PREVALENCE OF PATHOGENICITY GROUPS OF THE BLACKLEG SPECIES COMPLEX ON CANOLA IN NORTH DAKOTA

A Paper
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Sciences

By

Shanna Andrea Mazurek

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

October 2011

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Prevalence of Pathogenicity Groups of the Blackleg Species Complex on Canola in North Dakota

By

Shanna A. Mazurek

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

MASTER OF SCIENCE

ABSTRACT

Mazurek, Shanna Andrea, M.S., Department of Plant Pathology, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, October 2011. Prevalence of Pathogenicity Groups of the Blackleg Species Complex on Canola in North Dakota. Major Professors: Drs. Luis del Rio and Sam Markell.

North Dakota is the leading producer of canola (*Brassica napus)* in the United States. Blackleg, caused by two species of *Leptosphaeria*, *L. maculans* (Desm.) Ces & De Not and *L. biglobosa*, is an economically important disease wherever canola is grown. The virulence profile of these species has been classified in six pathogenicity groups (PG) based on inoculations on three differential cultivars, 'Westar', 'Glacier', and 'Quinta'. A PG-1 reaction is caused only by *L. biglobosa* isolates. By 2001, PG-2 was the predominant profile in North Dakota, although PG-1 isolates were also present. Between 2007 and 2008 canola leaves with characteristic blackleg lesions were collected from 54 fields located in the major canola growing regions of North Dakota. The virulence profile of 280 isolates retrieved from these leaves was characterized on the above mentioned set of differentials. The predominant species was *L. maculans* with approximately 74% of all isolates retrieved. Virulence profiles typical of PG-2, PG-3, PG-T, PG-4, and PG-5, which were previously described, were observed among the isolates evaluated. The virulence profile of almost 32% of all isolates did not fit within the previously described PGs and were assigned to new groups. The most prevalent PG among *L. maculans* isolates were PG-6 and PG-T. PG-6 causes a resistant reaction on differential 'Quinta'. PG-2, previously regarded as the most prevalent, was identified in only 13% of isolates. These results suggest a shift in the population of the blackleg species complex in North Dakota.

ACKNOWLEDGEMENTS

I would like to express my gratitude towards my advisory committee: Drs. Sam Markell, Luis del Rio, Brian Jenks, Neil Gudmestad, and Richard Horsley. You all played a significant role in my success and learning and I am grateful. Special thanks to Drs. del Rio and Markell for your continued support, guidance, time, understanding, and most importantly, your patience. This has been a marathon and I cannot thank you enough for running by my side the entire time. It is about time we finish this race!

Susan Ruud and Pratisara Bajracharya, all of the isolations would have been impossible without your help and guidance. Susan, I really appreciate all the time you spent teaching me proper techniques.

Dr. Brian Jenks, thank you for being such a great boss and supporting my interests outside of weed science. The Canola agronomic and disease survey would not have been possible without your support. Data collection was possible with the help of Jordan Hoefing, Tammy Duchsherer, and Danielle Delzer. I appreciate all the long hours you ladies put in. It was a blast teaching you all I could about canola!

The Northern Canola Growers, US Canola Association, and SBARE provided additional funding for the survey portion of this study.

To the twenty canola growers who participated in this two year study, thanks for your time, cooperation, and patience while putting up with my nagging for information!

Thank you to my parents for always encouraging me to follow my dreams and reminding me that things worth working for are never easy.

Jonathan, my amazing husband, thank you for never letting me give up. Without you by my side, with your checkbook open, this dream would not have been possible.

DEDICATION

Art Lamey is someone who will always inspire me. If I can be half the plant pathologist he was I will be doing well. Even though he is no longer with us and will not be able to watch my exit seminar or hear about my defense, I know he will be watching from above and taking notes.

This is for you Art. Thank you for all the things you taught me about life and science. I did it! It was a long, hard journey like you said it would be, but I never gave up and persevered! I hope I made you proud.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

CHAPTER 1. INTRODUCTION

Phoma stem canker or blackleg is an economically important disease worldwide on oilseed rape (rapeseed, canola, *Brassica rapa* L., *B. juncea* L.) especially in Australia, Europe, and North America (mainly in Canada and North Dakota) (West *et al.*, 2001). In the early 1970's in Australia, a severe epidemic stalled the oilseed rape industry when it was just emerging (Bokor *et al.*, 1975). Epidemics have also impacted production in Europe and Canada. Oddly, the disease is rare in Scotland and China where a large amount of oilseed rape is grown (Gugel and Petrie, 1992). Severity of epidemics varies greatly between different regions, growing season, crop rotation, and type of oilseed rape cultivated. When the disease does occur, yield losses are usually between five and fifteen percent but on some occasions results in total crop loss (Bokor *et al.*, 1975; Barbetti and Khangura, 1999).

Phoma stem canker was first found and described on dead red cabbage, *Brassica oleracea* var. *capitata* f. *rubra*, in 1791 by Tode as *Sphaeria lingam.* Desmazieres found the same fungus in 1849 in wild cabbage, *B. oleracea,* and reclassified it as *Phoma lingam*, which became known as an important pathogen of crucifer crops all over the world. *P. lingam* Tode ex. Fr. is most commonly known for being the causal agent of blackleg (Punithalingam and Holliday, 1972). In 1957, the telomorph (sexual stage) was found in New Zealand (Anonymous, 1957) and confirmed as *Leptosphaeria maculans* (Desm.) Ces. & de Not. in 1972 by Punithalingam and Holliday.

Canola is an important oilseed crop grown in North Dakota. Annually, North Dakota produces between 88-92 percent of the canola grown in the United States (NASS, 2010). Canola production is spread across the state but the majority of the acres are grown

in north eastern part of the state, especially Cavalier, Towner, and Rolette counties (Figure 1). Cavalier county normally accounts for between 22-25 percent of North Dakota's production (NASS, 2010).

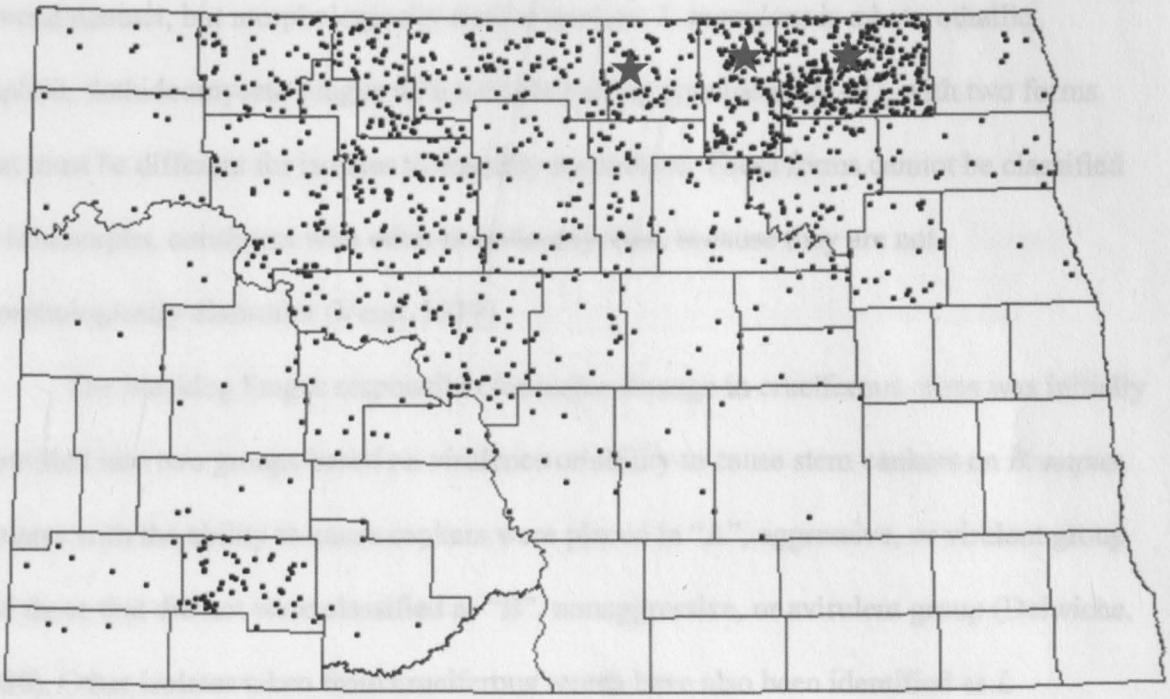

Figure 1. Harvested canola acres in North Dakota in 2009. 1 dot ≅ 405 hectares. Dots are randomly placed within each county. Counties with no dots represent none harvested or undisclosed data. Stars represent the three counties with highest annual production. Figure adapted from NASS, 2010.

CHAPTER 2. SCHOLARLY PAPER

Biology of the Pathogen

Classification of the *Leptosphaeria* sp. complex

Nomenclature and taxonomy of this fungus are confusing since it is associated with several distinct, but morphologically similar species. *L. maculans* is a heterothallic, haploid, dothideomycete fungus with a single mating type locus (*MAT*), with two forms that must be different for isolates to sexually recombine. These forms cannot be classified as idiomorphs, consistent with other dothideomycetes, because they are not morphologically dissimilar (Venn, 1979).

The blackleg fungus responsible for major damage in cruciferous crops was initially classified into two groups based on virulence or ability to cause stem cankers on *B. napus*. Isolates with the ability to cause cankers were placed in "A", aggressive, or virulent group and those that did not were classified as "B", nonaggressive, or avirulent group (Delwiche, 1980). Other isolates taken from cruciferous weeds have also been identified as *L. maculans* and are genetically dissimilar to the other two groups (Purwantara *et al.*, 2000). Although isolates from groups A and B have the ability to infect the same host and produce spores with similar morphology, colonies produced look different in culture (Cunningham, 1927), have genetic differences (Taylor *et al.*, 1991), and produce different stem and leaf symptoms (Johnson and Lewis, 1994; Brun *et al.*, 1997). Further, Somda *et al.* (1997) demonstrated that isolates from these groups were sexually incompatible with each other (1997).

The above information lead to the realization that blackleg is actually caused by a complex made of two distinct species; *L. maculans* and *L. biglobosa.* Group A or the

virulent group is *L. maculans* and the avirulent group (group B) is *L. biglobosa* sp. nov (Shoemaker and Brun, 2001). Isolates from both species have been further classified into pathogenicity groups (PG) based on differential infection severity on cotyledons of three canola cultivars: Westar, Glacier, and Quinta (Mengistu *et al.,* 1991). *L. maculans* isolates are classified as either PG-2, PG-3, PG-4 or PG-T (Chen and Fernando, 2006). *L. biglobosa* is confined to PG-1 and further divided into three distinct sub-groups; NA1, NA2, and NA3. Only one isolate of NA3 has been reported (Koch *et al.,* 1991). Epidemic severity is affected by the population structure of *Leptosphaeria* sp. complex. Survey results from Australia show severity increasing as the proportion of isolates found belonging to PG-3, PG-4, and PG-T increases (Ballinger and Salisbury, 1996). All 5 PGs are currently found in North Dakota (Chen and Fernando, 2006). Distinctions between the species and different pathogenicity groups are also supported by genetic work that will be discussed later.

Epidemiology and disease spread

Blackleg is generally considered to be monocyclic in North America, Europe, and Canada (Mahuku *et al.*, 1997). Epidemics are usually initiated by the spread of airborne ascospores that overwintered as pseudothecia on crop residues (Hall, 1992). These spores serve as primary inoculum and reach maximum discharge rates two years after the infected crop was harvest (Markell, S., *et al.,* 2008). Infections may also begin by infected seed, contact with infected stubble, or from rain-splashed conidia (Kharbanda and Stevens, 1993). Ascospore release occurs after wetting from rain or dew. Timing varies between regions (spring or winter cultivars), but always coincides with presence of young, susceptible plants (Pérès *et al.,* 1999). Infection generally results from penetration through

stomata but may also enter via wounds (Chen and Howlett, 1996). Both *L. maculans* and *L. biglobosa* require a minimum of four hours of leaf wetness for infection to occur (Wood and Barbetti, 1977; Biddulph *et al.,* 1999).

Disease cycle

Each species initially colonize plant tissue biotrophically and grow symptom-less through the lamella into the petiole, hypocotyl, and stem (Figure 2). Once established in the plant, cell death is induced and followed by production of asexual fruiting structures, pycnidia (Hammond and Lewis, 1987).

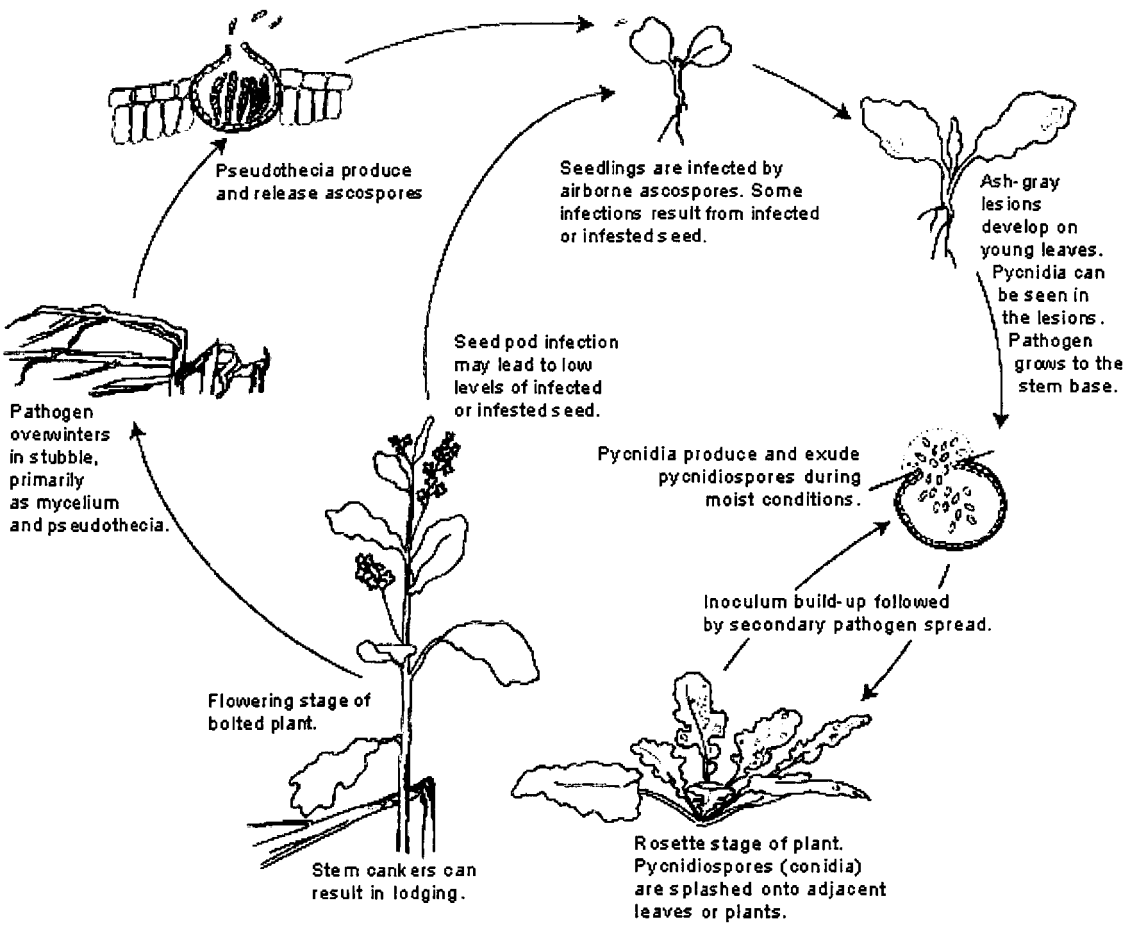

Figure 2. Blackleg disease cycle courtesy of American Phytopathological Society, 2000 (www.apsnet.org).

Phoma leaf spots or leaf lesions have different appearance based on species present, host resistance, and lesion stage. Lesions of both species develop similarly in all geographic regions where the disease is found. Lesions caused by *L. maculans* appear as small (< 1 cm), pale green spots that grow in diameter (up to 3 cm), change color to pale brown, and contain many dark pycnidia, the fruiting structures, that produce pycnidiospores or conidia (Figure 3A). Lesions of *L. biglobosa* tend to be darker in color with few to no pycnidia (Brun *et al.*, 1997). Secondary infections caused by conidia are more common in Western Australia than anywhere else. Here conidia can spread up to one meter from original foci (Wood and Barbetti, 1977). In any case, secondary infections generally do not lead to yield losses (Hall, 1992).

Hypocotyl infections generally occur above ground but below the cotyledons. Lesions usually have a distinct, dark margin and pycnidia. Only infection from *L. maculans* will result in hypocotyl lesions or cankers. These lesions can lead to complete stem sever if the infection is established before the stem becomes woody. This is more common in Australia and Canada (Barbetti and Khangura, 1999).

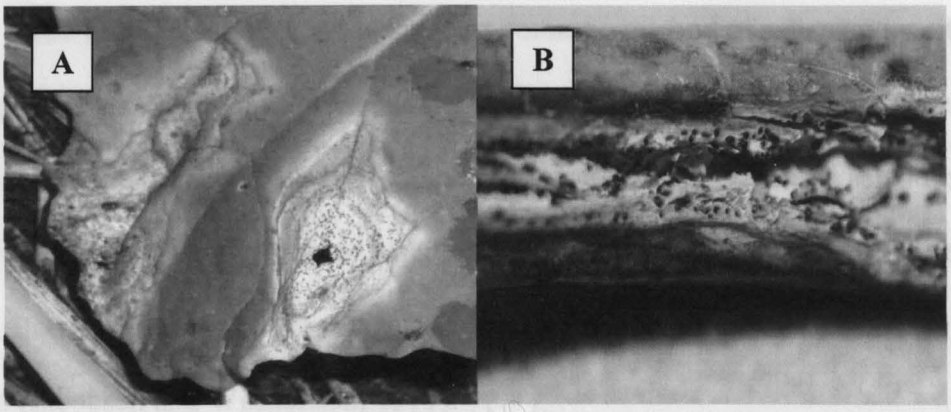

Figure 3. Signs and symptoms of blackleg disease on canola. **A,** Leaf lesion on adult plant. **B,** Stem canker on adult plant. (Photos courtesy of Sam Markell and Shanna Mazurek, NDSU).

Lesions at the stem base are typically associated with leaf scars where the lesion originated earlier in the season and can occur anywhere along the length of the plant. During pod development, stem lesions may spread and grow together, eventually cracking open to form a dry rot or canker (Figure 3B). This leads to girdling of the stem, which restricts water and nutrient movement and will cause premature ripening and lodging in severe cases. Both result in yield loss (Paul and Rawlinson, 1992).

After harvest, crop residue is colonized by *L. maculans* and *L. biglobosa* and pycnidia are produced. Conidia released during this saprophytic stage colonize stubble and raise inoculum levels. When pseudothecia mature they begin releasing ascospores that can infest crops and residue up to 3 km away (Petrie, 1978). The greatest risk of infection occurs within 500 m of the source of ascospore release. While ascospores can remain viable for about six weeks (Paul and Rawlinson, 1992), infected crop residue can remain an inoculum source for up to four years in Western Australia and north central North America (Barbettie and Khangura, 1997; Petrie, 1986). In Europe, residue degrades more rapidly due to climate, and is viable for no longer than two years (West *et al.,* 1999).

Seed-borne contamination is the least common method of infection by either *Leptosphaeria* species. Infected seed is the result of pod lesions that develop late in the season while seed is ripening. Seed-borne infection is most important in spreading disease to new areas over greater distances where the disease may have been nonexistent or less severe. Both species can be present as dormant mycelia under the seed coat or in the embryo (Jacobsen and Williamson, 1971).

Cultural practices

In all canola/oilseed rape (OSR) producing regions, proper stubble management and crop rotation help reduce the risk of infections from ascospores released from residues. Canola/OSR should be sown once in a four year rotation with non-host crops, such as cereal grains (Markell *et al.*, 2008). Ascospore survival rate is best in drier areas using minimum tillage, such as Western Australia and North America. Wetter climates and conventional tillage practices in Europe help residue break down. Residue left on the surface does not break down as rapidly (West *et al.,* 2000).

The risk of blackleg epidemics increases when producers use rotations tighter than canola once every four years in combination with no-till practices (Barbetti and Khangura, 1999). Cultural practices such as whole plant removal for harvest and field flooding for rice production allows countries like China and India to plant OSR in tighter rotations without high risk of epidemics (West *et al.,* 2000).

Chemical control

Control using chemicals varies depending on region, epidemiology, and crop economics. Most canola/oilseed rape producing areas have options for chemical control in the form of fungicide seed, soil, or foliage treatments. Using any of these options can be recommended when a number of factors may influence the crop at one time; high yield potential, residue with high inoculum level, or low cultivar resistance. Foliar fungicides provide a small amount of control and act for a short period of time as a protectant (Gugel and Petrie, 1992). Timing is critical for successful foliar application. North Dakota currently has two products registered for use as foliar protectants against blackleg

(McMullen and Markell, 2011). In North Dakota, foliar fungicide applications are not economical, especially if a resistant cultivar is grown (Markell, S. G., personal communication). A study by Gladders *et al.* (1998) indicated foliar fungicides to be effective in Europe on winter canola when sprayed at the seedling stage prior to winter kill, up to 6 months before any symptoms on the stems appear. A forecasting model would useful to improve timing and application efficacy. Forecasting models are not available because leaf lesions presence is not a good indicator for timing. Once lesions are present, the fungus may already be growing systemically in the plant (West *et al.,* 2001).

Genetic resistance

Breeding for resistant cultivars to *L. maculans* is a critical requirement for sustainable management of blackleg (West *et al.,* 2001). The genetic basis of blackleg resistance is most likely different between seedling and adult plants. Field resistance for blackleg has three components. First is genetic resistance, usually assessed in seedlings and young plants. Second is disease escape, in which plants are able to outgrow the pathogen as it is spreading. Lastly is disease tolerance, the ability of the plant to yield well even though it has been infected in some degree by the pathogen (Rimmer and van den Berg, 1992; West *et al.*, 2001).

There are two types of genetic resistance in *Brassica napus*, single-gene/specific resistance, and multi-gene/polygenic resistance. Ten avirulence genes (*AvrLm)* have been identified in *L. maculans* (Balesdent *et al.,* 2001), and ten corresponding resistance genes (*Rlm)* in *B. napus* (Delourme *et al.,* 2004). *Rlm* genes only give effective disease control if *L. maculans* population present is dominated by corresponding *AvrLm*. If the gene does not match, control will fail. Large-scale production of a cultivar with single gene resistance in

an area of high disease pressure will force the pathogen population to "shift" and break-down the resistance, resulting in no disease control (Li *et al.,* 2003). Shifts can occur very quickly under optimal conditions and have happened in Australia. A resistance gene moved from *B. rapa* subsp. *sylvestris* into *B. napus* was overcome within three years of its introduction into a commercial cultivar (Sprague *et al.,* 2006). Consequently, research for resistant cultivars is now focused on polygenic resistance.

Polygenic/quantitative resistance is more effective in the long-term even though the resistance level is lower than provided by a single gene. Multiple genes are responsible for plants being more resilient to resisting infection; therefore, it takes longer for the pathogen to break down the resistance. Resistance is normally overcome gene by gene, as the pathogen changes (Sprague *et al.*, 2006). Plants with polygenic resistance can still produce acceptable yields even if one resistance gene is overcome. Polygenic resistance for blackleg is thought to be race non-specific which helps maintain higher levels of resistance for longer periods of time (Delourme *et al.* 2006).

Genetic Structure of the *Leptosphaeria* sp. Complex

Genetic analysis

Information about genetic variation of *L. maculans* worldwide and the role evolutionary processes play in rapid population shifts is still limited. Up until 2000, not much was known about the genetics of the *Leptosphaeria* sp. complex. The size of its genome and chromosomes make it an ideal candidate for many molecular and genetic studies. Realization of the potential for *L. maculans* genetic maps to be used as a model system for future genetic and host-pathogen interactions of other Ascomycetes fungi led to

a flurry of projects resulting in large amounts of new information regarding phylogenetic relationships and taxonomic classification.

Since most of this information is recent, additional studies were conducted to test the validity. Mutations can occur in ribosomal DNA (rDNA) without having an immediate effect on the functional level of the gene, which would directly influence selection (Voigt *et al.*, 2005). Phylogenetic associations established using rDNA are often limited so additional characteristics can be used to improve validity. Voigt *et al.* (2005) re-examined the phylogenetic relationships between 28 *L. maculans* species complex isolates and twenty other dothideomycetes, previously inferred by Mendes-Pereira *et al.* (2003), using sequences from the mating type (*MAT*) 1-2 locus as well as from actin and β-tubulin. Using *MAT* genes as additional determinants of phylogenetic relationships for closely related species often increases accuracy (Turgeon, 1998). The phylogenetic relationships previously determined between members of the *L. maculans* sub-groups and the sub-clades of *L. biglobosa* using rDNA were consistent with the findings using sequences from *MAT1-2*, actin and β-tubulin (Voigt *et al.*, 2005).

Mating type

Sexual reproduction is very important in the *L. maculans* disease cycle. Ascospores, source of primary inoculum, result from sexual reproduction. Sexual recombination produces genetic variation within the species, helping it overcome resistance and adapt to changing conditions in order to proliferate (Sprague *et al.*, 2006). An example of the high degree of sexual out-crossing exhibited by this pathogen was presented by Barrins *et al.*(2002) who found 70 different genotypes in 84 isolates collected from one canola field in Australia.

In 2006, Gout *et al.* conducted a study in France that determined genetic diversity and distribution of mating type alleles in different field populations of *L. maculans*. Isolates were collected using three different spatial scales within fields; leaf, two square meter field plots, and whole field. They hoped to determine the actual distribution of isolates within a field. Of the 401 analyzed isolates, 309 or 78% had unique genotypes. Even at the smallest spatial scale, separate isolates from single leaf, the number of different genotypes was very close to the number of isolates. Only two of the fifty isolates collected from the same leaf had the same genotype. Both mating type alleles were found in all spatial scales in all fields. This showed sexual recombination occurred regularly in field populations and is an important part of *L. maculans* life and disease cycle (Gout *et al.,* 2006).

Studies similar to this one need to be conducted on a world-wide scale to determine the genetic structure of *L. maculans* population and see the process of species evolution. Such studies will also help explain how the rapid breakdown of the *Rlm* resistance genes occurs and show the level of gene migration among different *L. maculans* field populations (Gout *et al.*, 2006). Using mating type alleles will also help gain a better understanding of speciation events (Voigt *et al.*, 2005).

Like other dothideomycetes, *L. maculans* has a single mating type locus with two forms that must be different in order to mate (Venn, 1979). The mating type locus was found on an AFLP marker amplified from a *L. maculans* 'brassicae' isolate and was designated mat - and its corresponding part mat + (Mengistu *et al.,* 1994). Further sequence analysis of this fragment revealed similarity to the *MAT1-2* locus of other loculoascomycetes and it was suggested to re-designate mat- to *MAT1-2* and mat+ to *MAT1-1*, to conform to nomenclature suggested earlier from Turgeon and Yoder (2000).

In 2003, Cozijnsen and Howlett unsuccessfully attempted to clone the *MAT* locus using heterologous clones from other dothideomycetes. When building a genetic map for *L. maculans*, the mating type locus was found amplified on an isolate. This isolate was deemed mat-. The amino acid sequence from this AFLP primer had four conserved amino acids of the high mobility group (HMG) DNA-binding group for the mating type *MAT1-2* locus in other Ascomycetes as previously suggested by Turgeon and Yoder in 2000.

This AFLP marker was used to isolate the *MAT1-2* locus in *L. maculans* 'brassicae' and clone the *MAT1-1* locus. It was also used to characterize the mating type allele and help develop a PCR assay to distinguish between different mating type alleles (Cozijnsen and Howlett, 2003).

Discussion

Further differentiation of the *L. maculans* species complex will help in many ways. The *L. maculans* and *B. rapa* interaction is proving to be a valuable model for studying host-pathogen interactions and will be of more value once the complete genome sequences of these two organisms are available. Aside from this, all information about *L. maculans* will continue to direct disease management practices to maximize canola/oilseed rape yields.

Objective

The objective of this study was to determine the prevalence of pathogenicity groups of the causal agents of blackleg of canola in North Dakota. This goal was set based on reports of new pathogenicity groups being reported with increasing frequency. Changes in the population structure of these pathogens could increase the probability that more severe blackleg epidemics develop in the future.

CHAPTER 3. MATERIALS AND METHODS

Leaf Sampling Procedure

Leaves showing symptoms of *L. maculans* infection were collected from 54 naturally infected commercial canola fields in seven North Dakota counties during 2007 and 2008 (Figure 4A). Counties selected consistently had high annual canola acreage (> 16,150 hectares). A minimum of two fields were sampled in each county. These fields belonged to canola growers who agreed to collaborate in this and other canola projects. Along with sample collection, each grower provided us with the history of each field observed. Information from the growers included crop rotation, canola cultivar and seeding rate, current and past chemical applications, planting and harvest dates, and yield data.

A large scale hierarchical sampling strategy modified from McDonald *et al.* (1999) was used in the study. Hierarchical sampling is very useful for seed-borne or splash dispersed pathogens like *L. maculans* (Markell and Milus, 2008). The sampling procedure had a 10-site ordered hierarchy (Figures 4B, 4C). When possible, ten infected leaves from different plants were obtained at each site following a circular pattern with an average diameter of 3 m. Distances between sampling sites within a field were based on field size. Sites were never less than 350 m apart. This ensured locations selected were a representative sample of the entire field. Fields were sampled along the perimeter, a minimum of 33 m from field edge. GPS coordinates were recorded at each sample site using a Lowrance iFinder® H20 handheld mapping GPS receiver (Lowrance, Tulsa, Oklahoma, USA). Symptomatic leaf samples were taken in June when plants were at the 4-6 leaf stage. Blackleg lesion appearance ranged from white to gray in color with small, black pycnidia in concentric rings.

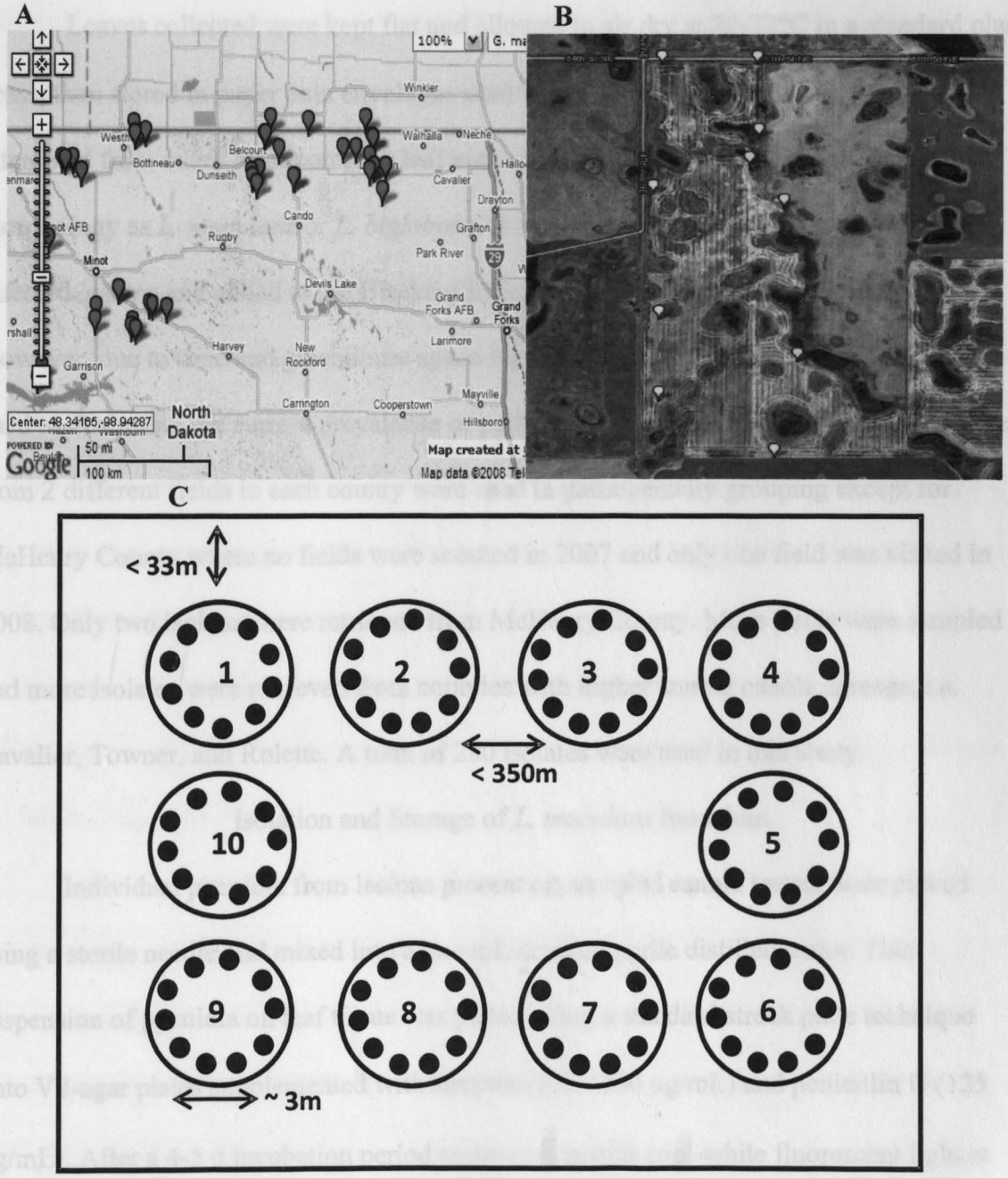

Figure 4. Diagrams for sample collection of *L. maculans* from canola fields in North Dakota. **A,** GPS locations of fields sampled (Map courtesy of Google Earth). **B,** GPS coordinates of each sample site were recorded in fields (Map courtesy of Google Earth). **C,** Ten-site ordered hierarchy (not to scale). Ten infected leaves were obtained at regular intervals in a circular pattern at each site. Ten sites were spaced according to field size along the perimeter of the field. Diagram drawn not to scale.

Leaves collected were kept flat and allowed to air dry at 20-22°C in a standard plant press, then stored in paper coin envelopes until used for isolation. Isolations were attempted from every lesion on each leaf but not all resulted in cultures identifiable by morphology as *L. maculans* or *L. biglobosa.* A total of 915 isolates were retrieved from infected lesions and added to the Blackleg library at North Dakota State University. However, due to time and greenhouse space limitations, only a subset of the isolates were used to determine the current prevalence of pathogenicity groups. A minimum of 4 isolates from 2 different fields in each county were used in pathogenicity grouping except for McHenry County where no fields were scouted in 2007 and only one field was visited in 2008. Only two isolates were retrieved from McHenry County. More fields were sampled and more isolates were retrieved from counties with higher annual canola acreage, i.e. Cavalier, Towner, and Rolette. A total of 280 isolates were used in this study.

Isolation and Storage of *L. maculans* Inoculum

Individual pycnidia from lesions present on sampled canola leaves were picked using a sterile needle and mixed into a one mL drop of sterile distilled water. This suspension of pycnidia on leaf tissue was plated using a standard streak plate technique onto V8-agar plates supplemented with streptomycin (200 µg/mL) and penicillin G (125 µg/mL). After a 4-5 d incubation period under continuous cool-white fluorescent light at room temperature (20-24°C), *L. maculans* was identified using pycnidia and colony morphology. Three to five day old colonies of *L. maculans* isolated via streak technique were picked as single-spore colonies and re-plated onto fresh V8-agar plates to maintain purity. The small colony was incubated under continuous cool-white fluorescent light for 10-14 d, until pycnidia were present and profusely sporulating. Pycnidiospores were

harvested and stored prior to inoculation following a protocol previously described by Chen and Fernando (2006). All inoculum was stored at -20°C for a minimum of 48 h before being used for pathogenicity group evaluation.

L. maculans Inoculation

Interaction phenotype (IP) and pathogenicity group (PG) of all isolates were determined using a standard set of canola differentials: 'Westar' (spring rape), 'Glacier' (winter rape), and 'Quinta' (winter rape). In addition, isolates were evaluated on two commercial winter rape cultivars, 'Bristol' and 'Columbus'. The former had a different combination of avirulence genes than the standard three-differential set, whereas the latter has resistance genes similar to those in 'Quinta' (Balesdent, *et. al.,* 2006). Although IP on Bristol and Columbus have no impact on named PGs, supplementation of the standard differential set with these cultivars provides data for interpretation of pathogen genetics in the future (Appendix A).

Differential and supplemental cultivars were planted and grown in 48-welled flats on greenhouse benches in MetroMix® (SunGro Horticulture, Bellevue, Washington, USA), a soil-less potting medium, with a photoperiod of 16 h light and eight h dark. All plants were watered daily. A shade curtain was hung in the greenhouse to help maintain a more stable temperature.

Plants were inoculated 10 d after sowing when the cotyledons were fully expanded. Inoculum was prepared from concentrated stock of frozen pycnidiospores. Three to seven drops of pycnidiospore stock were mixed into 10 mL of sterile distilled water. The concentration of the spore suspension was adjusted to 1.0×10^7 using a hemacytometer. Prior to inoculation, each lobule of the cotyledon was wounded using a sterile needle. A 10

μL drop of inoculum was applied to each wounding site on each cotyledon half. Six seedlings of each differential were inoculated for every isolate. After inoculation, spore suspension droplets were allowed to evaporate completely before plants were placed into a mist chamber set at saturation for 24 h, and then returned to the greenhouse. Primary leaves were pinched off as they developed in order to maintain integrity of cotyledons during evaluation. A set of purified and classified isolates previously obtained in North Dakota were used as positive controls (obtained from Dr. Dilantha Fernando, University of Manitoba, Winnipeg, Canada). Sterile distilled water blanks were used as negative controls. Both were run alongside unknown isolates. Isolates were run from April through July, 2009. A validation experiment was conducted each month using known isolates, a water control, and a subset of unknown isolates to check repeatability of the observed IPs.

Pathogenicity Group Evaluation of *L. maculans* Isolates

Ten days after inoculation, IPs were assessed by measuring lesion size, amount of tissue collapse and blackening, and sporulation using a 0-9 scale (Figure 5) (Delwiche, 1980): 0 = no darkening around wound, as in water controls; 1 = limited blackening around wound, lesion diameter 0.5-1.5 mm, faint chlorotic halo may be present, sporulation absent; 3 = dark necrotic lesions, 1.5-3.0 mm, chlorotic halo may be present, sporulation absent; 5 = non-sporulating 3-6 mm lesions, sharply delimited by dark necrotic margin, may show gray-green tissue collapse as in IP 7 and 9 or dark necrosis throughout; 7 = gray-green tissue collapse 3-5 mm diameter, sharply delimited, non-darkened margin; 9 = rapid tissue collapse at about 10 d, accompanied by profuse sporulation in large, more than 5 mm, lesions with diffuse margins.

An IP on this scale of 0-2 was deemed resistant, 3-6 was intermediate, and 7-9 was susceptible. Isolates were assigned to individual PGs according to the mode of the IPs observed on each differential; Westar, Glacier, and Quinta (Table 1) (Mengistu *et al.* 1991). Bristol and Columbus were evaluated using the same rating scale; however, the IPs observed did not affect pathogenicity grouping. Data was processed by determining mean, median, mode, and range.

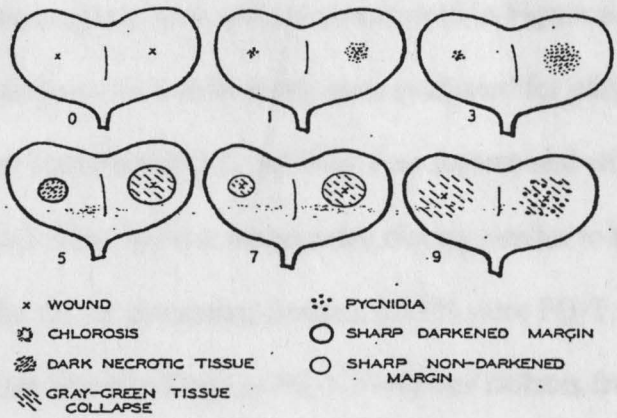

Figure 5. Rating scale used to determine IPs on infected cotyledons of differentials. (Figure taken from Delwiche, 1980).

Table 1. Known *Leptosphaeria maculans* and *L. biglobosa** pathogenicity groups (PGs) based on interaction phenotypes on three differential canola cultivars.

	Westar	Glacier	Quinta
PG-1 [a]	R (0)	R (0)	R (0)
PG-2	S (7-9)	R (0-2)	I (3-6)
PG-3	S (7-9)	S (7-9)	I (3-6)
PG-T	S (7-9)	I (3-6)	S (7-9)
PG-4	S (7-9)	S (7-9)	S (7-9)
PG-5[b]	I (3-6)	I (3-6)	I (3-6)

Note: A 0-9 scale (Delwiche, 1980) was used to assess the interaction phenotypes. Description of scale and rating protocol can be found in Materials and Methods section. R= resistant; S= susceptible; I= intermediate. Table adapted from Mengistu *et al.* (1991) and Chen and Fernando (2006).

[a]PG-1 is recognized at *L. biglobosa* by Shoemaker and Brun (2001).

[b]PG-5 was identified as intermediate on all differentials by Mahuku (1997).

CHAPTER 4. RESULTS

Prevalence of *L. maculans* and *L. biglobosa* Pathogenicity Groups

A total of 134 isolates were obtained from 22 canola fields in North Dakota in 2007 (Table 3). Of these, 23.1% were PG-1, 12.7% were PG-2, 8.2% were PG-3, 13.4% were PG-T and 6% were PG-4 (Figure 6A). One isolate was classified as PG-5, which accounted for 0.7% prevalence. The interaction phenotypes of the remaining 48 isolates did not fit into the current classification system used and are represented in Figure 6A as "undefined".

In 2008, 146 isolates from 32 canola fields were evaluated for pathogenicity. Of the isolates tested, 28.8% were confirmed PG-1. All PGs were represented with at least one isolate each. PG-2 and PG-3 were found at frequencies closely similar to those of 2007, 13% and 9.6% respectively. Of the remaining isolates, 15.1% were PG-T, 4.1% were PG-4 (Figure 6B). And one isolate was identified as PG-5. Forty one isolates from 2008 did not fit the current classification system and are represented in Figure 6B as "undefined".

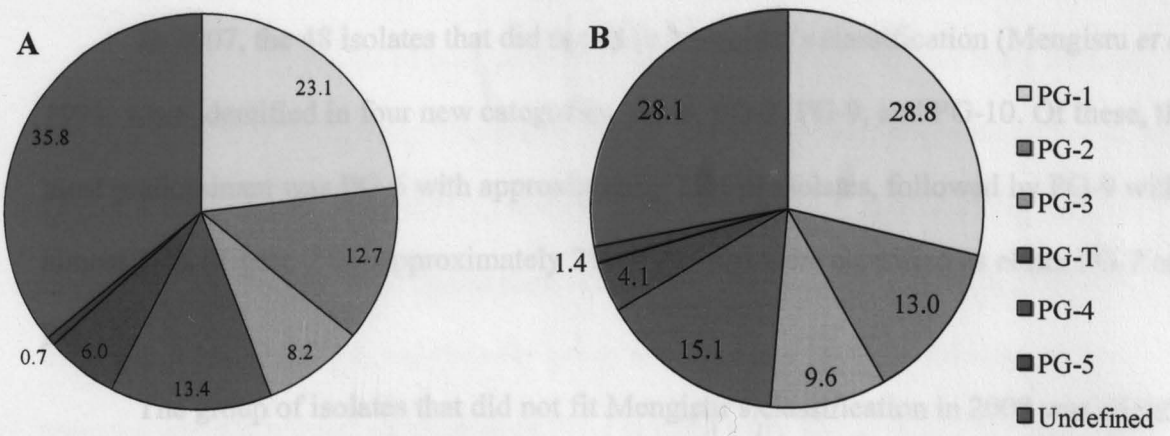

Figure 6. Distribution of pathogenicity groups of *L. maculans* and *L. biglobosa* in **A.** 2007 and in **B.** 2008.

Proposed New *L. maculans* Pathogenicity Groups

The virulence profile of approximately 48 and 41 isolates collected in 2007 and 2008, respectively, did not fit the classification proposed by Mengistu *et al.* (1991). Thus, a set of six additional pathogenicity groups were developed to accommodate the reactions these isolates produced on the same differentials (Table 2).

Table 2. Proposed new *Leptosphaeria maculans* pathogenicity groups (PGs) based on interaction phenotypes on three differential canola cultivars.

Pathogenicity groups	Westar	Glacier	Quinta
PG-6	S (7-9)	R (0-2)	R (0-2)
PG-7	S (7-9)	I (3-6)	R (0-2)
PG-8	I (3-6)	R (0-2)	R (0-2)
PG-9	S (7-9)	I (3-6)	I (3-6)
PG-10	S (7-9)	R (0-2)	S (7-9)
PG-11	I (3-6)	R (0-2)	I (3-6)

Note: A 0-9 scale (Delwiche, 1980) was used to assess the interaction phenotypes. Description of scale and rating protocol can be found in Materials and Methods section. R= resistant; S= susceptible; I= intermediate. Table adapted from Mengistu *et al.* (1991) and Chen and Fernando (2006).

In 2007, the 48 isolates that did not fit in Mengistu's classification (Mengistu *et al.*, 1991) were identified in four new categories, PG-6, PG-7, PG-9, and PG-10. Of these, the most predominant was PG-6 with approximately 73% of isolates, followed by PG-9 with almost 23% (Figure 7A). Approximately 2% of isolates were classified as either PG-7 or PG-10.

The group of isolates that did not fit Mengistu's classification in 2008 was slightly smaller than that of 2007, but more diverse (Figure 7B). As with the 2007 sample, the most predominant groups were PG-6 and PG-9 although in 2008 the prevalence of PG-6 was

reduced while that of PG-9 was increased compared to 2007. Prevalence of PG-7 isolates increased from 2.1 in 2007 to 7.2% in 2008 but PG-10 remained almost unchanged. PG-8 had two confirmed isolates and PG-7 had three isolates. One isolate each was found in PG-10 and PG-11 (Table 3). At least one isolate of every PG was found in Cavalier County in 2008.

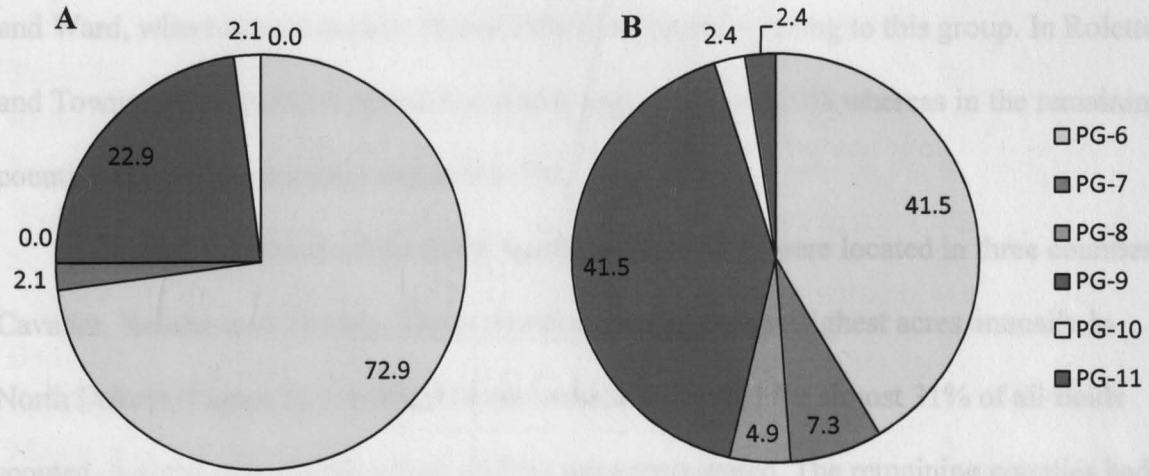

Figure 7. Distribution of proposed new pathogenicity groups of *L. maculans* and *L. biglobosa* in **A.** 2007 and in **B.** 2008.

Distribution of Observed *L. maculans* and *L. biglobosa* Pathogenicity Groups

Considering results of both years, the most predominant PG was PG-1 with an average of 26% of all isolates. The second, third, and fourth most prevalent pathogenicity groups were PG-6, PG-T, and PG-2 with an average 18.9, 14.3, and 12.9% of isolates, respectively. However, their distribution was not always uniform across the area with exception of PG-1, which was the only group retrieved from every field scouted. The counties with the highest prevalence of PG-1 isolates were Cavalier and Bottineau, and McLean where an average of approximately 40, 21, and 15% of all isolates, respectively, belong to this group. The counties with the highest prevalence of PG-2 were Cavalier,

Rolette, and Bottineau, where approximately 49, 17, and 12% of all isolates, respectively, belong to this group. In the remaining counties PG-2 representation ranged between approximately 0 and 9%. The counties with the highest prevalence of PG-T were Cavalier, Rolette, and Towner, where approximately 29, 21, and 14% of all isolates belong to this group (Table 4). In the remaining counties PG-T representation ranged between approximately 0 and 14%. The counties with the highest prevalence of PG-6 were Cavalier and Ward, where approximately 38 and 19% of all isolates belong to this group. In Rolette and Towner counties PG-6 prevalence was at approximately 10% whereas in the remaining counties PG-6 representation was below 7%.

Almost two-thirds of the fields scouted in this study were located in three counties, Cavalier, Rolette, and Towner. These counties account for the highest acres annually in North Dakota (Figure 1). Cavalier County, which accounted for almost 31% of all fields scouted, was the only county where all PGs were represented. The remaining counties had isolates representing between five and eight pathogenicity groups each. The relative frequency of all PGs except PG-4 and PG-6 increased from 2007 to 2008.

Table 3. Proposed new pathogenicity groups (PGs) of *L. maculans* isolates collected in North Dakota canola fields during 2007 and 2008.

County	No. Fields	Isolates in each pathogenicity group					
		PG-6	PG-7	PG-8	PG-9	PG-10	PG-11
2007							
Bottineau	3	5	0	0	1	0	0
Cavalier	5	4	1	0	5	0	0
McHenry	0	0	0	0	0	0	0
McLean	3	6	0	0	0	0	0
Renville	2	5	0	0	1	1	0
Rolette	4	5	0	0	3	0	0
Towner	3	5	0	0	0	0	0
Ward	2	5	0	0	1	0	0
Total	22	35	1	0	11	1	0
2008							
Bottineau	2	0	0	0	1	0	0
Cavalier	11	11	2	2	7	1	1
McHenry	1	0	0	0	0	0	0
McLean	3	0	0	0	1	0	0
Renville	2	0	0	0	1	0	0
Rolette	5	1	0	0	0	0	0
Towner	5	1	1	0	7	0	0
Ward	3	4	0	0	0	0	0
Total	32	17	3	2	17	1	1

Table 4. Known pathogenicity groups (PGs) of *Leptosphaeria maculans* or *L. biglobosa** isolates collected in North Dakota canola fields during 2007 and 2008.

County	No. Fields	Isolates in each pathogenicity group					
		PG-1*	PG-2	PG-3	PG-T	PG-4	PG-5
2007							
Bottineau	3	5	3	1	3	0	0
Cavalier	5	18	4	4	3	3	1
McHenry	0	0	0	0	0	0	0
McLean	3	1	1	0	2	1	0
Renville	2	2	1	0	2	0	0
Rolette	4	1	3	0	6	0	0
Towner	3	3	2	6	1	4	0
Ward	2	1	3	0	1	0	0
Total	22	31	17	11	18	8	1
2008							
Bottineau	2	11	1	0	1	0	0
Cavalier	11	9	14	5	9	3	2
McHenry	1	2	0	0	0	0	0
McLean	3	11	0	0	3	0	0
Renville	2	1	1	0	2	1	0
Rolette	5	5	3	3	2	1	0
Towner	5	2	0	6	5	1	0
Ward	3	1	0	0	0	0	0
Total	32	42	19	14	22	6	2

*PG-1 has been recognized as *L. biglobosa* by Shoemaker & Brun (2001).

CHAPTER 5. DISCUSSION

Results of this study show isolates of both *L. maculans* and *L. biglobosa* are present in North Dakota in McLean, Ward, Renville, Bottineau, Rolette, Towner, and Cavalier counties. Frequencies at which PG-1 was observed were more comparable to those of PG-2 in past studies conducted in North Dakota and Southern Canada (Chen and Fernando, 2006). It is possible that this difference is due to the fact that most isolates used in this study were retrieved from foliar lesions rather than stem cankers as was the case in Chen and Fernando's study.

Of the two species associated with blackleg, *L. maculans* was the most prevalent with approximately 74% of all isolates belonging to this species. Among *L. maculans* isolates, however, the most predominant groups were PG-6 and PG-T. PG-2 which was considered the most prevalent group in previous studies (Bradley *et al.*, 2005; Fernando and Chen, 2006; Kutcher *et al.*, 2007) came in third position. Most commercial cultivars planted in North Dakota are either resistant or moderately resistant to PG-2.Isolates from PG-2 are more virulent on differential Quinta than isolates from PG-6.

A total of 89 isolates evaluated in this study did not fit properly in the current classification system (Mengistu, 1991). This is an indication that the use of only three differentials is not enough to represent the range of virulence that is being created in canola fields. This observation is further supported by the fact that multiple PGs were found in all fields and on all sampling levels. Similar diversity within a field has been described in France (Gout *et al.*, 2006). Establishment of a larger, new set of differentials is required.

Data collected in 2007 and 2008 when compared to data from similar studies conducted in North Dakota or Canada (Bradley *et al.,* 2005; Chen and Fernando, 2006) indicates that the virulence of *L. maculans* is not only capable of change but has changed resulting in the observation of new PGs. A few factors most likely play a role in this phenomenon. *L. maculans* has the ability to easily complete sexual reproduction in nature which results in genetic recombination. This gives the pathogen a high potential to develop virulence to resistance genes (Sprague *et al.,* 2006). Many canola growers in North Dakota have been pushing rotation recommendations in order to maximize profits. Canola should be grown once every four years in rotation with non-host crops such as wheat (*Triticum* sp.) and barley (*Hordeum vulgare* L.) (Lamey, 1995). Of the fields sampled in Cavalier County, North Dakota's highest producing county (NASS, 2010), the most common rotation was a two year rotation of canola and wheat (Mazurek, data not presented). This along with favorable weather, reduced tillage, and use of cultivars with similar resistance genes creates selection pressure towards development of new virulence profiles capable of overcoming the resistance in use. Evolutionary potential coupled with poor crop management is ideal for a change in PGs.

While it is clear from the results of this study that the blackleg population in North Dakota is changing, the extent of the change may not be established accurately. More surveys that cover larger areas and include samples from stems of adult plants are necessary. Further, changes in virulence can sometimes affect the fitness of the pathogen (Williams, 1992). If that has occurred, the probability of detecting less fit isolates would be relatively small compared to that of a fit isolate. Comparing the relative fitness of the isolates used in this study, however, would give us a good idea of what is happening in the

fields. Production of cultural mutants, mutants produced by repeated culturing in artificial medium, has been used to explain the detection of PG-5 isolates (Mahuku et al, 1997); however, this is hardly the case of our PG-5 isolates. Although *L. maculans* has been documented to lose virulence in culture (Williams, 1992), isolates used in the studies reported here were retrieved from plant materials and were not sub-cultured long enough as to expect that changes in virulence could have occurred. Isolates used in this study were transferred no more than twice with little opportunity for loss of virulence. Hence the observation of intermediate IPs.

Emergence of pathogens with new virulence profiles will affect canola production in the state of North Dakota and possibly Canada. Populations with more virulent PGs are now well established in the state and with continued poor management and intense cropping will only become more prevalent. Growers need to follow rotation recommendations, growing canola once every four years and also rotating resistant cultivars (Lamey, 1995). The identification of new resistance genes will play a major role in the continued success of North Dakota's canola industry.

LITERATURE CITED

Anonymous. 1957. 31st Annu. Rpt, pp 32-33. New Zealand Dept. of Scientific and Industrial Research. 84pp.

Balesdent, M. H., Attard, A., Ansan-Melayah, D., Delourme, R., Renard, M., and Rouxel, T. 2001. Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*. Phytopathology 91: 70-76.

Balesdent, M. H., Louvard, K., Pinochet, X., and Rouxel, T. 2006. A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. Eur. J. Plant Pathol. 114: 53-65.

Ballinger, D. J., and Salisbury, P. A. 1996. Seedling and adult plant evaluation of race variability in *Leptosphaeria maculans* on *Brassica* species in Australia. Aust. J. of Exp. Ag. 36: 485-488.

Barbetti, M. J., and Khangura, R. K. 1997. Developments for better management of blackleg disease in Western Australia. Proceedings of 11th Australian Research Assembly on *Brassicas.* Ag West. Aust. 11-14.

Barbetti, M. J., and Khangura, R. K. 1999. Managing blackleg in the disease-prone environment of Western Australia. Proceedings of 10th International Rapeseed Congress. http://www.regional.org.au/papers/index.htm

Barrins, J. M., Purwantara, A., Ades, P. K., Salisbury, P. A., and Howlett, B. J. 2002. Genetic diversity of isolates of *Leptosphaeria maculans* from a canola paddock in Australia. Aust. Plant Pathol. 31: 129-135.

Biddulph, J. E., Fitt, B. D. L., Gladders, P., Jedryczka, M., West, J. S., and Welham, S. J. 1999. Conditions for infection of oilseed rape leaves by ascospores of UK (A group) and Polish (B group) *Leptosphaeria maculans* (stem canker). *Groupe Consultatif International de Recherche Sur le Colza Bulletin.* 16: 82-3.

Bokor, A., Barbetti, M. J., Brown, A. G. P., MacNash, G. C., and Wood, P. 1975. Blackleg of rapeseed. J. of Ag West. Aust. 16: 7-10.

Bradley, C. A., Parks, P. S., Chen, Y., and Fernando, W. G. D. 2005. First report of pathogenicity groups 3 and 4 of *Leptosphaeria maculans* on canola in North Dakota. Plant Dis. 89: 776.

Brun, H., Levivier, S., Eber, F., Renard, M., and Chèvre, A. M. 1997. Electrophoretic analysis of natural populations of *Leptosphaeria maculans* directly from leaf lesions. Plant Pathol. 46: 147-54.

Chen, Y., and Howlett, B. J. 1996. Rapid necrosis of guard cells is associated with the arrest of fungal growth in leaves of Indian mustard (*Brassica juncea)* inoculated with avirulent isolates of *Leptosphaeria maculans*. Physiol. Mol. Plant Pathol. 48: 73-81.

Chen, Y., and Fernando, W. G. D. 2006. Prevalence of pathogenicity groups of *Leptosphaeria maculans* in western Canada and North Dakota, USA. Can. J. Plant Pathol. 28: 1-7.

Cozjinsen, A. J., and Howlett, B. J. 2003. Characterization of the mating-type locus of the plant pathogenic ascomycete *Leptosphaeria maculans.* Curr. Genet. 43:351-357.

Cunningham, G.H. 1927. Dry rot of swedes and turnips: its cause and control. New Zealand Dept of Ag. Bulletin 133.

Delourme, R., Chevre, A. M., Brun, H., Rouxel, T., Balesdent, M. H., Dias, J. S., Salisbury, P., Renard, M., and Rimmer, S. R. 2006. Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus).* Eur J. Plant Pathol. 114: 41-52.

Delwiche, P. A. 1980. Aspects of blackleg (*Leptosphaeria maculans)* resistance to rapeseed *(Brassica napus).* Ph.D. Thesis, University of Wisconsin. 144pp.

Gladders, P., Symonds B. V., Hardwick, N. V., and Sansford, C. E. 1998. Opportunities to control canker (*Leptosphaeria maculans)* in winter oilseed rape by improving spray timing. International Organization for Bio Control Bulletin. 18: 1-11.

Gout, L., Eckert, M., Rouxel, T., and Balesdent M. H. 2006. Genetic variability and distribution of mating type alleles in field populations of *Leptosphaeria maculans* from France. Appl. Environ. Microbiology. 72: 185-191.

Gugel, R. K., and Petrie, G. A. 1992. History, occurrence, impact, and control of blackleg of rapeseed. Can. J. Plant Pathol. 14: 36-45.

Hall, R. 1992. Epidemiology of blackleg of oilseed rape. Can. J. Plant Pathol. 14: 46-55.

Hammond, K. E. and Lewis, B. G. 1987. Variation in stem infections caused by aggressive and non-aggressive isolates of *Leptosphaeria maculans* on *Brassica napus* var. *oleifera.* Plant Pathol. 36: 53-65.

Jacobsen, B. J. and Williams, P. H. 1971. Histology and control of *Brassica oleracea* seed infection by *Phoma lingam.* Plant Dis. Rep. 55: 934-8.

Johnson, R. D. and Lewis, B. G. 1994. Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans.* Plant Pathol. 43: 269-277.

Kharbanda, P.D. and Stevens, R.R. 1993. Seed testing for blackleg of canola. Vegreville, AB, Canada: AECV93-R5.

Koch, E., Song, K., Osborn, T. C., and Williams, P. H. 1991. Relationship between pathogenicity and phylogeny based on restriction fragment length polymorphism in *Leptosphaeria maculans.* Mol. Plant-Microbe Inter. 4: 341-349.

Kutcher, H. R., Keri, M., McLaren, D. L., and Rimmer, S. R. 2007. Pathogenic variability of *Leptosphaeria maculans* in western Canada. Can. J. of Plant Pathol. 29: 388-393.

Lamey, H. A. 1995.Survey of Blackleg and Sclerotinia Stem Rot of Canola in North Dakota in 1991 and 1993. Plant Dis. 79: 322-324.

Li, H., Sivasithamparam, K., and Barbetti, M. J. 2003. Breakdown of a *Brassica rapa* subsp. *sylvestris* single dominant blackleg resistance gene in *B. napus* rapeseed by *Leptosphaeria maculans* in field isolates in Australia. Plant Dis. 87: 752-754.

Mahuku, G. S., Goodwin, P. H., Hall, R., Hsiang, T. 1997. Variability in the highly virulent type of *Leptosphaeria maculans* within and between oilseed rape fields. Can J. Bot. 75: 1485-1492.

Markell, S., del Rio, L., Halley, S., Mazurek, S., Matthew, F., and Lamey, A. 2008. Blackleg of Canola. North Dakota State University. Extension publication. PP-1367.

Markell, S. G. and Milus, E. A. 2008. The emergence of a novel population of *Puccinia striiformis* f. sp. *tritici* in eastern United States. Phytopathology. 98: 632-639.

McDonald, B. A., Zhan, J., and Burdon, J. J. 1999. Genetic structure of *Rhynochosporium secalis* in Australia. Phytopathology. 89: 639-645.

McMullen, M. P., and Markell, S. G. 2011. North Dakota Field Crop Fungicide Guide. North Dakota State University. Extension Publication. PP-622: 20-22.

Mendes-Perira, E., Balesdent, M. H., Brun, H., and Rouxel, T. 2003. Molecular phylogeny of the *Leptosphaeria maculans-L. biglobosa* species complex. Mycol. Res. 107:1287-1304.

Mengistu, A., Rimmer, S.R., Koch, E., and Williams, P. H. 1991. Pathogenicity grouping of isolates of *Leptosphaeria maculans* on *Brassica napus* cultivars and their disease reaction profiles on rapid-cycling *Brassica.* Plant Dis. 75: 1279-1282.

North Dakota Agricultural Statistics (NAAS). 2010. North Dakota Agriculture. Ag. Statistics N° 79. Aug. 2010. NDSU, Fargo, ND. 144p.

Paul, V. H., and Rawlinson, C. J. 1992. Diseases and Pests of Rape. Gelsenkirchen-Buer, Germany: Verlag Th. Mann.

Pérès, A., Poisson, B., Le Sourne, V., and Maisonneauve C. 1999. *Leptosphaeria maculans:* effect of temperature, rainfall, and humidity on the formation of pseudothecia. Proceedings of 10th International Rapeseed Congress. http://www.regional.org.au/papers/index.htm

Petrie, G. A. 1978. Occurrence of a highly virulent strain of blackleg (*Leptosphaeria maculans*). Can. Plant Dis. Surv. 58: 21-25.

Petrie, G. A. 1986. Consequences of survival of *Leptosphaeria maculans* in canola stubble residue through an entire crop rotation sequence. Can. J. Plant Pathol. 8: 353.

Punithalingham, E., and Holliday, P. 1972. *Leptosphaeria maculans*, Comm. Mycol. Inst. Descriptions of pathogenic fungi and bacteria. Set 34, No. 331.

Purwantara, A., Barrins, J. M., Cozjinsen, A. J., Ades, P. K., and Howlett, B. J. 2000. Genetic diversity of isolates of the *Leptosphaeria maculans* species complex from Australia, Europe, and North America using amplified fragment length polymorphism analysis. Mycol. Res. 104: 772-781.

Rimmer, S. R. and van den Berg, C. G. J. 1992. Resistance of oilseed *Brassica* spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 56-66.

Shoemaker, R. A., and Brun, H. 2001. The telomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. Can. J. Bot. 79: 412-419.

Somda, I., Harkous, S., and Brun, H. 1997. Bipolar heterothallism in B-group isolates of *Leptosphaeria maculans*. Plant Pathol. 46: 890-896.

Sprague, S. J., Balesdent, M. H., Brun, H., Hayden, H. L., Marcroft, S. J., Pinochet, X., Rouxel, T., and Howlett, B. J. 2006. Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence populations of *Leptosphaeria maculans* in France and Australia. Eur. J. of Plant Pathol. 114: 33-40.

Sprague, S. J., Marcroft, S. J., Hayden, H. L., and Howlett, B. J. 2006. Major gene resistance to blackleg in *Brassica napus* overcome within three years of commercial production in southeastern Australia. Plant Dis. 90: 190-198.

Taylor, J. L., Borgmann, I., and Séguin-Swartz, G. 1991. Electrophoretic karyotyping of *Leptosphaeria maculans* differentiates highly virulent from weakly virulent isolates. Curr. Gen. 19: 273-275.

Turgeon, B. G. 1998. Application of mating type gene technology to problems in fungal biology. Annu. Rev. Phytopathology. 36: 115-137.

Turgeon, B. G. and Yoder, O. C. 2000. Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet. Biol. 31: 1–5.

Venn, L. A. 1979. The genetic control of sexual compatibility in *Leptosphaeria maculans*. Aust. J. Plant Pathol. 8: 5-6.

Voigt, K., Cozijnsen, A. J., Kroymann, J., Pöggeler, S., and Howlett, B. J. 2005. Phylogenetic relationships between members of the crucifer pathogenic *Leptosphaeria maculans* species complex as shown by mating type (MAT1-2), actin, and β-tubulin sequences. Mol. Phylogenetics and Evol. 37: 541-557.

West, J. S., Biddulph, J. E., Fitt, B. D. L., and Gladders, P. 1999. Epidemiology of *Leptosphaeria maculans* in relation to forecasting stem canker severity on winter oilseed rape in the UK. Ann. Appl Biol. 135: 535-546.

West, J. S., Evans, N., Liu, S., Hu, B., and Peng, L. 2000. *Leptosphaeria maculans* causing stem canker of oilseed rape in China. New Disease Reports. http://www.bspp.org.uk/ndr/2000/2000-3.htm

West, J. S., Kharbanda, P. D., Barbetti, M. J., and Fitt, B. D. L. 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada, and Europe. Plant Pathol. 50:10-27.

Williams, P. H. 1992. Biology of *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 30-35.

Wood, P. McR. and Barbetti, M. J. 1977. A study on the inoculation of rape seedlings with ascospores and pycnidiospores of the blackleg disease causal agent *Leptosphaeria maculans*. J. Aust. Inst. Ag. Sci. 43: 79-80.

APPENDIX A

Table A. Interaction phenotype mode (IPM) and range (IPR) for differential cultivars of *B. napus* to isolates of *Leptosphaeria maculans* and *L. biglobosa** ten days post inoculation.[1]

	Westar		Glacier		Quinta		Columbus		Bristol		
R Gene	*none*		*rlm 2, 3*		*rlm 1, 4*		*rlm 1, 3*		*rlm 2,9*		
Isolate	IPM	IPR	IPM	IPR	IPM	IPR	IPM	IPR	IPM	IPR	PG
731	7,9	7 to 9	7	5 to 9	3	1 to 5	5	5 to 7	5	1 to 5	3
731	9	7 to 9	7	5 to 9	5	5 to 7	7	5 to 9	7	7 to 9	3
735	7	7 to 9	1	0 to 3	3	1 to 5	1	1 to 3	3	1 to 5	2
736	7	5 to 9	7	5 to 7	7	5 to 7	1	0 to 3	1,3	1 to 3	4
736	9	7 to 9	7	7 to 9	7	7 to 9	7	7 to 9	5	5 to 9	4
737	9	7 to 9	3	3 to 7	7	7 to 9	7	7 to 9	3	3 to 5	T
737	7	5 to 9	3	1 to 5	7	5 to 7	1,3	1 to 3	3	1 to 5	T
922	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	1	0 to 1	1*
923	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	0	0 to 0	1
924	0	0 to 1	0	0 to 0	0	0 to 0	0	0 to 1	0	0 to 0	1
925	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	1
926	0	0 to 1	0	0 to 1	0	0 to 0	0	0 to 1	0	0 to 1	1
927	7	7 to 9	1	0 to 3	3	1 to 5	3	0 to 3	3	1 to 5	2
929	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
930	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
933	0	0 to 3	0	0 to 0	0	0 to 0	0	0 to 1	1	0 to 1	1
935	7	5 to 9	7	5 to 9	3	3 to 5	5	3 to 7	7	7 to 9	3
937	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
938	7	5 to 9	5,7	5 to 7	1	1 to 5	5	3 to 9	5	5 to 7	7,12
939	9	7 to 9	1	0 to 3	0	0 to 0	0	0 to 0	3	0 to 3	6
940	7	5 to 7	1	1 to 7	1	0 to 5	0	0 to 0	3	1 to 5	6
940	5,7	5 to 7	3	1 to 5	3	3 to 5	3	1 to 5	3	3 to 5	9,5
941	7	7 to 9	5	3 to 7	5	3 to 7	7	3 to 7	3	1 to 5	9
942	7	7 to 9	1	0 to 1	5	0 to 5	0	0 to 5	3	0 to 3	2
942	9	7 to 9	0	0 to 1	3	1 to 5	1,3	0 to 3	0	0 to 0	2
943	0	0 to 3	0	0 to 0	0	0 to 0	0	0 to 0	3	1 to 3	1
945	7	5 to 7	0	0 to 0	0	0 to 0	0	0 to 1	3	0 to 3	6
946	9	7 to 9	3	0 to 5	7	5 to 7	7	5 to 9	5	3 to 7	T
949	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
950	7	5 to 9	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	6
951	7	5 to 9	1	0 to 5	3	0 to 5	3	0 to 5	5	0 to 5	2
952	7	5 to 9	5	3 to 5	7	5 to 9	7	7 to 9	5	1 to 7	T

954	7	7 to 9	1	0 to 1	1	0 to 3	1	0 to 3	0	0 to 3	6
967	7	7 to 9	1	0 to 3	3	1 to 5	3	1 to 5	3	1 to 3	2
969	0	0 to 0	1	0 to 1	0	0 to 1	0	0 to 1	0	0 to 1	1
988	7	7 to 9	0	0 to 1	3	1 to 3	0	0 to 3	0	0 to 3	6
991	9	7 to 9	1	0 to 3	3	1 to 3	1	1 to 3	5	3 to 7	2
992	9	7 to 9	7	5 to 9	7	5 to 9	7	5 to 9	0	0 to 1	4
994	3	1 to 5	3	1 to 5	3	1 to 5	1	0 to 5	7,9	7 to 9	5
995	9	7 to 9	5	3 to 5	7	7 to 9	7	5 to 7	3,5	3 to 5	T
999	7	7 to 9	0	0 to 3	1	1 to 3	3	1 to 5	3	1 to 5	6
1001	7,9	7 to 9	0	0 to 1	1	1 to 5	0	0 to 3	0	0 to 1	6
1008	7	7 to 9	7	5 to 9	7	5 to 7	1	0 to 5	7	7 to 9	4
1008	7	7 to 9	7	7 to 9	7	5 to 9	7	5 to 7	7	5 to 7	4
1010	7	7 to 9	7	5 to 7	5	3 to 7	5	3 to 7	3	3 to 5	3
1015	7	7 to 9	7	7 to 9	7,9	7 to 9	7	7 to 9	7	7 to 9	4
1015	7	7 to 9	7	7 to 9	7	5 to 7	7	5 to 7	7	5 to 7	4
1017	7	5 to 7	1	1 to 3	5	5 to 7	1	0 to 3	1	1 to 3	2
1018	7	3 to 7	3	1 to 5	3	1 to 5	0,1	0 to 3	3	1 to 5	9
1021	7	7 to 7	0	0 to 1	0	0 to 3	0	0 to 1	1	1 to 3	6
1024	7	7 to 9	0	0 to 1	0	0 to 3	0	0 to 3	1	0 to 3	6
1025	9	7 to 9	7,9	7 to 9	9	7 to 9	7	0 to 9	9	7 to 9	4
1027	9	7 to 9	9	7 to 9	7	7 to 9	7	0 to 9	9	7 to 9	4
1030	9	5 to 9	1,3	0 to 3	5,7	1 to 7	0	0 to 3	3	1 to 3	9,T,10
1031	5	1 to 5	1	0 to 3	1,3	0 to 3	0	0 to 3	5	0 to 7	8, 11
1031	1	0 to 3	0	0 to 1	0	0 to 1	0	0 to 1	3	1 to 3	1
1032	7	5 to 9	1	1 to 5	3	1 to 5	3	1 to 7	1,3	0 to 3	2
1033	7	5 to 7	0	0 to 0	0	0 to 1	0	0 to 1	3	1 to 5	6
1034	7	3 to 9	7	5 to 7	3	3 to 7	1	0 to 1	5	0 to 7	3
1035	0	0 to 1	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	1
1036	9	7 to 9	0	0 to 1	0	0 to 3	0	0 to 1	1	0 to 5	6
1036	7	7 to 9	0	0 to 1	1,3	0 to 3	0	0 to 3	3	1 to 5	6,2
1038	9	5 to 9	7	5 to 7	5	1 to 5	3	1 to 5	7,9	7 to 9	2
1040	7	5 to 7	3	0 to 3	7	0 to 7	5	0 to 7	3	0 to 5	T
1041	0	0 to 3	0	0 to 1	0	0 to 1	0	0 to 0	0	0 to 1	1
1041	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1043	7	5 to 9	0	0 to 3	0	0 to 7	0	0 to 1	0	0 to 3	6
1044	9	9 to 9	3	1 to 5	9	1 to 9	7	5 to 9	5	3 to 7	T
1045	7	3 to 7	3	1 to 5	3,5	0 to 7	3	1 to 5	3	0 to 5	9
1045	7	3 to 7	3	1 to 5	7	3 to 7	7	5 to 7	3	3 to 5	T
1046	7,9	7 to 9	1	0 to 3	3	0 to 3	3	0 to 5	1	0 to 3	2

1047	3	0 to 5	0	0 to 1	0	0 to 1	0	0 to 1	0	0 to 3	8
1048	9	7 to 9	0	0 to 1	0	0 to 1	0	0 to 0	0	0 to 1	6
1049	0	0 to 1	0	0 to 0	0	0 to 1	0	0 to 1	0	0 to 1	1
1050	9	9 to 9	1	0 to 5	3	1 to 3	0	0 to 1	5,7	3 to 7	2
1051	9	7 to 9	3	1 to 3	7	5 to 9	5	0 to 7	1	0 to 5	T
1052	9	7 to 9	3	1 to 3	7	3 to 9	5	1 to 7	5	5 to 7	T
1053	9	9 to 9	0	0 to 1	0,1	0 to 3	0	0 to 0	0	0 to 3	6
1054	9	9 to 9	7	5 to 9	3	3 to 5	0	0 to 5	7	5 to 9	3
1055	9	9 to 9	3	0 to 3	3	3 to 5	3	1 to 5	3	1 to 5	9
1056	9	9 to 9	1	0 to 3	1	0 to 3	0	0 to 1	0	0 to 3	6
1056	7	7 to 9	0	0 to 1	0,1	0 to 3	1	0 to 3	1	0 to 5	6
1057	9	7 to 9	3	1 to 3	7	5 to 9	7	5 to 7	3	1 to 5	T
1059	9	9 to 9	3	0 to 7	9	5 to 9	9	7 to 9	3	1 to 7	T
1060	9	7 to 9	0	0 to 1	3	0 to 3	3	0 to 3	3	3 to 5	2
1062	9	7 to 9	7	5 to 9	3	1 to 3	3	0 to 3	5,7	5 to 7	3
1062	7	7 to 9	7	5 to 7	5	3 to 5	5	1 to 7	7	3 to 7	3
1062	9	7 to 9	7	5 to 9	7	5 to 7	5	5 to 7	7	5 to 7	4
1063	9	7 to 9	1	0 to 1	0	0 to 3	0	0 to 1	3	1 to 3	6
1064	9	7 to 9	1	0 to 1	1	0 to 3	0	0 to 1	3	1 to 5	6
1065	9	3 to 9	3	1 to 5	3	3 to 5	5	3 to 7	3	1 to 5	3
1066	9	5 to 9	1	0 to 3	1	0 to 3	0	0 to 0	7	5 to 7	6
1067	7	3 to 9	3	1 to 5	5	1 to 5	1	1 to 5	3	0 to 3	3
1068	7,9	7 to 9	3	0 to 3	3	1 to 5	5	1 to 5	3	3 to 5	3
1069	9	7 to 9	3	1 to 3	5	3 to 7	3	3 to 3	3	3 to 7	3
1070	7	5 to 9	1	0 to 3	0	0 to 0	0	0 to 0	3	1 to 3	6
1071	9	7 to 9	0	0 to 1	3	1 to 5	3	0 to 5	1	0 to 1	2
1072	0	0 to 3	0	0 to 1	0	0 to 1	0	0 to 0	3	1 to 3	1
1074	7	3 to 9	3	3 to 5	1	1 to 3	0	0 to 1	3	1 to 3	7
1074	0	0 to 1	0	0 to 0	0	0 to 0	0,1	0 to 1	0	0 to 0	1
1074	7	7 to 9	3	1 to 3	1	0 to 3	0	0 to 1	3	1 to 5	7
1075	7	5 to 9	0	0 to 1	3	1 to 5	1	0 to 5	0	0 to 1	2
1075	7	7 to 9	3	1 to 3	7	5 to 7	5	3 to 7	1	1 to 5	T
1075	9	7 to 9	1	1 to 3	7	5 to 9	5	3 to 7	3	1 to 7	10
1076	0	0 to 1	0	0 to 1	0	0 to 1	0	0 to 1	1	0 to 1	1
1077	9	9 to 9	3	0 to 5	7	5 to 9	7	5 to 7	0	0 to 3	T
1079	7	5 to 9	0	0 to 3	5	0 to 5	1	0 to 1	1	0 to 3	2
1080	7	7 to 9	1	0 to 3	0	0 to 3	0	0 to 0	0	0 to 0	6
1081	9	7 to 9	3	1 to 3	7	5 to 7	7	3 to 7	1	0 to 3	T
1081	7	7 to 9	3	1 to 7	7	5 to 7	7	5 to 7	3	0 to 5	T

1082	3	0 to 3	0	0 to 1	1	1 to 5	0	0 to 1	1	0 to 3	8
1083	7	5 to 9	1	0 to 5	5	0 to 5	3	0 to 5	0	0 to 3	2
1084	9	5 to 9	1	1 to 3	5,7	3 to 7	0	0 to 0	0	0 to 0	2,10
1085	5	3 to 7	3	1 to 5	5	0 to 5	3,5	0 to 5	1	0 to 5	5
1085	5,7	5 to 7	3,5	1 to 5	5	5 to 7	5	3 to 5	1	0 to 5	5,9
1086	7	5 to 9	1	0 to 5	5	0 to 5	3	3 to 7	3	1 to 5	2
1087	7	7 to 9	3	0 to 3	1,3	1 to 5	0	0 to 0	3	0 to 3	7,9
1088	7	5 to 9	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	6
1088	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1089	9	9 to 9	3	0 to 3	5	0 to 9	7	3 to 7	3	0 to 3	9
1090	7	5 to 9	0	0 to 1	0	0 to 1	0	0 to 1	0	0 to 1	6
1090	7	5 to 7	0	0 to 3	0	0 to 0	0	0 to 1	1	0 to 1	6
1091	7	5 to 9	1	0 to 3	3	0 to 5	3	0 to 5	0	0 to 3	2
1092	0	0 to 3	0	0 to 1	0	0 to 1	0	0 to 1	0	0 to 1	1
1093	7	5 to 9	1	0 to 3	5	1 to 7	1	0 to 5	1	0 to 3	2
1094	7	7 to 9	5	0 to 5	7	5 to 9	3	0 to 5	5	0 to 5	T
1095	7	5 to 9	1,3	0 to 3	1	0 to 3	0	0 to 3	3	3 to 5	6,7
1096	9	7 to 9	5	3 to 5	3	1 to 5	0	0 to 1	3	0 to 3	9
1097	9	5 to 9	3	1 to 5	3	1 to 5	0	0 to 1	3	1 to 5	9
1097	7	7 to 9	7	5 to 7	5	5 to 7	5	0 to 7	7	5 to 7	3
1097	9	7 to 9	3,5,7	3 to 7	3	1 to 5	3	1 to 5	5	3 to 7	9,3
1098	7	5 to 7	3	1 to 5	3	1 to 3	0	0 to 0	3	1 to 3	9
1098	5,7	5 to 7	5,7	5 to 7	5	5 to 7	5	5 to 7	5	3 to 7	3,19,13
1099	7	7 to 9	3,5	3 to 5	7	5 to 9	3	3 to 7	1	1 to 5	T
1100	7	5 to 7	0	0 to 3	3	0 to 5	0	0 to 3	3	0 to 5	2
1101	9	7 to 9	0	0 to 1	0	0 to 1	0	0 to 1	3	0 to 3	6
1102	7	7 to 9	3	1 to 3	3	1 to 5	0	0 to 3	3	1 to 5	9
1103	7	0 to 9	3	0 to 7	5	5 to 7	0	0 to 3	7	0 to 7	9
1104	7	3 to 9	3	1 to 5	3	0 to 3	1	0 to 3	5	1 to 5	9
1104	7	7 to 7	7	3 to 7	3	1 to 3	3	0 to 5	5	3 to 7	3
1105	7	0 to 9	3,5	0 to 5	3	1 to 5	0	0 to 0	0	0 to 0	9
1106	7	7 to 9	7	5 to 9	3	1 to 7	0	0 to 0	0	0 to 0	3
1108	7,9	5 to 9	5	0 to 7	3	1 to 5	3	0 to 5	3	3 to 7	9
1109	7	7 to 9	7	5 to 9	7	5 to 7	3	1 to 3	7	5 to 7	4
1110	7	5 to 9	7	5 to 7	3	0 to 5	1	0 to 3	7	0 to 7	3
1111	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	0	0 to 1	1
1111	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1112	0	0 to 0	0	0 to 0	0	0 to 0	5	3 to 7	3	0 to 5	1
1113	0	0 to 0	0	0 to 0	0	0 to 0	5	5 to 9	7	5 to 9	1

1115	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1116	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1117	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1118	0	0 to 0	0	0 to 0	0	0 to 0	1	0 to 3	5	0 to 5	1
1119	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1120	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 7	0	0 to 0	1
1122	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1123	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1124	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1125	9	7 to 9	7	5 to 9	7	5 to 9	0	0 to 0	0	0 to 0	4
1126	7	7 to 9	0	0 to 0	3	0 to 5	3	3 to 5	5	3 to 5	2
1127	7	5 to 7	7	5 to 9	3	0 to 5	3	1 to 5	7	0 to 7	3
1128	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1131	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1132	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1133	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1136	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1137	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1137	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1138	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1139	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1140	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1141	0	0 to 0	0	0 to 1	0	0 to 0	0	0 to 0	0	0 to 0	1
1143	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1143	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1144	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1145	0	0 to 0	0	0 to 0	0	0 to 1	0	0 to 0	0	0 to 1	1
1146	0	0 to 1	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1148	0	0 to 0	0	0 to 0	0	0 to 0	1	0 to 3	1	0 to 5	1
1149	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1153	7	5 to 7	3	1 to 5	7	5 to 9	3	0 to 5	5	1 to 7	T
1154	7	7 to 9	1	0 to 3	3	3 to 5	1	0 to 3	0	0 to 3	2
1158	7	7 to 9	7	5 to 7	7	0 to 9	7	7 to 9	7	5 to 7	4
1159	0	0 to 1	0	0 to 0	0	0 to 1	0	0 to 1	0	0 to 1	1
1167	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1168	7	7 to 9	3	1 to 5	5	0 to 5	1	1 to 5	1	1 to 5	9
1169	7	5 to 9	0	0 to 1	0	0 to 1	0	0 to 0	1	0 to 3	6
1170	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1171	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1

1172	0	0 to 3	0,1	0 to 1	0	0 to 3	1	1 to 3	3	3 to 7	1
1173	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1174	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1176	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 1	0	0 to 1	1
1176	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1177	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1178	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1179	0	0 to 0	0	0 to 1	0	0 to 0	0	0 to 0	0	0 to 0	1
1180	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1181	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1184	0	0 to 1	0	0 to 1	1	0 to 5	0	0 to 0	0,1	0 to 5	1
1185	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1186	7	5 to 7	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	6
1187	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1190	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1191	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1193	7	7 to 9	3	1 to 3	7	5 to 7	7	7 to 7	3	1 to 5	T
1194	7	5 to 7	5	3 to 7	3	1 to 5	0	0 to 3	5	0 to 7	9
1195	7	5 to 7	5	0 to 5	3	3 to 5	3	1 to 5	1,3	1 to 3	9
1197	9	7 to 9	5	3 to 5	5	5 to 7	7	5 to 7	3	0 to 3	9
1198	7	7 to 7	5	3 to 5	3	1 to 3	5	3 to 7	3	1 to 5	9
1199	7	0 to 9	3	1 to 5	1	1 to 5	1	0 to 3	3	3 to 5	7
1204	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1205	7	5 to 7	3	1 to 5	7	5 to 9	3	3 to 5	1	1 to 3	T
1206	7	7 to 9	3,5	3 to 5	5	5 to 7	5	3 to 7	3	1 to 5	9
1207	7	5 to 7	3	1 to 3	7	5 to 9	3	1 to 5	1,3,5	1 to 5	T
1209	7	7 to 7	3	1 to 5	5	3 to 7	1,3	1 to 3	1	1 to 3	9
1213	0	0 to 3	0	0 to 3	0	0 to 1	0	0 to 1	1	0 to 3	1
1217	7	7 to 9	3	3 to 7	7	5 to 7	3	1 to 5	3	1 to 5	T
1219	7	7 to 9	0,1	0 to 1	3	0 to 3	0	0 to 3	3	1 to 5	2
1223	7	7 to 9	5	3 to 5	5	5 to 7	5	3 to 7	3	0 to 5	9
1225	0	0 to 3	0	0 to 1	0	0 to 1	0	0 to 1	0	0 to 3	1
1231	9	7 to 9	5	5 to 7	7	5 to 9	7	5 to 7	3	1 to 5	T
1232	7	7 to 9	3	1 to 5	3	1 to 5	3	1 to 5	5	0 to 7	9
1233	7	7 to 9	7	5 to 7	5	3 to 5	7	5 to 7	3	3 to 5	3
1233	7	7 to 9	7	5 to 9	5	3 to 7	3	1 to 5	5	3 to 7	3
1235	7	7 to 9	1	0 to 1	3	0 to 3	0	0 to 0	0,1	0 to 1	2
1236	9	7 to 9	7	7 to 9	7,9	7 to 9	7	5 to 9	9	5 to 9	4
1237	0	0 to 0	1	0 to 3	0	0 to 0	0	0 to 0	5	3 to 7	1

1239	7	7 to 9	0	0 to 0	1	0 to 3	0	0 to 3	0	0 to 3	6
1241	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1245	7	5 to 9	0	0 to 1	3	1 to 5	1	0 to 3	1	0 to 3	2
1246	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1247	7	5 to 7	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	6
1254	7	7 to 9	0	0 to 0	0	0 to 3	0	0 to 0	0	0 to 3	6
1256	7	5 to 9	3	1 to 5	3	0 to 5	0	0 to 3	1	0 to 3	9
1259	0	0 to 7	0	0 to 0	0	0 to 1	0	0 to 0	0	0 to 3	1
1260	7	7 to 9	7	5 to 9	3	1 to 3	1	0 to 1	5	3 to 7	3
1268	7	7 to 9	5	5 to 7	5	5 to 7	7	5 to 7	5	5 to 7	9
1269	7	5 to 9	0	0 to 0	0	0 to 3	0	0 to 0	1	0 to 3	6
1270	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	1
1272	7	5 to 7	0	0 to 1	0	0 to 3	0	0 to 0	1	0 to 3	6
1273	9	7 to 9	7	5 to 9	3	0 to 5	1	1 to 3	3	0 to 5	3
1274	7	5 to 7	7	7 to 9	7	5 to 7	5	3 to 5	5	3 to 5	4
1275	7	7 to 9	7	5 to 7	3,5	3 to 5	1	0 to 3	5	3 to 7	3
1276	7	5 to 7	3	1 to 5	3	0 to 5	5	3 to 7	3	0 to 3	9
1277	7	7 to 9	0	0 to 1	0	0 to 0	0	0 to 1	1	0 to 3	6
1281	0	0 to 0	0	0 to 1	1	0 to 3	1	0 to 1	0	0 to 1	1
1288	7	7 to 9	3,5	3 to 5	7	5 to 7	7	5 to 7	3	1 to 5	T
1291	7	7 to 9	3	0 to 5	7	5 to 7	7	5 to 7	3	1 to 5	T
1291	9	7 to 9	5	5 to 7	7	5 to 9	3	1 to 5	3	1 to 5	T
1298	7	7 to 9	1	0 to 3	1	0 to 3	1	0 to 3	5	0 to 5	6
1304	7	7 to 9	0	0 to 1	0	0 to 3	1	0 to 3	3	1 to 5	6
1313	7	7 to 9	7	0 to 9	7	5 to 7	5	3 to 5	7	5 to 7	4
1319	7	7 to 9	0	0 to 1	0	0 to 5	0	0 to 0	3	0 to 5	6
1323	7	0 to 9	1	0 to 1	1	0 to 1	1	1 to 3	3	0 to 5	6
1324	7	7 to 9	0	0 to 1	3	0 to 5	5	3 to 7	3	0 to 3	2
1326	7	7 to 9	3	1 to 5	9	5 to 9	5	3 to 5	3	1 to 5	T
1327	7	7 to 9	3	0 to 5	3	1 to 3	3	1 to 3	3	1 to 5	9
1328	7	7 to 9	1	0 to 3	0	0 to 3	0	0 to 3	5	0 to 5	6
1331	7	5 to 9	5	3 to 5	9	7 to 9	7	7 to 9	3	1 to 5	T
1332	9	7 to 9	3,5	3 to 5	9	7 to 9	5	3 to 7	7	7 to 9	T
1332	7	5 to 7	3	1 to 3	7	5 to 7	3	1 to 5	1	0 to 3	T
1333	7	5 to 9	1	0 to 5	1	0 to 3	1	1 to 3	3	1 to 5	6
1334	7	7 to 9	3	1 to 5	7	5 to 9	7	3 to 7	1	0 to 3	T
1335	7	7 to 9	5	3 to 7	7	5 to 7	7	5 to 7	5	5 to 7	T
1338	7	7 to 9	1	0 to 3	1	1 to 5	1	0 to 3	3	1 to 3	6
1340	7	7 to 7	0	0 to 0	0	0 to 3	1	0 to 3	0	0 to 0	6

1341	9	7 to 9	1	0 to 3	1	1 to 5	0	0 to 0	5	0 to 5	6
1342	7	5 to 9	7	7 to 9	3	1 to 5	1	0 to 3	5	0 to 5	3
1343	7	5 to 7	7	5 to 9	5	1 to 5	0	0 to 3	3	1 to 5	3
1344	7	7 to 9	7	5 to 7	3	1 to 5	0	0 to 3	5	0 to 7	3
1345	7	5 to 7	1	0 to 3	3	1 to 5	0	0 to 1	3	1 to 5	2
1347	7	7 to 9	5	3 to 5	7	7 to 9	7	7 to 9	5	3 to 7	T
1347	7	7 to 9	5	3 to 7	7	5 to 9	7	5 to 7	3	1 to 5	T
1348	7	7 to 7	0	0 to 1	0	0 to 1	0	0 to 3	0	0 to 1	6
1349	7	7 to 9	0	0 to 1	3	3 to 7	0	0 to 3	1	0 to 3	2
1350	7,9	7 to 9	3	1 to 3	3	0 to 5	3	1 to 5	5	3 to 7	9
1352	7	7 to 9	1	1 to 5	7	5 to 7	5	3 to 5	7	5 to 7	10
1353	7	5 to 9	0	0 to 3	0	0 to 3	0	0 to 3	0	0 to 3	6
1355	7	5 to 9	0	0 to 0	1	0 to 3	0	0 to 3	1	0 to 3	6
1356	7,9	7 to 9	3	3 to 7	7	7 to 9	7	7 to 9	5	3 to 5	T
1357	9	7 to 9	0	0 to 3	1	0 to 3	0	0 to 3	5	3 to 7	6
1359	7	7 to 9	3	1 to 5	7	5 to 9	7	7 to 9	3	1 to 5	T
1360	7,9	7 to 9	0	0 to 3	0	0 to 3	0	0 to 3	3,5	1 to 5	6
1362	7	7 to 9	3	1 to 3	7	7 to 7	7	5 to 7	5	1 to 5	T
1363	0	0 to 5	0	0 to 3	0	0 to 3	0	0 to 1	0	0 to 3	1
1366	7	5 to 7	0	0 to 1	1	0 to 3	0	0 to 1	1,3	1 to 3	6
1372	7	7 to 9	7	5 to 7	3	1 to 5	1	0 to 3	5	1 to 7	3
1373	7	7 to 9	7	7 to 9	7	7 to 9	7	7 to 9	7,9	7 to 9	4
1374	9	7 to 9	3	1 to 5	7	5 to 9	7	5 to 9	5	5 to 7	T
1375	9	9 to 9	5	1 to 5	7	5 to 9	7	5 to 7	5	3 to 5	T
1376	7	7 to 9	1	0 to 3	3	1 to 5	5	1 to 5	3	1 to 5	2
1377	9	5 to 9	1,3	1 to 3	3	1 to 5	3	1 to 5	3	1 to 5	2,9
1379	7	7 to 9	5	3 to 7	5	3 to 7	5	3 to 7	3	0 to 5	9
1380	7	7 to 7	5	3 to 7	7	5 to 9	3	1 to 3	3	1 to 5	T
1381	7	7 to 9	3	0 to 3	5	1 to 5	3	0 to 3	1	1 to 3	9
1384	7	7 to 7	5	5 to 7	7	5 to 7	7	0 to 7	5	3 to 7	T
1388	7	5 to 9	1	0 to 3	1	1 to 3	1	1 to 3	1	0 to 1	6
1389	7	5 to 9	3	1 to 3	5	3 to 5	3	1 to 3	1	1 to 3	9
1398	7	5 to 9	7	5 to 7	3	1 to 5	1	0 to 3	3	1 to 5	3
1407	7	7 to 9	7	5 to 9	5	3 to 7	5	3 to 7	5	3 to 7	3
1408	7	5 to 9	3	0 to 3	7	5 to 9	1,3	1 to 3	1	0 to 3	T
1409	7	5 to 7	7	5 to 9	3	1 to 5	3	1 to 5	3	1 to 5	3
1410	7	7 to 9	7	5 to 9	7	5 to 7	7	5 to 7	7	5 to 7	4
1420	7	7 to 9	1	0 to 3	1	0 to 3	0	0 to 1	3	1 to 3	6
1424	7	5 to 7	0	0 to 1	1	0 to 3	0,1	0 to 1	1	1 to 3	6

1427	9	0 to 7	5,7	5 to 7	7	5 to 9	3	3 to 5	7	5 to 7	T,4
1436	9	7 to 9	5	3 to 5	7	7 to 9	7	7 to 9	5	3 to 5	T
1437	7	7 to 9	7	5 to 9	3,5	1 to 5	1	0 to 3	5	1 to 5	3
1439	7	7 to 9	3	1 to 5	7	5 to 9	5	1 to 5	1,3	1 to 3	T
1442	7	7 to 9	3	1 to 5	7	5 to 9	5	1 to 7	1	0 to 3	T
1450	7	5 to 9	5	0 to 7	7	3 to 9	3	1 to 7	3	0 to 5	T
1459	7	7 to 7	0	0 to 1	3	3 to 5	1	0 to 3	0,1	0 to 1	2
1460	7	7 to 9	1	0 to 3	0	0 to 3	0	0 to 3	3	1 to 5	6
1462	7	7 to 9	1	0 to 1	3	1 to 5	1	0 to 5	1	0 to 3	2
1463	7	7 to 9	0	0 to 3	3	1 to 5	1	0 to 3	1	0 to 3	2
1471	9	7 to 9	0	0 to 1	3	1 to 5	0	0 to 1	3	0 to 3	2
1474	7	7 to 9	7	0 to 9	7	1 to 9	3	0 to 5	5	1 to 7	4
1475	7	7 to 9	7	7 to 9	7	5 to 9	5	3 to 7	7	5 to 7	4
WC[b]	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	NA
WC	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	0	0 to 0	NA

[a]IPMs are based on Delwiche's (1980) zero to nine scale. Interactions highlighted in gray represent bi or tri-modal data in which the PG actually changes depending on the mode used. Interactions highlighted in yellow were positive controls obtained from University of Winnipeg, Manitoba, Canada. *R gene* is the recognized resistance gene present in each differential cultivar.

[b]WC (water control) were inoculated with sterile distilled water blanks and used as a negative control. *PG-1 has been classified as *L. biglobosa* by Shoemaker and Brun (2001).