bailey 1923; Gulya et al. 1997). Rust overwinters as teliospores on wild and volunteer sunflowers in the northern U.S., which serves as a source of primary inoculum for the next cropping season. Under optimal conditions of moderate to warm temperatures (13-30°C) and free moisture (dew), rust severity can rapidly increase. This is due in part to the polycyclic nature of *P. helianthi*, where repeating urediniospores repeatedly infect healthy foliar, stem, and head tissues. Ultimately, photosynthetic processes are inhibited, leading to reductions in yield and quality (Friskop et al. 2011). Yield reductions of up to 80% have been documented in confection sunflower when urediniospores appear early in the vegetative growth stages (Markell et al. 2009). Since the early 2000s, surveys coordinated by the National Sunflower Association (NSA) and conducted by North Dakota State University (NDSU) have reported increases in the prevalence and severity of sunflower rust, especially in the Northern Great Plains (Friskop et al. 2011). In 2013 and 2015, 66 and 62% of fields surveyed across eight U.S. states were found to have rust (Kandel 2014; Kandel and Gulya 2016).

Rust is managed primarily with genetic resistance and the use of fungicides (Friskop et al. 2015a; Friskop et al. 2011; Harveson 2010). Of these, resistance is the more economically and environmentally friendly management tool (Putt and Sackston 1957). Since sunflower first emerged as a commercial crop, many private and public sunflower breeding programs have worked to find sources of rust resistance and incorporate the genes conferring that resistance into commercial hybrids to help prevent yield losses (Hulke et al. 2010; Putt and Sackston 1963).

Incorporation of resistance genes is also widely used to manage other economically important diseases of sunflower, in particular, downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni (Sackston 1981). Often, a single dominant resistance gene is incorporated into commercial hybrids to manage rust (Quresh et al. 1990). The first example of rust being controlled with resistance genes occurred in the early 1960's, when Putt and Sackston (1963) incorporated the R_1 and R_2 rust resistance genes into cultivated sunflower hybrids. Both of these sources of resistance were derived from wild annual sunflowers collected from Renner, TX (Putt and Sackston 1963; Seiler 1992). Unfortunately, deployment of single-gene resistance is not very durable and is often overcome by the pathogen, rendering the associated resistance gene largely ineffective in areas where virulence to the gene commonly occurs. Frequent changes in virulence can be a result of sexual recombination events or random mutations that occur within the *P. helianthi* population (Kong et al. 1999). Extensive surveys to determine pathogen virulence have been performed over many years and locations, and provides a large body of knowledge on which races of *P. helianthi* are prevalent in certain geographic regions. Notable changes in virulence have been detected in the U.S. over the past 20 years. In the 1990s, there were 20 virulence combinations in bulk collections known to exist (Gulya 2006; Gulya and Viranyi 1994); however, more recent surveys in 2011 and 2012 detected 29 races (Friskop et al. 2015b). Similarly, numerous races/virulence phenotypes have been reported in South America and Asia (Jing et al. 2015; Moreno et al. 2012). These surveys are very informative and can help pathologists and breeders determine which resistance genes may or may not be useful in a particular area.

Because of host-pathogen co-evolution, there is a continued need to find novel sources of potentially new resistance to rust. Historically, cultivated and wild germplasm have provided

sources of new resistance genes. One notable source of sunflower germplasm is the Unites States Department of Agriculture (USDA) sunflower germplasm collection in the National Plant Germplasm System, maintained at the USDA North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. There are approximately 3,200 annual *Helianthus* spp. accessions at the NCRPIS with 3,062 accessions available to the public for screening (Marek et al. 2012). Previously, Gulya et al. (2000) screened some of the North American collection of annual wild *Helianthus* accessions and identified accessions resistant to common and highly virulent races of *P. helianthi* occurring in the 1990s. Interestingly, a disproportionate amount of the resistant accessions identified had originated from TX (Gulya et al. 2000). In 2013, Friskop (2013) screened a statistically derived cross-section of the NCRPIS collection ('core-set') and identified two accessions considered highly resistant to the most virulent races at the time. Of these two accessions, one had originated from the southern U.S. The objective of this study was to evaluate wild *Helianthus* germplasm derived from TX for new potential sources of resistance to commonly detected and highly virulent races of *P. helianthi*.

Materials and Methods

Host material. One hundred eighty-two wild *H. annuus* and 33 wild *H. argophyllus* accessions, all of which are annuals and collected from TX, were obtained from the USDA NCRPIS. Additionally, the nine internationally accepted rust differentials (7350, MC90, MC29, P386, HA-R1, HA-R2, HA-R3, HA-R4, and HA-R5) were obtained from the USDA-Agricultural Research Service (ARS) Sunflower Research Unit in Fargo, ND (Gulya and Masirevic 1996). All of the germplasm screenings in this study were planted and conducted in the North Dakota Agricultural Experiment Station (NDAES) greenhouse complex in Fargo.

Pathogen material and inoculation. All isolates of *P. helianthi* used in the studies were collected in ND from a 2011 and 2012 survey performed by Friskop et al. (2015b) and virulence phenotyped (race-typed) on the nine international rust differentials previously mentioned. The initial greenhouse screening of all 215 wild *Helianthus* accessions was performed using a single pustule isolate ND11_25J, race-typed as a 336; which was selected because it was one of the most commonly detected races in ND (Friskop et al. 2015b; Gulya and Markell 2009). For the advanced and additional screenings, six single pustule isolates including ND11_10H (race 300), ND11_06E (race 304), ND11_25J (race 336), ND11_29B (race 337), ND11_05C (race 776), and ND12_06A (race 777) were combined in an attempt to create an aggregate virulence phenotype of 777; which is virulent on all resistance genes represented in the nine differentials.

To ensure high viability, inoculum was used throughout this study, an increase of urediniospores was timed to coincide with each screening experiment. In order to increase *P. helianthi* isolates, seeds of a susceptible sunflower hybrid ‘Jaguar’ (Seeds 2000, Breckenridge, MN) were planted in 6.4 cm deepot cells (Stuewe and Sons, Tangent, OR) filled with potting soil (Sunshine Mix, Sun Gro, Canada). Fourteen days after planting, isolates were taken out of storage in the -80°C freezer and heat shocked in a 40°C water-bath. Inoculations were performed by suspending *P. helianthi* urediniospores in Soltrol 170 (Chevron Philips LLC., The Woodlands, TX), a petroleum based oil product, at approximately 275,000 spores ml^{-1}. The urediniospore solution was sprayed onto the susceptible plants using a Preval CO_2 sprayer (Chicago Aerosol, Coal City, IL). Inoculated plants were allowed to dry for 45-60 min and placed into humidity chambers at 100% humidity and 23°C and left in the dark for 20-24 h to facilitate an infection period. After 24 h, the inoculated plants were then moved to the greenhouse where they were bottom-watered for approximately 10-14 days. Rust urediniospores

were collected into pill gel capsules (Gallipot Inc., St. Paul, MN) using a mini-cyclone collector (G-R Manufacturing, Manhattan, KS) attached to a piston vacuum pump (Welch, Niles, IL). Urediniospores stored in capsules were desiccated in indicating drierite (W.A. Hammond Company, Xenia, OH) and temporarily stored at 3.5^{o}C until the urediniospores were needed for subsequent inoculations.

Initial screening. Most wild *Helianthus* accessions have inherent seed dormancy and therefore must undergo a special process to break dormancy to facilitate consistent germination across accessions. Seventy-two seeds from each of the 215 accessions were soaked in a 20% bleach solution for approximately five minutes for surface sterilization, then rinsed off with distilled water and placed into sterile 60 x 15 mm petri dishes (Falcon brand, Corning, Corning, NY). Approximately, 15-18 ml of a 1.71 x 10^{-2}% ethephon (Florel brand, Monterey Lawn and Garden, Fresno, CA) solution was added to the petri dishes after which, seeds were soaked in the dark at 3.5^{o}C for 24 h. Next, the seeds were rinsed again with distilled water, placed on moistened germination blotter paper and stored in the dark at 3.5^{o}C for 10-14 days to mimic a cold stratification period (Harada 1982; Marek et al. 2012).

For the initial greenhouse screening, the trial was arranged as a complete randomized design with six replicates. After breaking the dormancy, 72 seeds of each accession were planted into a 27.94 x 53.90 cm, 50 cell plug tray (5 x 10 cells, product description: PL-50, T.O. Plastics Inc., Clearwater, MN) with individual cell dimensions of 4.83 x 4.83 x 6.03 cm and filled with potting soil. Four sunflower seeds were planted in three cells per replicate for a total of 12 seeds per replicate. Additionally, three cells per tray were planted with a susceptible check. Plants were grown in the greenhouse at a constant temperature of 22 ± 2°C under a 14 h photoperiod with supplemental light. Plants were manually thinned out 12 days after planting, so each accession

was left with five plants per replicate for the inoculation. Sunflowers were inoculated with isolate ND11_25J (race 336) using a Preval CO_2 sprayer as described previously, 13-16 days after planting when the first true leaves were fully emerged.

Five plants were rated for each accession within a replicate, with up to a total of 30 plants rated for each accession. The infection types on the two inoculated true leaves were evaluated 14-15 days after inoculation using a rating scale developed by Sackston (1962) and modified by Yang et al. (1986), where, 0 = no infection or hypersensitive flecks; ; = hypersensitive fleck response; 1 = very small pustules <0.2 mm in diameter; 2 = small pustules 0.2-0.4 mm; 3 = pustules 0.4-0.6 mm; 4 = pustules 0.6-0.8 mm; and 5 = pustules >0.8 mm. Infection types of 0, ;, 1, and 2 were all considered to be a resistant reaction, while infection types of 3, 4, and 5 were considered to be a susceptible reaction (Yang et al. 1986). Due to high heterozygosity that existed within each accession, some individual plants had a mixed infection type, where both resistant and susceptible reactions were present on the same leaf; these plants were categorized as mixed. All plants with a mixed infection type were considered susceptible. When possible, the infection types of five plants for each accession per replicate were evaluated. Additionally, chlorosis and necrosis visible around rust pustules were evaluated according to Sackston (1962), where C = chlorosis and N = necrosis.

Advanced screening. Based on initial screening results, all *H. annuus* accessions where greater than 95% of all the plants were resistant (n=22) were selected for advanced screening. *Helianthus argophyllus* accessions where 100% of all the plants were resistant (n=2) were also selected.

For this experiment, the trial was arranged as a complete randomized design with four replicates. After breaking the seed dormancy, the seed was planted in 3.8 cm Ray Leach cone-

tainers (Stuewe and Sons, Tangent, OR) filled with potting soil. For each accession, two sunflower seeds were planted in six cone-tainers for a total of 12 seeds for each replicate. Eight accessions were grown together in a rack with six additional cone-tainers planted with a susceptible check. Plants were grown in the NDAES greenhouse under the same temperature and light conditions described previously. Plants were manually thinned out so each accession would have six plants per replicate for inoculation. Six *P. helianthi* isolates previously mentioned of races 300, 304, 336, 337, 776, and 777 were combined in equal proportions, suspended in soltrol, and inoculated as previously described. Evaluations of six plants (if available) per rep were conducted using the modified infection type scale previously described. A total of up to 24 plants per each accession were evaluated. If no pustules were observed on a plant's true leaves (0 and ; reactions), then cotyledons were evaluated for presence of pustules. This additional rating of the cotyledons was not performed on plants with infection types of 1 or greater.

Additional accessions of interest. An additional 20 *H. annuus* and three *H. argophyllus* accessions previously determined to confer high levels of resistance to *Plasmopara halstedii* were also included with the advanced screening (Humann et al. *unpublished*). These additional accessions were screened in order to determine if any accessions conferred high levels of resistance to both *P. helianthi* and *P. halstedii*.

Initial screening analysis. Plants from the initial screening were categorized as susceptible, mixed, or resistant based off of the infection type observed. Resistance was calculated by dividing the number of resistant (infection type: 0, ;, 1, and 2) plants in an accession by the total number of plants screened in an accession and multiplying the quotient by 100 to convert it to a percent. Additionally, the number of susceptible, mixed, and resistant

plants for an accession were converted to a percent of the total number of plants screened for that accession.

Advanced and additional accessions of interest screening analysis. Due in part to a highly variable host population and large number of plants screened for each accession (n=24), a wide range of infection types was observed on many accessions. In order to present the most meaningful data, a mechanism to present frequency of infection types was developed. Each plant evaluated was given a total value of one and that value was placed into the infection type category observed on the plant. For plants with multiple infection types, the value was divided evenly among these infection types occurring. For example, if a plant's infection type was IT4, then a one was assigned to IT4. If a plant's infection type was IT3/4, then a value of 0.5 was given to IT3 and IT4. If a plant's infection type was IT2/3/4, then a value of 0.33 was given to IT2, IT3, and IT4. This method gave equal weight to every infection type observed on a plant. Next, the accession's overall total for each infection type category was summed across all plants rated in an accession. An accession's infection type totals combined was equal to the total number of plants rated for that accession. An accession's overall infection type totals were converted to a percent of the total number of plants rated for that accession. For example, if an accession's 24 plants had infection type values of 2 (IT0), 14.5 (IT;), 4.5 (IT1), 1.66 (IT2), 1.33 (IT3), 0 (IT4), and 0 (IT5), then the corresponding percentages for each infection type category would be 8% (IT0), 60% (IT;), 19% (IT1), 7% (IT2), 6% (IT3), 0% (IT4), and 0% (IT5).

The necrosis and chlorosis observation for every plant's infection type was separated into two categories: N and C. If an N or C was included in the plant's infection type rating, then a value of one was assigned to the respective category. For example, if a plant's infection type was 1C, then a one was assigned to category C. If a plant's infection type was 1N2C, then a one was

assigned to categories N and C. Next, an accession's overall total for N and C observations was summed across all plants rated in that accession and was converted to a percent of the total number of plants rated for that accession. For example, if an accession with 24 plants had a necrosis value of 10 and a chlorosis value of two, this means that 42% of plants showed necrosis and 8% of plants showed chlorosis for the accession.

All plants with no signs of pustules (IT0 or IT; reaction) on the true leaves were evaluated for signs of pustules on the cotyledons. For all accessions, the number of plants with pustules on their cotyledons was divided by the total number of plants with no pustules (0 or ; reaction) on their first true leaves. All plants with an infection type of 1 or greater on their true leaves were excluded from this analysis.

Results

Initial screening. Germination of accessions was good, but variable. Of the 182 *H. annuus* accessions, 82 had all 30 plants evaluated, 93 had 21-29 plants evaluated, and at least 12 plants were evaluated on the remaining seven accessions (Table C.1). As a result of this unevenness, the susceptible (IT 3, 4 and 5), mixed (IT 0, ;, 1, or 2 plus 3, 4, or 5), and resistant (IT 0, ;, 1, and 2) number of plants was converted to percentages of the total number of plants in order to make comparisons (Fig. 3.1). One hundred percent of plants of the susceptible check, P386, were susceptible, while 100% of plants of the resistant check, HA-R2, were resistant. Eleven accessions had 100% of plants resistant, but no accessions with 100% susceptible. Collectively, 72 accessions had at least one plant categorized as mixed, but the maximum percent of mixed plants in an accession was only 17%. Twenty-two accessions with 95% of their plants or greater resistant were included in the advanced screening.

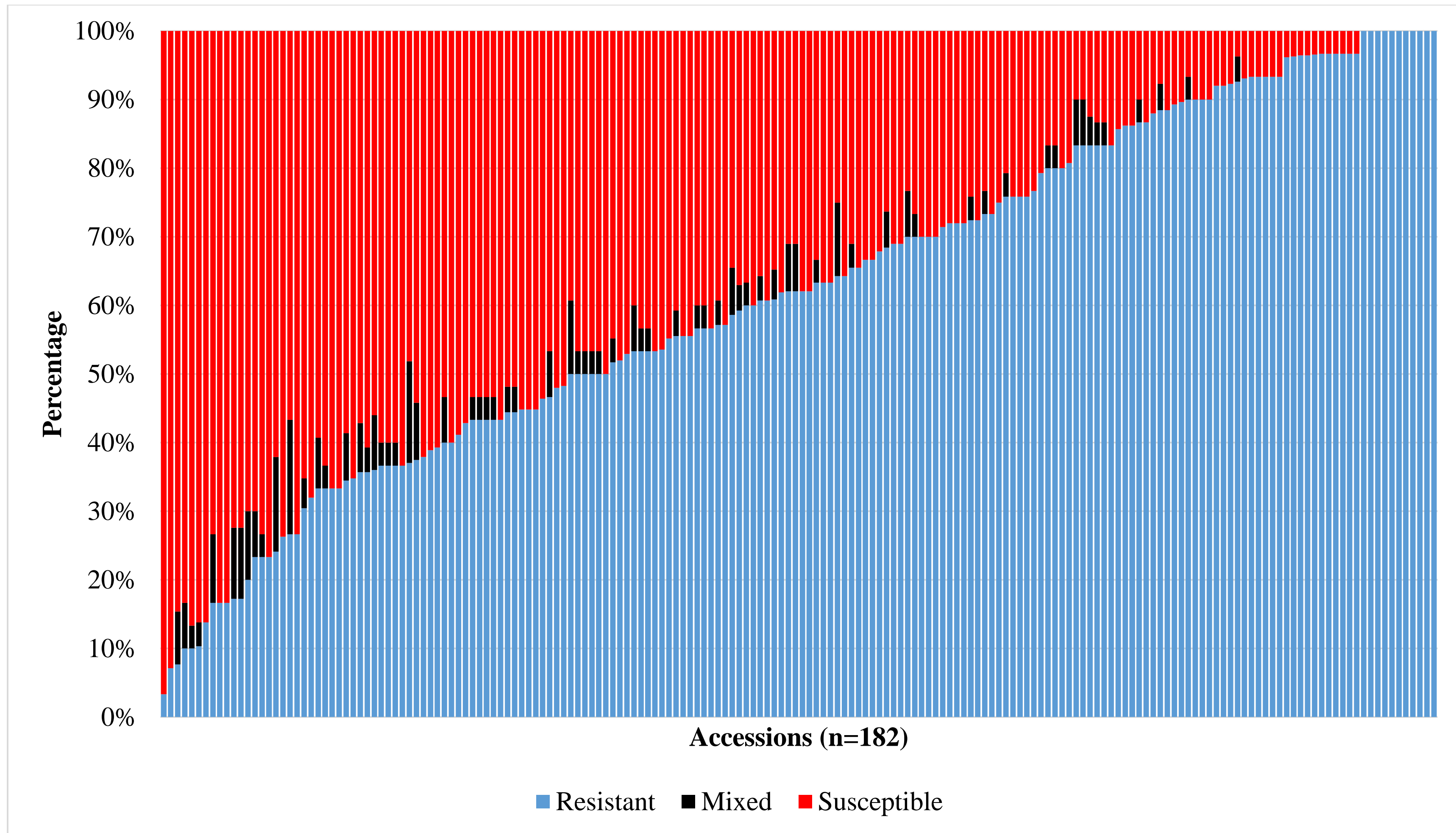

Figure 3.1. Percent of resistant, mixed, and susceptible plants for each of the 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336.

Of the 33 *H. argophyllus* screened, nine accessions had all 30 plants evaluated, 22 had 21-29 plants evaluated, and at least 17 plants were evaluated on the remaining two accessions (Table C.2). All 33 accessions had greater than 75% of their plants resistant and 15 accessions had 100% resistant (Fig. 3.2). Six accessions had at least one plant categorized as mixed, but the maximum percent of mixed plants in an accession was only 4%. Two accessions with 100% of their plants resistant and good germination were included in the advanced screening.

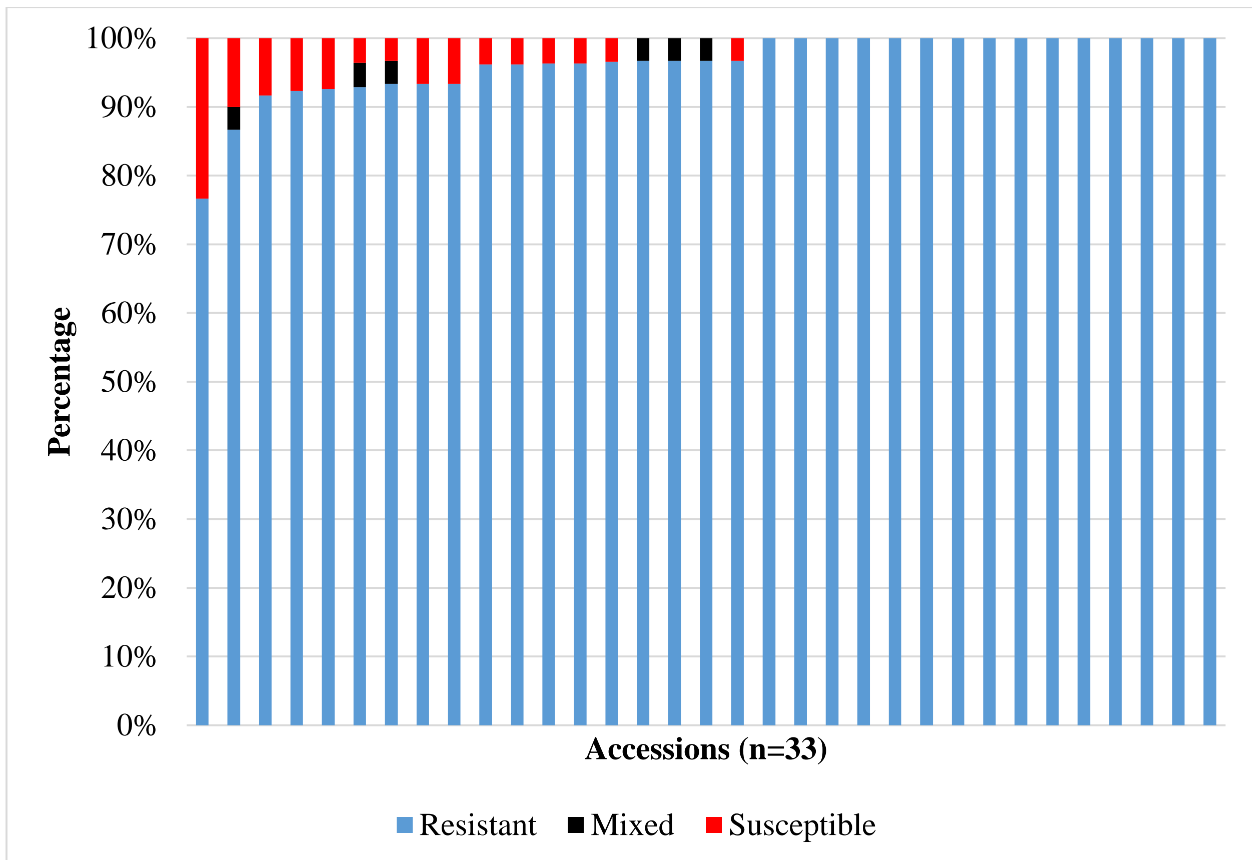

Figure 3.2. Percent of resistant, mixed, and susceptible plants for each of the 33 wild *Helianthus argophyllus* accessions in the initial screening to *Puccinia helianthi* race 336.

Advanced screening. Of the 22 *H. annuus* and two *H. argophyllus* accessions, 17 had all 24 plants evaluated, five had 17-23 plants evaluated (including both *H. argophyllus* accessions), and two accessions had at least four plants evaluated (Table 3.1). All 24 accessions had greater than 95% of their plants resistant, of which, 22 accessions had 100% resistance, including both *H. argophyllus* accessions. One hundred percent of plants of the susceptible check, 7350, were susceptible. Infection types of 4 and 5 occurred on 93% of those 7350 plants, with infection type 3 making up the remaining 7% (Fig. 3.3). In each case, chlorosis was visually observed surrounding pustules on every plant (Fig. 3.4). One hundred percent of plants of the resistant check, HA-R2 were resistant. An infection type of ; (fleck) was observed on 100% of those HA-R2 plants. Similarly necrosis was visually observed on every plant.

Table 3.1. Susceptible, mixed, and resistant plants and percent resistance for 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates.

Item No.	Accession No. (PI)	Species	Susceptible	Mixed	Resistant	Total	Resistance (%)
41	435429	*H. annuus*	0	0	24	24	100
44	435432	*H. annuus*	0	0	24	24	100
46	435434	*H. annuus*	1	0	22	23	96
56	435444	*H. annuus*	0	0	9	9	100
101	435850	*H. annuus*	0	0	24	24	100
107	468450	*H. annuus*	0	0	24	24	100
108	468451	*H. annuus*	0	0	24	24	100
110	468453	*H. annuus*	0	0	24	24	100
111	468454	*H. annuus*	0	0	24	24	100
112	468455	*H. annuus*	0	0	24	24	100
113	468456	*H. annuus*	0	0	24	24	100
115	468458	*H. annuus*	0	0	24	24	100
152	468512	*H. annuus*	1	0	23	24	96
157	468517	*H. annuus*	0	0	24	24	100
159	468519	*H. annuus*	0	0	24	24	100
160	468520	*H. annuus*	0	0	24	24	100
161	468521	*H. annuus*	0	0	21	21	100
163	468523	*H. annuus*	0	0	24	24	100
164	468524	*H. annuus*	0	0	4	4	100
185	494568	*H. annuus*	0	0	24	24	100
200	613728	*H. annuus*	0	0	24	24	100
203	649810	*H. annuus*	0	0	21	21	100
199	494582	*H. argophyllus*	0	0	23	23	100
213	649864	*H. argophyllus*	0	0	23	23	100
Susc. Check	7350	*H. annuus*	12	0	0	12	0
Res. Check	HA-R2	*H. annuus*	0	0	24	24	100

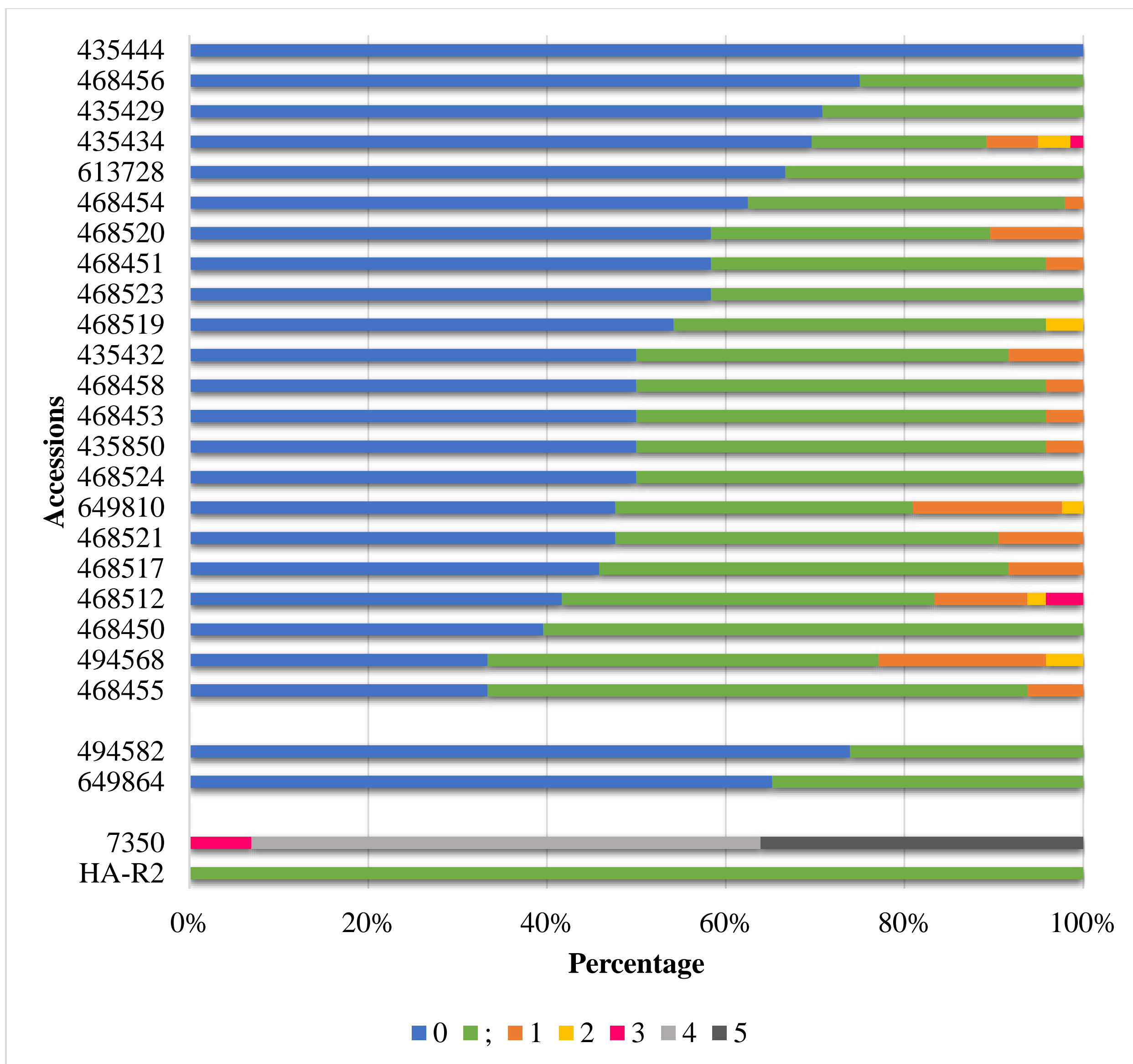

Figure 3.3. Percent of infection types (0, ;, 1, 2, 3, 4, and 5) for each of the 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates.

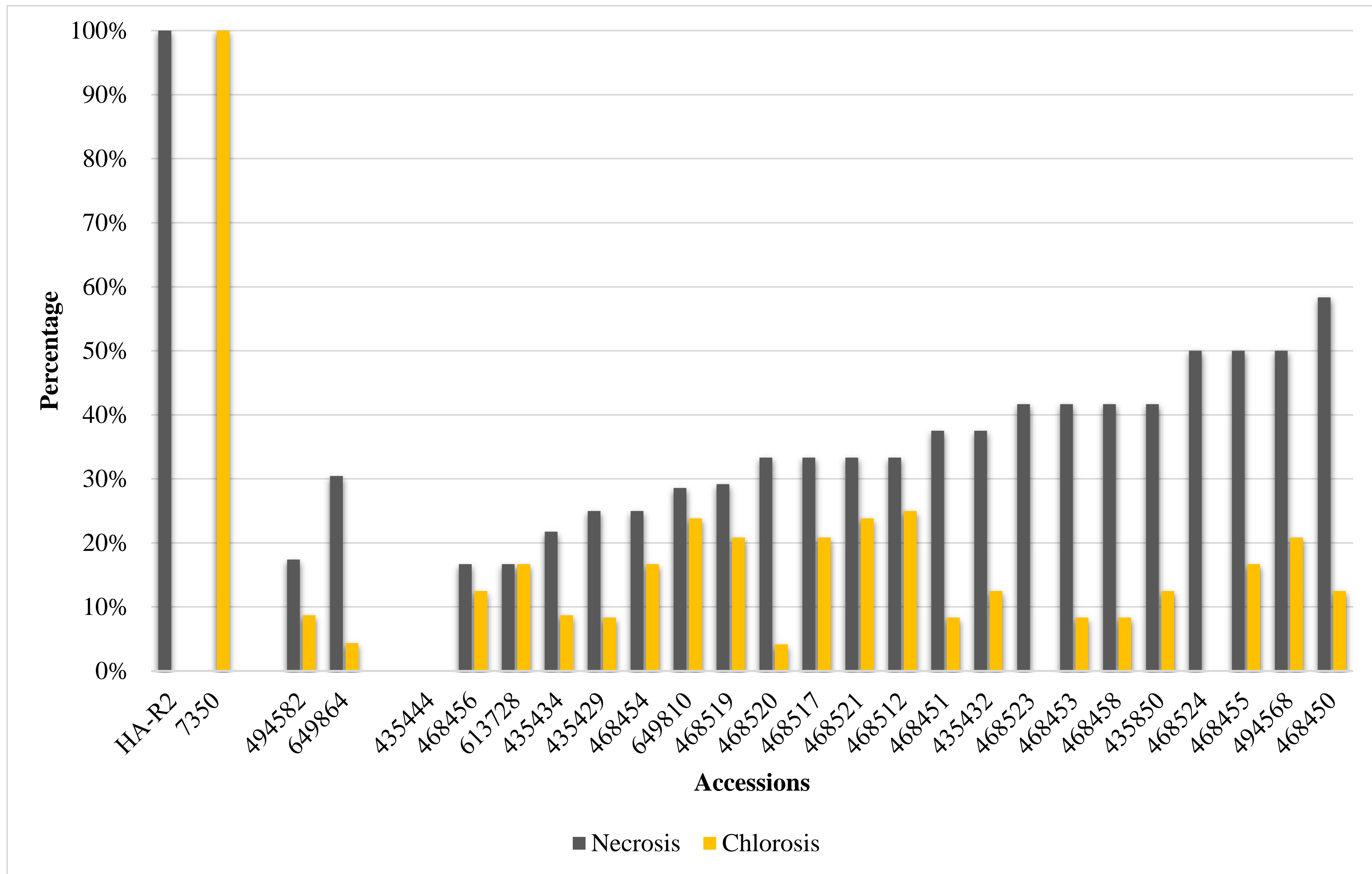

Figure 3.4. Percent of plants showing symptoms of necrosis or chlorosis for 22 wild *Helianthus annuus* and two wild *H. argophyllus* accessions in the advanced screening of accessions to a bulk mixture of six *Puccinia helianthi* isolates.

The infection type category 0 accounted for at least 33% of the total infection types observed for all 24 accessions. The infection types of 0 and ; combined, accounted for 77% of the total infection types observed for all 24 accessions. Seven *H. annuus* accessions were composed entirely of 0 and ; infection types and four of the seven accessions had no pustules on true leaves or cotyledons. Both *H. argophyllus* accessions were composed entirely of 0 and ; infections types and both had no pustules on true leaves or cotyledons. One *H. annuus* accession showed no symptoms of necrosis, the other 23 accessions had necrosis occur on 17-58% of plants (Fig. 3.4). Three *H. annuus* accessions showed no symptoms of chlorosis, the other 21 accessions had chlorosis occur on 4-25% of plants.

<u>Additional accessions of interest.</u> The additional screening of 20 *H. annuus* and three *H. argophyllus* accessions conferring high levels of resistance to *P. halstedii* were conducted at the same time as the previously mentioned 24 accessions. Therefore, all values of the susceptible and resistant checks are the same for this screening of 23 total additional accessions of interest.

Of the 20 *H. annuus* and three *H. argophyllus* accessions, 10 had all 24 plants evaluated, eight had 17-23 plants evaluated, and five accessions had at least four plants evaluated (Table 3.2). The infection types of 0, ;, 1, and 2 accounted for at least 80% of all infection types recorded for the 23 accessions (Fig. 3.5). One *H. annuus* accession was composed entirely of 0 and ; infections types, however it did have pustules on the cotyledons. Two of the three *H. argophyllus* accessions were composed entirely of 0 and ; infection types and both had no visible pustules on the true leaves or cotyledons. Two *H. annuus* accessions showed no necrosis, while the other 21 accessions had 13-67% of plants with necrosis (Fig. 3.6). All 23 accessions had plants with chlorosis ranging from 8-80%.

Table 3.2. Susceptible, mixed, and resistant plants and percent resistance for 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions of interest to a bulk mixture of six *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*.

Item No.	Accession No. (PI)	Species	Susceptible	Mixed	Resistant	Total	Resistance (%)
2	413161	*H. annuus*	1	0	4	5	80
27	435414	*H. annuus*	1	0	3	4	75
32	435419	*H. annuus*	1	1	11	13	85
33	435420	*H. annuus*	2	0	18	20	90
34	435421	*H. annuus*	3	1	20	24	83
68	435482	*H. annuus*	3	0	5	8	63
71	435485	*H. annuus*	1	1	21	23	91
102	468445	*H. annuus*	0	0	24	24	100
105	468448	*H. annuus*	0	0	21	21	100
106	468449	*H. annuus*	0	0	24	24	100
117	468460	*H. annuus*	0	0	21	21	100
126	468474	*H. annuus*	1	1	15	17	88
129	468477	*H. annuus*	0	0	21	21	100
142	468502	*H. annuus*	0	1	23	24	96
151	468511	*H. annuus*	2	1	21	24	88
165	468525	*H. annuus*	1	0	23	24	96
179	468543	*H. annuus*	0	1	23	24	96
183	494566	*H. annuus*	0	0	24	24	100
201	613746	*H. annuus*	0	0	15	15	100
207	649847	*H. annuus*	1	1	19	21	90
195	494578	*H. argophyllus*	0	0	24	24	100
196	494579	*H. argophyllus*	0	0	24	24	100
212	649863	*H. argophyllus*	0	0	18	18	100
Susc. check	7350	*H. annuus*	12	0	0	12	0
Res. check	HA-R2	*H. annuus*	0	0	24	24	100

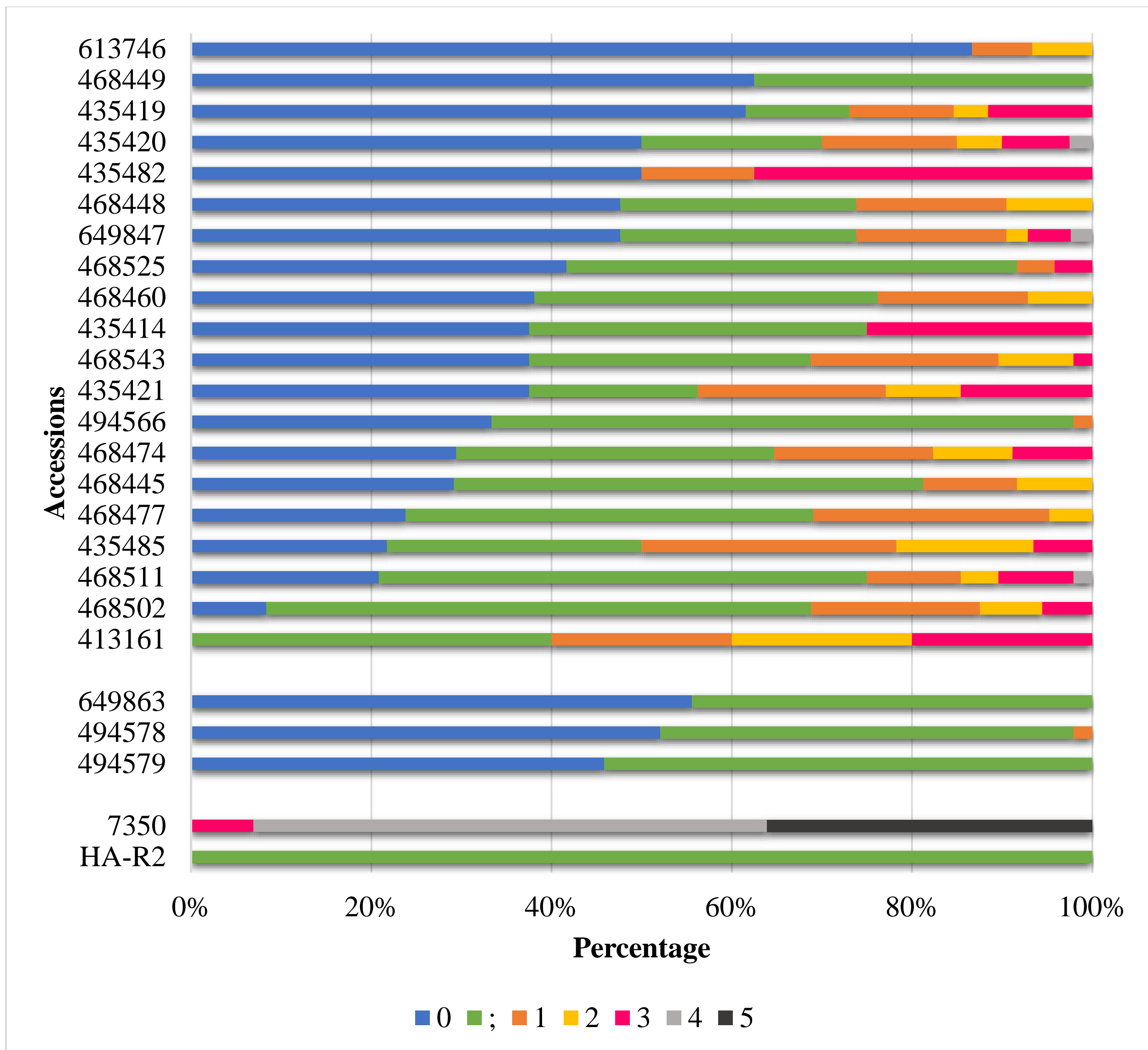

Figure 3.5. Percent of infection types (0, ;, 1, 2, 3, 4, and 5) for each of the 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions of interest to a bulk mixture of six *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*.

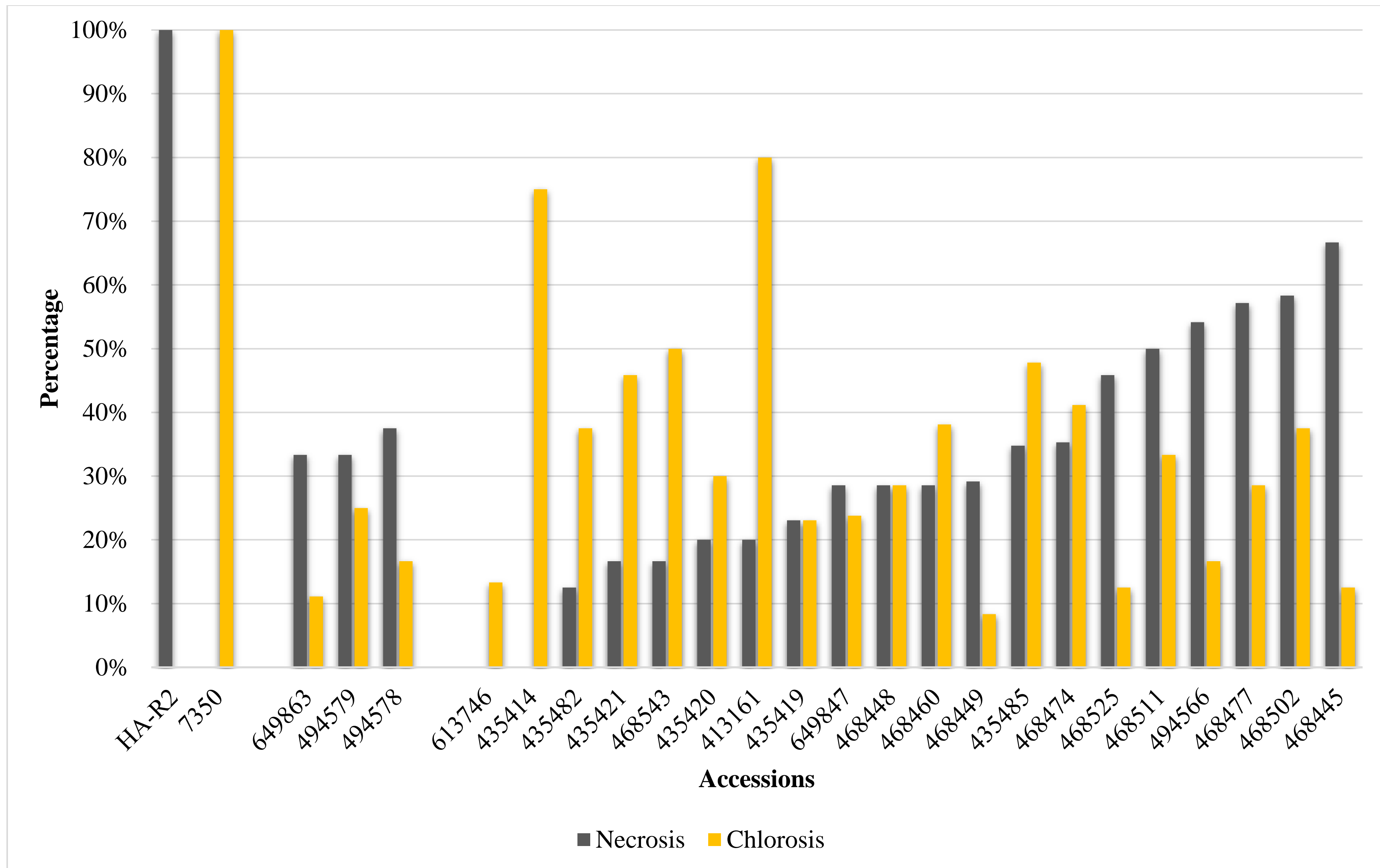

Figure 3.6. Percent of plants showing symptoms of necrosis or chlorosis for 20 wild *Helianthus annuus* and three wild *H. argophyllus* accessions in the screening of additional accessions to a bulk mixture of *Puccinia helianthi* isolates. All 23 additional accessions of interest were previously determined to confer high levels of resistance to *Plasmopara halstedii*.

Discussion

In this study we screened over 200 accessions from two species in an effort to identify potentially novel sources of resistance. To do so most effectively, a relatively large number of plants per accession were used and a high level of detail was given to methods of evaluation (e.g. infection types, necrosis/necrosis symptoms, cotyledon infections). After screening all accessions to a common pathogen race, and a subset of accessions to multiple isolates in attempt to create a more virulent race combination, all plants from two dozen accessions remained resistant. The accessions identified and the associated data may be useful for managing sunflower rust for many years.

The strategy used in this study was to first screen all accessions to a single-pustule isolate of a common race of *P. helianthi* (race 336), and then advance a small percentage of those accessions to be screened by a more virulent combination of isolates and races. Screening lines to bulk collections has been used effectively in this pathosystem to screen lines quickly, but it is not without risk (Gulya 2000). The isolates selected for the advanced screening were all single-pustule derived, race-typed on the standard differential set, and then combined into a bulk mixture of inoculum. While we expected the aggregate virulence phenotype of the bulk inoculum to be a 777 (which confers virulence on all differentials), the bulk inoculum conferred only minimal levels of virulence on several differentials lines, and subsequently expressed the virulence phenotype of race 336. Although the advanced screening was done with a collection of multiple isolates from different origins and the pre-screening was done with a single-pustule derived isolate, the initial screening and advanced screening were essentially done with the same race. The loss of virulence to several differentials during the study could be explained by the loss of viability to one or more isolates used in the bulk inoculum. Additionally, Friskop et al.

(2015b) found that isolates with virulence on many resistance genes are very rare in nature, while isolates with limited virulence are much more common, suggesting a fitness penalty for additive virulence may exist in *P. helianthi* (Friskop et al. 2015b). Consequently, it is possible that the more virulent isolates used in this study could have been outcompeted by other isolates with less virulence. In future screening, the authors recommend only the use of single isolates used as inoculum, this would eliminate either the possibility of the loss of viability of an isolate going unnoticed or a fitness advantage by less virulent isolates.

Previous studies had indicated that a higher percentage of accessions originating from TX may be resistant to rust than from other locations. When Gulya et al. (2000) screened 128 accessions to a bulk isolate/race mixture in 2000, he considered six accessions resistant, with each accession having greater than 70% of the plants resistant. Similarly, Friskop (2013) screened 112 accessions to race 336 and considered five accessions resistant, with each accession having greater than 80% of the plants resistant. In our study, we found 70 and 54 *H. annuus* accessions with greater than 70% and 80% the plants resistant to race 336, respectively. Two primary reasons could explain why a higher frequency of accessions appeared to be resistant in our study. First, the isolates used to screen the whole populations tested were different. Gulya et al. (2000) used a bulk collection of isolates with mixture of races collected in the 1990s, potentially increasing the chance that the pathogen would have been virulent on more germplasm. Friskop (2013) used isolates of race 336, but the isolates were different, and could have had virulence differences that could not be accounted for in the differentials. Secondly, the origin of germplasm was different. Gulya et al. (2000) screened germplasm from seven geographic regions of the U.S. and Friskop (2013) screened a statistical cross-section of accessions originating from six continents, while this study screened only TX derived

germplasm. While it was beyond the scope of the study to determine if and what origin may result in higher frequency of native resistance, the higher frequency of resistance in this study may circumstantially support previous indications that a higher frequency of resistant germplasm may be present in TX than in other locations. Given that the center of origin and domestication of *H. annuus* is the continental U.S., wild and commercial *Helianthus* species are very well adapted to the U.S. southern Great Plains and TX is the largest state in the Great Plains by orders of magnitude (Putt 1997). The suggestion that a large amount of resistance in TX is not biologically unreasonable. Additionally, the warm TX climate is conducive for *P. helianthi* to go through many secondary reproduction cycles throughout the year, and in some cases, without being interrupted by a killing frost. It is possible that this favorable climate may have allowed both the pathogen to adapt to native germplasm quicker than in other areas.

Previously, Gulya et al. (2000) identified six wild *H. annuus* accessions derived from TX that all had greater than 70% of plants resistant to a bulk mixture of races. These same six accessions, which included PI 468451 (108), PI 468455 (112), PI 435435 (47), PI 468519 (159), PI 435428 (40), and PI 468457 (114), were screened against races common only to North America in 2011 and 2012 in this study. PI accessions 468451 (108), 468455 (112), and 468519 (159) were identified as being among the most resistant accessions identified in the initial screening, whereas PI 435435 (47), PI 435428 (40), and PI 468457 (114) were not. PI 468451 (108), 468455 (112), and 468519 (159) were screened in the advanced screening and all found to be 100% resistant. Of PI 468451 (108), 468455 (112), and 468519 (159), the infection types 0 and ; accounted for 96%, 93%, and 96% of all infection types observed for each accession, respectively. This data indicates that three of the six accessions that were highly resistant to races

in the 1990's are still highly resistant to races in the 2010's, indicating that they still contain a viable source of resistance to current U.S. *P. helianthi* populations.

Examples of rust resistance genes identified from wild *Helianthus* germplasm incorporated into cultivars and hybrids exist (Jan et al. 1991; Jan et al. 2004; Putt and Sackston 1963; Quresh 1990; Sendall et al. 2006). However, choosing which of the accessions to advance into the breeding process can be difficult, especially when a large amount of accessions have a high percentage of resistance. This is particularly true in our study, where all 24 accessions included in the advanced screening had greater than 95% resistance to the most virulent combination of races detected in North America. In order to aid in selection of elite accessions, there are several very important things to consider; including the frequency of infection types, presence of pustules on the cotyledons, symptoms of necrosis and chlorosis, the possibility that selected accessions could be resistant to other pathogens (such as *Plasmopara halstedii*), and used internationally.

The frequency of infection types was considered for each of the 24 accessions included in the advanced screening. Accessions with plants that only showed infection types of 0 and ; included seven *H. annuus* accessions: PI 435444 (56), PI 468456 (113), PI 435429 (41), PI 613728 (200), PI 468523 (163), PI 468524 (164), and PI 468450 (107), and two *H. argophyllus* accessions: PI 494582 (199) and PI 649864 (213). Additionally, plants with infection types of only 0 or ; on the true leaves were evaluated for presence of pustules on cotyledons. Of the previous nine accessions, only six accessions including: PI 435444 (56), PI 468523 (163), PI 468524 (164), PI 468450 (107), PI 494582 (199), and PI 649864 (213) had plants that did not display any signs of pustules on the true leaves or cotyledons.

Previously, Sackston (1962) noted that the cereal rust infection type scale was not directly applicable for evaluating sunflower rust and suggested that in addition to the infection type rating, a phenotypic description of chlorosis or necrosis associated with rust pustules or flecking should also be evaluated. Results showed that 22 of the 23 accessions had either symptoms of chlorosis and/or necrosis at varying levels. This variability could be partly due to the high levels of heterozygosity that exists within the wild *Helianthus* germplasm. The presence of chlorosis and necrosis seemed to correlate broadly with virulence and avirulence, where chlorosis tended to be more common on virulent reactions and necrosis tended to be more common on avirulent reactions. However, necrosis and chlorosis did not seem to correlate closely within infection type reactions which were categorized as resistant (IT = 0, ;, 1, and 2) or susceptible (IT = 3, 4, and 5). Consequently, the chlorosis and necrosis evaluations did not provide useful information for differentiating accessions.

In a companion research project, these same 182 *H. annuus* and 33 *H. argophyllus* accessions were screened to *Plasmopara halstedii*, which causes downy mildew of sunflower. The most resistant accessions from that study were included in this study as additional accessions of interest. While a majority of these accessions were determined to be resistant to rust, many had numerous plants that showed susceptible infection types. The most promising accessions of interest in this study included two *H. annuus* (PI 468456 (113) and PI 468449 (106)) and two *H. argophyllus* (PI 649863 (212), and PI 494579 (196)) accessions. These four accessions only had plants showing infection types of 0 and ; and have the most promise for possessing novel resistance genes to both pathogens. To the best of our knowledge these accessions have not been explored for resistance to either pathogen.

Another consideration is advancing accessions that can be used for international production, particularly in South America, Europe and Asia, where hectarage surpasses that in the U.S (Sandbakken and Kleingartner 1997). The scope of this study was to screen germplasm to North American pathogen races, but the virulence of pathogen populations in other countries is different from what is found in North America (Friskop et al. 2015b). For example, different races are known to exist in South America and China, but because not all the same differentials have been used to virulence phenotype isolates, comparisons are difficult to make between the *P. helianthi* populations (Jing et al. 2015; Moreno et al. 2012). Moreno et al. (2012) identified an isolate in Argentina that was virulent on HA-R6, which is a line that has been known to be resistant to the most virulent North American race 777. Consequently, evaluation of accessions from an international collection of isolates may be prudent before making the final selection of accessions to be advanced into the breeding process.

The results from this study are promising for the future long-term management of *P. helianthi* in the U.S. Not only were high levels of resistance detected within the population of TX germplasm, there were a large number of accessions with a high percent of the plants showing resistance. Additionally, a couple of these accessions could be harboring sources of resistance to both *P. helianthi* and *P. halstedii*.

Literature Cited

Bailey, D. L. 1923. Sunflower Rust. University of Minnesota Tech. Bull. 16.

Berglund, D. 2007. Introduction. Pages 1-5 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Friskop, A. J. 2013. Developing management tools for sunflower rust (*Puccinia helianthi*). Ph.D. dissertation, North Dakota State University. 80 pp.

Friskop, A. J., Gulya, T. J., Halley, S. A., Schatz, B. G., Schaefer, J. P., Jordahl, J. G, Meyer, S. M., Misek, K. W., Hendrickson, P., and Markell, S. G. 2015a. Effect of fungicide and timing of application on management of sunflower rust. Plant Dis. 99:1210-1215.

Friskop, A. J., Gulya, T. J., Harveson, R. M., Humann, R. M., Acevedo, M., and Markell, S. G. 2015b. Phenotypic diversity of *Puccinia helianthi* (sunflower rust) in the United States from 2011 and 2012. Plant Dis. 99:1604-1609.

Friskop, A., Markell, S., Gulya, T., Halley, S., Schatz, B., Schaefer, J., Wunsch, M., Meyer, S., Kandel, H., Acevedo, M., Venette, J., and Venette R. 2011. Sunflower rust. North Dakota State Univ. Coop. Ext. Serv., Publ. PP-1557. Fargo, ND.

Gulya, T. 2006. The sunflower rust situation: Current races in the northern and central Great Plains, and resistance in oilseed and confection hybrids. Proc. 28th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/251/Gulya_Rust_06.pdf

Gulya, T., Kong, G., and Brothers, M. 2000. Rust resistance in wild *Helianthus annuus* and variation by geographic origin. Proc. 15th Int. Sunflower Conf., International Sunflower Association, Toulouse, France. Online Publication. http://isasunflower.org/fileadmin/documents/aaProceedings/15thISCToulouse2000/PosterWorkshopI-O/I-USA7.pdf

Gulya, T. J. and Markell, S. 2009. Sunflower rust status – 2008: race frequency across the Midwest and resistance among commercial hybrids. Proc. 31st Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/resources/195/gulya_ruststatus_09.pdf

Gulya, T. J. and Masirevic, S. 1996. Inoculation and evaluation methods for sunflower rust. Proc. 18th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/638/1996_gulya_inoculation_evaluation methods-.pdf

Gulya, T., Rashid, K. Y., and Masirevic, S. M. 1997. Sunflower diseases. Pages 263-379 in: Sunflower Technology and Production. A. A. Schneiter ed. American Society of Agronomy, Madison, WI.

Gulya, T. and Viranyi, F. 1994. Virulent new races of sunflower rust (*Puccinia helianthi*) from the southern great plains. Proc. 16th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/719/1994_gulya_virulentracessfrust.pdf

Harada, W. S. 1982. The effects of ethephon on dormant seeds of cultivated sunflower (*Helianthus annuus* L.). Proc. 10th Int. Sunflower Conf., International Sunflower Association, Surfers Paradise, Australia. Online Publication. http://isasunflower.org/fileadmin/documents/Proceedings/10thISC1982/Agronomy/T1982AGR03.pdf

Harveson, R. M. 2010. Rust of sunflower in Nebraska. University of Nebraska-Lincoln Ext. Publ. G1995. Lincoln, NE.

Heiser, C. B., Smith, D. M., Clevenger, S. B., and Martin, W. C. 1969. The North American sunflowers (*Helianthus*). Mem. Torrey Bot. Club 22:1-213.

Hulke, B. S., Miller, J. F., and Gulya, T. J. 2010. Registration of the restorer oilseed sunflower germplasm RHA 464 possessing genes for resistance to downy mildew and sunflower rust. J. Plant Regist. 4:249-254.

Jan, C. C., Quresh, Z., and Gulya, T. J. 1991. Genetics of rust race 4 resistance derived from *Helianthus petiolaris* and *H. argophyllus* accessions. Proc. 13th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/807/1991_jan_geneticsrustraceresistance.pdf

Jan, C. C., Quresh, Z., and Gulya, T. J. 2004. Registration of seven rust resistant sunflower germplasms. Crop Sci. 44:1887-1888.

Jing, L., Xu, X., Jing, J., Li, L., and Navi, S. 2015. Determination of physiological races and evaluation of sunflower for resistance to *Puccinia helianthi* Schw. J. Phytopathol. 163: 507-512.

Kandel, H. 2014. 2013 National Sunflower Crop Survey. Proc. 36th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1227/kandel_2013.crop.survey_paper_2014.pdf

Kandel, H. and Gulya, T. 2016. 2015 National Sunflower Crop Survey. Proc. 38th Annu. Sunflower Res. Forum. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/1252/2015.sunflower.survey.report_web.pdf

Kong, G. A., Goulter, K. C., Kochman, J. K., and Thompson, S. M. 1999. Evolution of pathotypes of *Puccinia helianthi* on sunflower in Australia. Australas. Plant Path. 28:320-332.

Marek, L. F., Block, C. C., and Gardner, C. C. A. 2012. 2012 Update: New sunflower genetic resources in the U.S. national sunflower collection and potential use for crop improvement. Proc. 18th Intl. Sunflower Conf. International Sunflower Association. Mar del Plata, Argentina. Online Publication.

Markell, S., Gulya, T., McKay, K., Hutter, M., Hollingsworth, C., Ulstad, V., Koch, R., and Knudsvig, A. 2009. Widespread occurrence of the aecial stage of sunflower rust caused by *Puccinia helianthi* in North Dakota and Minnesota in 2008. Plant Dis. 93:6.

Moreno, P. S., de Romano, A. B., Romano, M. C., Vergani, P., Sposaro, M., Bulos, M., Altieri, E., Ramos, M. L., and Sala, C. A. 2012. A survey of physiological races of *Puccinia helianthi* in Argentina. Proc. 18th Int. Sunflower Conf. Mar del Plata, Argentina. Online Publication. http://www.asagir.org.ar/asagir2008/buscar_congreso.asp?

Putt, E. D. 1997. Early History of Sunflower. Pages 1-20 in: Sunflower Technology and Production. A.A. Schneiter, ed. American Society of Agronomy, Madison, WI.

Putt, E. D. and Sackston, W. E. 1957. Studies on sunflower rust. I. Some sources of rust resistance. Can. J. Plant Sci. 37:43-54.

Putt, E. D. and Sackston, W. E. 1963. Studies on sunflower rust. IV. Two genes, R_1 and R_2, for resistance in the host. Can. J. Plant Sci. 43:490-496.

Quresh, Z., Jan, C. C., and Gulya, T. J. 1990. Transferring rust resistant genes from wild *Helianthus* ssp. into cultivated line. Proc. 12th Sunflower Res. Workshop. National Sunflower Association, Mandan, ND. Online Publication. http://www.sunflowernsa.com/uploads/research/838/1990_quresh_transferringrustgenes.pdf

Sackston, W. E. 1962. Studies on sunflower rust. III. Occurrence, distribution, and significance of races of *Puccinia helianthi* Schw. Can. J. Bot. 40:1449-1458.

Sackston, W. E. 1981. Downy mildew of sunflower. Pages 545-576 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, London, England.

Sackston, W. E. 1992. On a treadmill: Breeding sunflower for resistance to disease. Annual Rev. Phytopathol. 30:529-551.

Sandbakken, J. and Kleingartner, L. 2007. U.S. Production. Pages 6-8 in: Sunflower Production Guide. D. R. Berglund, ed. North Dakota State Univ. Coop. Ext. Serv., Publ. A-1331. Fargo, ND.

Schilling, E. E. 2006. *Helianthus*. Pages 141-169 in: Flora of North America North of Mexico. Vol. 21. Flora of North America Editorial Committee, eds. Oxford University Press, New York, NY.

Seiler, G. J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Res. 30:195-230.

Sendall, B. C., Kong, G. A., Goulter, K. C., Aitken, E. A. B, Thompson, S. M., Mitchell, J. H. M., Kochman, J. K., Lawson, W., Shatte, T., and Gulya, T. J. 2006. Diversity in the sunflower: *Puccinia helianthi* pathosystem in Australia. Australas. Plant Path. 35:657-670.

USDA, National Agricultural Statistics Service. 2016. Sunflower quick statistics. Available at: http://quickstats.nass.usda.gov/results/C714A9DF-EFBF-3E42-902C-DE1316523C76?pivot=short_desc

Yang, S. -M., Antonelli, E. F., Luciano, A., and Luciani, N. D. 1986. Reactions of Argentine and Australian sunflower rust differentials to four North American cultures of *Puccinia helianthi* from North Dakota. Plant Dis. 70:883-886.

APPENDIX A. SUMMARY OF STATISTICAL ANALYSES FOR THE 2011-2014 SUNFLOWER DOWNY MILDEW FUNGICIDE SEED TREATMENT EFFICACY TRIALS

Table A.1. Analysis of variance for the sunflower downy mildew incidence rating 31 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	22.81	0.99	0.4125
Treatment	9	2393.90	103.86	<.0001

Table A.2. Analysis of variance for the sunflower downy mildew incidence rating 46 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	2.77	0.05	0.9851
Treatment	9	1135.87	20.33	<.0001

Table A.3. Analysis of variance for the sunflower downy mildew incidence rating 60 days after planting across ten fungicide seed treatments for the 2011 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	169.49	2.20	0.1108
Treatment	9	911.62	11.85	<.0001

Table A.4. Analysis of variance for the sunflower downy mildew incidence rating 26 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	290.84	1.30	0.2948
Treatment	9	4033.67	18.03	<.0001

Table A.5. Analysis of variance for the sunflower downy mildew incidence rating 37 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	276.87	1.93	0.1478
Treatment	9	4895.26	34.21	<.0001

Table A.6. Analysis of variance for the sunflower downy mildew incidence rating 51 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	387.69	1.65	0.2009
Treatment	9	2453.00	10.45	<.0001

Table A.7. Analysis of variance for the sunflower downy mildew incidence rating 65 days after planting across ten fungicide seed treatments for the 2011 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	477.51	2.10	0.1231
Treatment	9	1628.45	7.18	<.0001

Table A.8. Analysis of variance for the sunflower downy mildew incidence rating 36 days after planting across ten fungicide seed treatments for the 2011 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	69.61	1.64	0.2024
Treatment	9	876.63	20.71	<.0001

Table A.9. Analysis of variance for the sunflower downy mildew incidence rating 54 days after planting across ten fungicide seed treatments for the 2011 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	93.76	2.37	0.0926
Treatment	9	971.24	24.57	<.0001

Table A.10. Analysis of variance for the sunflower downy mildew incidence rating 37 days after planting across nine fungicide seed treatments for the 2012 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	5.74	0.31	0.8208
Treatment	8	49.98	2.66	0.0301

Table A.11. Analysis of variance for the sunflower downy mildew incidence rating 48 days after planting across nine fungicide seed treatments for the 2012 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	4.31	0.27	0.8471
Treatment	8	52.63	3.29	0.0112

Table A.12. Analysis of variance for the sunflower downy mildew incidence rating 45 days after planting across nine fungicide seed treatments for the 2012 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	94.48	3.99	0.0195
Treatment	8	1500.94	63.34	<.0001

Table A.13. Analysis of variance for the sunflower downy mildew incidence rating 52 days after planting across nine fungicide seed treatments for the 2012 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	151.93	5.48	0.0052
Treatment	8	1209.20	43.63	<.0001

Table A.14. Analysis of variance for the sunflower downy mildew incidence rating 34 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	37.36	1.84	0.1312
Treatment	7	5327.34	261.83	<.0001

Table A.15. Analysis of variance for the sunflower downy mildew incidence rating 48 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	47.21	1.33	0.2751
Treatment	7	5398.36	151.87	<.0001

Table A.16. Analysis of variance for the sunflower downy mildew incidence rating 59 days after planting across eight fungicide seed treatments for the 2013 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	37.26	1.14	0.3556
Treatment	7	4758.15	146.15	<.0001

Table A.17. Analysis of variance for the sunflower downy mildew incidence rating 23 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	97.31	0.63	0.6748
Treatment	7	7698.75	50.19	<.0001

Table A.18. Analysis of variance for the sunflower downy mildew incidence rating 38 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	138.26	0.86	0.5170
Treatment	7	7782.92	48.45	<.0001

Table A.19. Analysis of variance for the sunflower downy mildew incidence rating 52 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 1.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	190.06	0.92	0.4798
Treatment	7	7601.14	36.78	<.0001

Table A.20. Analysis of variance for the sunflower downy mildew incidence rating 28 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	20.68	0.86	0.4748
Treatment	7	1168.26	48.86	<.0001

Table A.21. Analysis of variance for the sunflower downy mildew incidence rating 40 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	54.37	1.49	0.2474
Treatment	7	987.19	26.96	<.0001

Table A.22. Analysis of variance for the sunflower downy mildew incidence rating 61 days after planting across eight fungicide seed treatments for the 2013 Fargo, ND field trial 2.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	3	84.47	1.55	0.2308
Treatment	7	1018.75	18.71	<.0001

Table A.23. Analysis of variance for the sunflower downy mildew incidence rating 27 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	32.67	0.84	0.5285
Treatment	7	2353.84	60.75	<.0001

Table A.24. Analysis of variance for the sunflower downy mildew incidence rating 35 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	141.99	2.74	0.0341
Treatment	7	2143.06	41.40	<.0001

Table A.25. Analysis of variance for the sunflower downy mildew incidence rating 46 days after planting across eight fungicide seed treatments for the 2013 Thompson, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	165.94	2.53	0.0467
Treatment	7	1972.95	30.09	<.0001

Table A.26. Analysis of variance for the sunflower downy mildew incidence rating 23 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	60.35	1.11	0.3885
Treatment	4	7611.65	139.47	<.0001

Table A.27. Analysis of variance for the sunflower downy mildew incidence rating 39 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	21.20	0.41	0.8354
Treatment	4	6852.81	132.88	<.0001

Table A.28. Analysis of variance for the sunflower downy mildew incidence rating 57 days after planting across five fungicide seed treatments for the 2014 Carrington, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	55.44	0.18	0.9680
Treatment	4	2267.97	7.25	0.0009

Table A.29. Analysis of variance for the sunflower downy mildew incidence rating 24 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	437.58	2.47	0.0677
Treatment	4	1510.32	8.51	0.0004

Table A.30. Analysis of variance for the sunflower downy mildew incidence rating 35 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	383.87	1.57	0.2138
Treatment	4	2623.09	10.73	<.0001

Table A.31. Analysis of variance for the sunflower downy mildew incidence rating 49 days after planting across five fungicide seed treatments for the 2014 Fargo, ND field trial.

Source of variation	Degrees of freedom	Mean squares	F value	*P* value
Rep	5	209.35	1.23	0.3330
Treatment	4	1956.69	11.47	<.0001

APPENDIX B. SUMMARY OF RESULTS OF SCREENING *HELIANTHUS* GERMPLASM TO *PLASMOPARA HALSTEDII*

Table B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714.

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
1	413160	32	23	55	42
2	413161	2	25	27	93
3	413162	30	25	55	45
4	413163	22	27	49	55
5	413164	23	23	46	50
6	413165	20	14	34	41
7	413166	11	23	34	68
8	413167	10	25	35	71
9	413168	15	44	59	75
10	413169	13	24	37	65
12	435357	25	27	52	52
13	435359	21	35	56	63
14	435363	38	34	72	47
15	435366	25	43	68	63
16	435367	19	20	39	51
17	435368	12	19	31	61
18	435369	22	31	53	58
19	435370	24	36	60	60
20	435407	18	42	60	70
21	435408	26	33	59	56
22	435409	18	30	48	63
23	435410	37	33	70	47
24	435411	46	20	66	30
25	435412	32	34	66	52
26	435413	23	25	48	52
27	435414	2	29	31	94
28	435415	35	24	59	41
29	435416	9	33	42	79
30	435417	13	39	52	75
31	435418	12	45	57	79
32	435419	5	40	45	89
33	435420	1	38	39	97
34	435421	9	55	64	86
35	435423	9	50	59	85

Table B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714 (continued).

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
36	435424	10	35	45	78
37	435425	26	27	53	51
38	435426	35	13	48	27
39	435427	7	15	22	68
40	435428	12	27	39	69
41	435429	32	35	67	52
42	435430	30	28	58	48
43	435431	20	51	71	72
44	435432	10	76	86	88
45	435433	21	37	58	64
46	435434	16	48	64	75
47	435435	16	48	64	75
48	435436	25	33	58	57
49	435437	14	42	56	75
50	435438	12	71	83	86
51	435439	11	63	74	85
52	435440	11	56	67	84
53	435441	34	44	78	56
54	435442	24	50	74	68
55	435443	11	44	55	80
56	435444	13	7	20	35
57	435445	31	52	83	63
58	435448	29	27	56	48
59	435455	34	26	60	43
60	435456	16	54	70	77
61	435457	16	24	40	60
62	435458	13	27	40	68
63	435459	29	31	60	52
64	435460	19	29	48	60
65	435461	29	39	68	57
66	435462	19	32	51	63
67	435463	33	23	56	41
68	435482	5	31	36	86
69	435483	32	33	65	51
70	435484	18	56	74	76
71	435485	6	73	79	92
72	435486	10	52	62	84
73	435487	8	50	58	86

Table B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714 (continued).

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
74	435488	24	41	65	63
75	435489	23	35	58	60
76	435494	21	31	52	60
77	435495	22	41	63	65
78	435497	20	32	52	62
79	435498	24	19	43	44
80	435504	16	53	69	77
81	435531	29	20	49	41
82	435532	15	55	70	79
83	435533	26	17	43	40
84	435534	28	54	82	66
85	435535	37	27	64	42
86	435536	17	8	25	32
87	435554	19	21	40	53
101	435850	16	60	76	79
102	468445	10	74	84	88
103	468446	26	51	77	66
104	468447	12	68	80	85
105	468448	6	60	66	91
106	468449	8	70	78	90
107	468450	16	62	78	79
108	468451	13	71	84	85
109	468452	13	56	69	81
110	468453	21	47	68	69
111	468454	31	36	67	54
112	468455	23	50	73	68
113	468456	5	65	70	93
114	468457	11	55	66	83
115	468458	14	43	57	75
116	468459	20	62	82	76
117	468460	8	69	77	90
118	468461	27	57	84	68
119	468462	20	50	70	71
120	468463	18	38	56	68
121	468464	11	62	73	85
122	468465	30	46	76	61
123	468466	20	64	84	76
124	468467	23	54	77	70

Table B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714 (continued).

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
125	468473	8	33	41	80
126	468474	8	45	53	85
127	468475	11	31	42	74
128	468476	23	60	83	72
129	468477	8	49	57	86
130	468478	15	53	68	78
131	468479	12	53	65	82
132	468480	8	27	35	77
133	468481	9	30	39	77
134	468482	19	43	62	69
135	468494	12	47	59	80
136	468495	15	33	48	69
137	468497	9	61	70	87
138	468498	9	29	38	76
139	468499	12	65	77	84
140	468500	15	65	80	81
141	468501	15	44	59	75
142	468502	8	78	86	91
143	468503	33	32	65	49
144	468504	16	31	47	66
145	468505	17	43	60	72
146	468506	36	29	65	45
147	468507	12	59	71	83
148	468508	11	64	75	85
149	468509	10	75	85	88
150	468510	16	63	79	80
151	468511	7	60	67	90
152	468512	19	63	82	77
153	468513	23	36	59	61
154	468514	17	51	68	75
155	468515	34	59	93	63
156	468516	17	43	60	72
157	468517	24	54	78	69
158	468518	19	54	73	74
159	468519	25	31	56	55
160	468520	14	65	79	82
161	468521	16	49	65	75
162	468522	15	62	77	81

Table B.1. Susceptible and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Plasmopara halstedii* race 714 (continued).

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
163	468523	12	40	52	77
164	468524	16	46	62	74
165	468525	10	59	69	86
166	468526	17	56	73	77
167	468527	30	34	64	53
168	468528	24	44	68	65
169	468529	35	32	67	48
170	468530	15	53	68	78
171	468531	11	21	32	66
172	468532	39	32	71	45
173	468533	24	30	54	56
174	468534	18	38	56	68
175	468535	27	36	63	57
176	468536	22	15	37	41
177	468541	22	31	53	58
178	468542	30	30	60	50
179	468543	6	54	60	90
180	468544	35	53	88	60
183	494566	10	79	89	89
184	494567	20	74	94	79
185	494568	17	48	65	74
200	613728	20	53	73	73
201	613746	4	47	51	92
202	613747	13	25	38	66
203	649810	8	21	29	72
204	649811	30	39	69	57
205	649845	14	29	43	67
206	649846	11	35	46	76
207	649847	5	44	49	90
208	649848	21	25	46	54
209	649849	11	42	53	79
210	649850	11	28	39	72
214	664613	10	33	43	77
215	664692	25	35	60	58
Check	HA335	170	5	175	3
Check	Myc 270	1769	75	1844	4

Table B.2. Susceptible and resistant plants and percent resistance for 33 wild *Helianthus argophyllus* accessions in the initial screening to *Plasmopara halstedii* race 714.

Item No.	Accession No. (PI)	Susceptible	Resistant	Total	Resistance (%)
11	413171	25	61	86	71
88	435623	14	49	63	78
89	435624	7	59	66	89
90	435625	20	48	68	71
91	435626	20	58	78	74
92	435627	14	37	51	73
93	435628	14	19	33	58
94	435629	12	24	36	67
95	435630	14	17	31	55
96	435631	3	47	50	94
97	435632	20	26	46	57
98	435633	17	21	38	55
99	435634	11	63	74	85
100	435635	10	57	67	85
181	468648	14	12	26	46
182	468649	16	20	36	56
186	494569	6	48	54	89
187	494570	15	15	30	50
188	494571	4	38	42	90
189	494572	10	21	31	68
190	494573	14	24	38	63
191	494574	13	55	68	81
192	494575	9	38	47	81
193	494576	10	59	69	86
194	494577	14	52	66	79
195	494578	2	58	60	97
196	494579	3	65	68	96
197	494580	4	62	66	94
198	494581	4	68	72	94
199	494582	7	70	77	91
211	649862	14	49	63	78
212	649863	3	62	65	95
213	649864	3	71	74	96
Check	HA335	30	0	30	0
Check	Myc 270	286	4	290	1

APPENDIX C. SUMMARY OF RESULTS OF SCREENING *HELIANTHUS* GERMPLASM TO *PUCCINIA HELIANTHI*

Table C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336.

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
1	413160	18	4	7	29	24
2	413161	7	0	18	25	72
3	413162	22	0	8	30	27
4	413163	29	0	1	30	3
5	413164	12	0	18	30	60
6	413165	22	3	5	30	17
7	413166	22	1	7	30	23
8	413167	21	2	7	30	23
9	413168	12	2	16	30	53
10	413169	20	0	10	30	33
12	435357	17	1	10	28	36
13	435359	3	0	27	30	90
14	435363	5	1	24	30	80
15	435366	18	0	11	29	38
16	435367	16	2	10	28	36
17	435368	14	0	5	19	26
18	435369	21	3	6	30	20
19	435370	17	2	10	29	34
20	435407	12	0	16	28	57
21	435408	13	4	10	27	37
22	435409	12	0	15	27	56
23	435410	8	0	16	24	67
24	435411	11	0	19	30	63
25	435412	14	1	12	27	44
26	435413	11	1	16	28	57
27	435414	5	1	13	19	68
28	435415	4	0	26	30	87
29	435416	13	1	15	29	52
30	435417	10	0	19	29	66
31	435418	7	0	22	29	76
32	435419	13	0	15	28	54
33	435420	17	5	8	30	27
34	435421	10	0	20	30	67
35	435423	10	1	19	30	63

Table C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336 (continued).

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
36	435424	8	1	14	23	61
37	435425	3	2	25	30	83
38	435426	4	0	25	29	86
39	435427	3	0	23	26	88
40	435428	2	1	27	30	90
41	435429	0	0	30	30	100
42	435430	16	1	13	30	43
43	435431	2	0	28	30	93
44	435432	0	0	30	30	100
45	435433	11	0	17	28	61
46	435434	1	0	27	28	96
47	435435	2	0	23	25	92
48	435436	2	0	23	25	92
49	435437	3	0	27	30	90
50	435438	6	0	24	30	80
51	435439	3	0	27	30	90
52	435440	7	1	22	30	73
53	435441	9	0	21	30	70
54	435442	14	1	15	30	50
55	435443	16	1	13	30	43
56	435444	0	0	12	12	100
57	435445	3	2	25	30	83
58	435448	9	0	21	30	70
59	435455	14	2	9	25	36
60	435456	11	1	18	30	60
61	435457	13	2	9	24	38
62	435458	12	0	12	24	50
63	435459	12	1	17	30	57
64	435460	13	1	16	30	53
65	435461	12	0	15	27	56
66	435462	14	0	7	21	33
67	435463	16	0	13	29	45
68	435482	15	1	7	23	30
69	435483	11	3	14	28	50
70	435484	3	1	26	30	87
71	435485	9	0	20	29	69
72	435486	14	1	15	30	50
73	435487	7	1	21	29	72

Table C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336 (continued).

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
74	435488	7	3	18	28	64
75	435489	15	0	13	28	46
76	435494	13	0	16	29	55
77	435495	7	0	21	28	75
78	435497	16	2	9	27	33
79	435498	16	0	13	29	45
80	435504	25	0	5	30	17
81	435531	8	0	9	17	53
82	435532	8	0	21	29	72
83	435533	10	0	7	17	41
84	435534	18	1	11	30	37
85	435535	18	0	12	30	40
86	435536	9	2	18	29	62
87	435554	12	0	13	25	52
101	435850	0	0	29	29	100
102	468445	10	2	17	29	59
103	468446	9	1	19	29	66
104	468447	7	0	23	30	77
105	468448	2	1	23	26	88
106	468449	2	0	27	29	93
107	468450	0	0	28	28	100
108	468451	0	0	29	29	100
109	468452	2	0	28	30	93
110	468453	1	0	29	30	97
111	468454	1	0	25	26	96
112	468455	0	0	30	30	100
113	468456	0	0	28	28	100
114	468457	2	0	24	26	92
115	468458	0	0	30	30	100
116	468459	2	0	28	30	93
117	468460	8	0	22	30	73
118	468461	4	1	25	30	83
119	468462	21	3	5	29	17
120	468463	25	0	5	30	17
121	468464	16	0	13	29	45
122	468465	13	1	16	30	53
123	468466	26	1	3	30	10
124	468467	19	0	11	30	37

Table C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336 (continued).

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
125	468473	17	0	8	25	32
126	468474	12	1	17	30	57
127	468475	8	0	13	21	62
128	468476	5	1	24	30	80
129	468477	3	0	22	25	88
130	468478	8	0	20	28	71
131	468479	14	1	12	27	44
132	468480	3	1	20	24	83
133	468481	7	0	18	25	72
134	468482	7	0	22	29	76
135	468494	14	1	15	30	50
136	468495	16	0	12	28	43
137	468497	25	2	3	30	10
138	468498	10	1	16	27	59
139	468499	14	1	15	30	50
140	468500	10	0	18	28	64
141	468501	6	1	22	29	76
142	468502	14	2	14	30	47
143	468503	7	0	18	25	72
144	468504	10	1	17	28	61
145	468505	16	1	13	30	43
146	468506	18	1	11	30	37
147	468507	15	0	14	29	48
148	468508	14	0	16	30	53
149	468509	16	1	13	30	43
150	468510	17	0	13	30	43
151	468511	9	0	20	29	69
152	468512	1	0	26	27	96
153	468513	11	0	18	29	62
154	468514	1	1	25	27	93
155	468515	4	0	25	29	86
156	468516	4	1	25	30	83
157	468517	0	0	30	30	100
158	468518	7	2	21	30	70
159	468519	1	0	29	30	97
160	468520	1	0	29	30	97
161	468521	0	0	30	30	100
162	468522	2	0	28	30	93

Table C.1. Susceptible, mixed, and resistant plants and percent resistance for 182 wild *Helianthus annuus* accessions in the initial screening to *Puccinia helianthi* race 336 (continued).

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
163	468523	1	0	29	30	97
164	468524	1	0	27	28	96
165	468525	5	0	21	26	81
166	468526	9	2	18	29	62
167	468527	5	0	25	30	83
168	468528	9	0	19	28	68
169	468529	7	0	22	29	76
170	468530	16	2	12	30	40
171	468531	11	1	1	13	8
172	468532	25	1	3	29	10
173	468533	23	0	7	30	23
174	468534	11	0	19	30	63
175	468535	13	0	17	30	57
176	468536	3	0	26	29	90
177	468541	15	0	8	23	35
178	468542	25	0	4	29	14
179	468543	11	1	15	27	56
180	468544	9	0	21	30	70
183	494566	2	0	28	30	93
184	494567	3	0	25	28	89
185	494568	1	0	29	30	97
200	613728	1	0	28	29	97
201	613746	8	1	21	30	70
202	613747	6	0	23	29	79
203	649810	1	0	29	30	97
204	649811	18	1	11	30	37
205	649845	26	0	2	28	7
206	649846	13	0	12	25	48
207	649847	17	0	11	28	39
208	649848	11	0	18	29	62
209	649849	4	0	24	28	86
210	649850	21	3	5	29	17
214	664613	11	0	7	18	39
215	664692	19	1	10	30	33
Res. Check	HA-R2	0	0	29	29	100
Susc. Check	P386	28	1	0	29	0

Table C.2. Susceptible, mixed, and resistant plants and percent resistance for 33 wild *Helianthus argophyllus* accessions in the initial screening to *Puccinia helianthi* race 336.

Item No.	Accession No. (PI)	Susceptible	Mixed	Resistant	Total	Resistance (%)
11	413171	2	0	28	30	93
88	435623	0	1	29	30	97
89	435624	7	0	23	30	77
90	435625	0	1	29	30	97
91	435626	2	0	28	30	93
92	435627	0	0	27	27	100
93	435628	0	0	28	28	100
94	435629	1	1	28	30	93
95	435630	0	0	17	17	100
96	435631	1	0	25	26	96
97	435632	1	0	25	26	96
98	435633	0	0	29	29	100
99	435634	2	0	22	24	92
100	435635	0	0	27	27	100
181	468648	0	0	20	20	100
182	468649	2	0	24	26	92
186	494569	0	0	26	26	100
187	494570	0	0	26	26	100
188	494571	0	0	28	28	100
189	494572	2	0	25	27	93
190	494573	3	1	26	30	87
191	494574	1	0	29	30	97
192	494575	1	0	28	29	97
193	494576	1	0	26	27	96
194	494577	0	0	27	27	100
195	494578	0	0	28	28	100
196	494579	1	1	26	28	93
197	494580	0	1	29	30	97
198	494581	1	0	26	27	96
199	494582	0	0	25	25	100
211	649862	0	0	23	23	100
212	649863	0	0	24	24	100
213	649864	0	0	29	29	100
Res. Check	HA-R2	0	0	29	29	100
Susc. Check	P386	28	1	0	29	0