CHARACTERIZATION OF GENETIC RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* AND EPIDEMIOLOGY OF THE DISEASE IN *BRASSICA NAPUS* L.

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

By

Fereshteh Shahoveisi

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

Major Department:
Plant Pathology

November 2020

Fargo, North Dakota

North Dakota State University
Graduate School

Title

CHARACTERIZATION OF GENETIC RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* AND EPIDEMIOLOGY OF THE DISEASE IN *BRASSICA NAPUS* L.

By

Fereshteh Shahoveisi

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. Luis E. del Río Mendoza
Chair

Dr. Samuel Markell

Dr. Zhaohui Liu

Dr. Md. Mukhlesur Rahman

Dr. Wenhao Dai

Approved:

11/12/2020
Date

Dr. Jack B. Rasmussen
Department Chair

ABSTRACT

This dissertation contains three research chapters conducted on Sclerotinia stem rot (SSR) of canola (*Brassica napus* L.). This disease is caused by the fungus *Sclerotinia sclerotiorum* and is considered endemic in canola-producing areas of North Dakota. The first research chapter presents results of a study that evaluated the role of eight phenotyping scoring systems and nine variant calling and filtering methods in detection of QTL associated with response to SSR. The study, conducted on two doubled-haploid mapping populations, showed that using multiple phenotypic data sets derived from lesion length and plant mortality and imputing missing genotypic data increased the number of QTL detected without negatively affecting the effect (R^2) of QTL. Nineteen QTL were detected on chromosomes A02, A07, A09, C01, and C03 in this study. The second research chapter presents results of a work that assessed the role of temperature regimes and wetness duration on *S. sclerotiorum* ascospore germination and ascosporic infection efficiency. This study showed that optimum ascospore germination occurred at 21 °C while it significantly decreased at 10 and 30 °C. Infection efficacy experiments indicated that extreme temperatures and interrupting wet periods were detrimental for the disease development. A logistic regression model with 75% accuracy was developed for the disease prediction. The third research chapter presents results of a study that evaluated the role of temperature on mycelial growth of 19 *S. sclerotiorum* isolates collected from different geographical regions and on SSR development on plant introduction (PI) lines with different levels of resistance. Mycelial growth and disease development peaked at 25 °C. While lesion expansion on resistant cultivars and the susceptible check was negatively affected at 30 °C, the disease developed significantly on the PI with a high level of susceptibility. Results of these studies provide insights into integrated management strategies of SSR.

ACKNOWLEDGEMENTS

My deep gratitude goes first to my major advisor, Dr. Luis del Río Mendoza, for his continuous support during my Ph.D. education, his enthusiasm, expertise, and motivation. I could not have imagined having a better advisor and mentor for my Ph.D. study. I also sincerely thank the members of my Ph.D. committee, Drs. Samuel Markell, Md. Mukhlesur Rahman, Zhaohui Liu, and Wenhao Dai, for their constant support, encouragement, and insightful comments.

Many thanks go to Dr. Atena Oladzad and Dr. Kishore Chittem who generously helped me to complete my experiments and data analyses. Also, my thanks are extended to the Plant Pathology Department Chair, Dr. Jack Rasmussen, who generously helped me to work on my professional development skills. I am also grateful for the help I received from members of our canola program, Susan Ruud, Dante Marino, and former members Seyedmojtaba Mansouripour, Bruna Rissato, and Sudha GC Upadhaya. Thanks to my wonderful friends, Zahra, Nakisa, Oveis, Samira, and Brandt, for their support and friendship during the past five years.

I am also thankful to the Sclerotinia Initiative (USDA-ARS), USDA-NIFA/ Hatch program, and the Northern Canola Growers Association for funding my Ph.D. projects.

Lastly and most importantly, I would like to thank my husband, Seyedali Hosseinirad, and my parents, Shahla Jedinejad and Farhang Shahoveisi, for their unconditional support and love in every step of the way.

DEDICATION

I dedicate this dissertation to my parents who supported me throughout my life. Thank you for your unconditional love and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Canola or rapeseed (*Brassica napus* L.) is an important oilseed crop that has been bred for low level of glucosinolates (<30 umol/g) and erucic acid (<2%) (Mag 1983) for animal and human consumptions (Lin et al. 2013). Total production of rapeseed in the world was more than 75 million tons in 2018. Leading countries in rapeseed production are Canada, China, India, France, Australia, Germany, Ukraine, Poland, United Kingdom, and Russian Federation (FAO 2018). United States is also one of the top canola producers with more than 3,402 million lb (> $ 494 million dollars) produced in 2019; with North Dakota accounting for more than 85% of the nation's production (USDA-NASS 2019). Despite this, the demand for canola oil in the country is almost three times the offer. Increasing production to meet this demand will require increasing canola acreage and planting cultivars with improved agronomic traits and resistance to diseases.

B. napus can be affected by several biotic and abiotic stresses. Among biotic stresses, plant diseases including Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, blackleg, caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not. , and clubroot, caused by *Plasmodiophora brassicae* Woronin could cause serious economic losses (del Río et al. 2007; Dixon 2009; del Rio Mendoza et al. 2012). SSR is endemic in canola-producing areas of North Dakota where its average annual incidence ranged between 4 and 19% from 1993 to 2004 causing an economic loss estimated at US$94 million (Lamey 1995; Lamey 2003; Bradley and Lamey 2005). On average, every unit increase in SSR incidence reduces the yield by 0.5% (del Río et al. 2007).

Several production practices are recommended for management of Sclerotinia stem rot. However, their isolated use in most cases is not enough to reduce the economic damage this disease causes. Crop rotations and other cultural practices are not sufficient to manage the

disease efficiently because the pathogen has a wide host range (more than 500 plant species) and its sclerotia remains viable in the soil for five years or longer (Peltier et al. 2012; Jain et al. 2012; Sharma et al. 2016). While biological control methods are environmentally friendly, they also are population-density dependent, which means they are less effective when levels of the host, i.e. propagules, are low but still capable of reducing yields. This makes the biological control practices not an effective management strategy when used individually (Fernando et al. 2004; Rothmann and McLaren 2018).

Fungicide applications at flowering time can be used to manage the disease; however, they represent an extra production cost that in some instances may not provide appropriate economic returns (Hind-Lanoiselet et al. 2005). Further, many of the fungicides used to manage Sclerotinia have a history of fungicide resistance in other pathosystems (Lehner et al. 2015) that may contribute to reduce even further the profitability and sustainability of canola production.

Since the issue of unnecessary fungicide applications occurs not just in canola but in many other crops, disease warning models have been developed to help farmers make better-informed spraying decisions. The majority of these models and risk maps have been generated using a range of independent variables including weather factors, cultural practices, and presence/absence of infected petals (Bom and Boland 2000; Workneh and Yang 2000; Harikrishnan and del Río 2008; Clarkson et al. 2014; Chilvers and Willbur 2018; del Río 2018). While these models may provide valuable information, their accuracy may be compromised by not considering the factors that influence “disease establishment on the leaf”. During disease development, the fungus colonizes casted petals first and from there enters the stem if in direct contact with it; more frequently, however, it moves from the petals into the leaves (disease establishment) and from there into the petiole and stem. The effects of environmental factors

such as temperature and moisture on this stage have not been studied thoroughly. To obtain more comprehensive and accurate models for assessment of the risk of disease development and planning for required fungicide applications, factors affecting disease onset need to be studied.

Cultivars with acceptable levels of genetic resistance against Sclerotinia stem rot are yet to become available to farmers. Genetic resistance is the most efficient tool for management of plant diseases (Mundt et al. 2002). However, development of highly resistant lines to diseases caused by *S. sclerotiorum* is challenging due to the quantitative nature of the resistance to the disease (Baswana et al. 1991; Zhao and Meng 2003; Zhao et al. 2004). Hence, the majority of *B. napus* commercial hybrids and breeding lines contain low levels of resistance to *S. sclerotiorum* (Bradley et al. 2006; Zhao et al. 2004) and development of highly resistant lines is yet a major goal of canola breeding programs (Derbyshire and Denton-Giles 2016).

Several studies have been conducted to identify plant materials including wild type germplasm, plant introduction (PI) accessions, and breeding lines that are resistant to *S. sclerotiorum* infection (Garg et al. 2008, 2010; Khot et al. 2011; Uloth et al. 2014; Taylor et al. 2015). Multiple studies have focused on detection of quantitative trait loci (QTL) associated with resistance to the disease; mapping studies including bi-parental, nested association, and genome wide association have been used to identify these genetic loci (Zhao & Meng 2003; Zhao et al. 2006; Yin et al. 2010; Wu et al. 2013; Wei et al. 2014; Gyawali et al. 2016; Wu et al. 2016; Wu et al. 2019; Qasim et al. 2020). These studies have resulted in identification of numerous molecular markers located on different *B. napus* chromosomes including A01, A02, A03, A06, A07, A08, A09, A10, C01, C02, C03, C04, C05, C06, C07, and C08. While various inoculation techniques such as agar plug, toothpick, mycelium on petals, and natural inoculation have been used in these studies, only one type of scoring system, mostly

quantitative, have been deployed to generate the phenotyping dataset. Recent studies in other pathosystems such as *Rhizoctonia solani*-common bean (Oladzad et al. 2019), *Fusarium solani*-common bean (Zitnick-Anderson et al. 2020), and *Leptosphaeria maculans*-canola (Mansouripour et al. unpublished) have shown the benefit of reducing phenotypic severity data with up to nine categories to two or three categories in genome wide association studies (GWAS). However, little information has been generated on their application on biparental mapping studies and in *S. sclerotiorum*-canola pathosystem.

More recently, *B. napus* mapping studies have been conducted using genotyping by sequencing (GBS) and single nucleotide polymorphism (SNP) arrays which currently are the most efficient genotyping methods (ArifUzZaman et al. 2016; Yu et al. 2017; Wu et al. 2019). However, the impact of some genotypic data processing steps, which are regularly taken prior to genome-wide association study (GWAS), e.g. use of variant calling and filtering methods such as imputation of missing data and use of uniquely mapped reads, on the ability to detect genomic regions associated with reaction to disease in biparental populations has not been explored. This is especially important because SNP calling could be a challenging proposition in polyploidy crops such as *B. napus* due to the presence of highly similar homoeologous chromosomes (Clevenger et al. 2015; Clevenger and Ozias-Akins 2015).

In general, the viability of using marker assisted selection (MAS) to integrate resistance genes into breeding lines will benefit from more comprehensive mapping studies that identify efficient markers, e.g. markers more tightly linked to them. The probability of identifying these markers might increase when different genotyping methods and phenotyping scoring systems are used.

The objectives of this study were:

I) To assess the impact of using different phenotyping score systems and variant calling methods in identification of QTL associated with response to *Sclerotinia sclerotiorum* on canola (*Brassica napus* L.)

II) To elucidate the effect of leaf wetness duration and incubation temperature on development of ascosporic infection by *S. sclerotiorum*.

III) To characterize the role of incubation temperature regimes on response of *B. napus* plant introduction materials to infection by *S. sclerotiorum*.

References

ArifUzZaman, M., Mamidi, S., McClean, P., and Rahman, M. 2016. QTL mapping for root vigor and days to flowering in *Brassica napus* L. Can. J. Plant Sci. 97:99-109.

Baswana, K. S., Rastogi, K. B., and Sharma, P. P. 1991. Inheritance of stalk rot resistance in cauliflower (*Brassica oleracea* var. Botrytis L.). Euphytica 57:93-96.

Bom, M., and Boland, G. J. 2000. Evaluation of disease forecasting variables for Sclerotinia stem rot (*Sclerotinia sclerotiorum*) of canola. Can. J. Plant Sci. 80:889-898.

Bradley, C. A., Henson, R. A., Porter, P. M., LeGare, D. G., Del Rio, L. E., and Khot, S. D. 2006. Response of canola cultivars to *Sclerotinia sclerotiorum* in controlled and field environments. Plant Dis. 90:215-219.

Bradley, C. A., and Lamey, H. A. 2005. Canola disease situation in North Dakota, USA. 1993-2004. Pages 33-34, in: Proceedings of the 14th Australian Research Assembly on Brassicas, Port Lincoln, Australia.

Chilvers, M., and Willbur, J. 2018. Sporecaster: New white mold risk prediction smartphone app. Michigan State University Extension.

Clarkson, J. P., Fawcett, L., Anthony, S. G., and Young, C. 2014. A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. PLoS One 9:e94049.

Clevenger, J., Chavarro, C., Pearl, S. A., Ozias-Akins, P., and Jackson, S. A. 2015. Single nucleotide polymorphism identification in polyploids: a review, example, and recommendations. Mol. Plant 8:831-846.

Clevenger, J., and Ozias-Akins, P. 2015. SWEEP: a tool for filtering high-quality SNPs in polyploid crops. G3-Genes Genom. Genet. 5:1797-1803.

del Río, L. 2018. Estimated risk of Sclerotinia stem rot development. North Dakota State University Extension. https://www.ag.ndsu.edu/sclerotinia/riskmap.html.

del Río, L. E., Bradley, C. A., Henson, R. A., Endres, G. J., Hanson, B. K., McKay, K., Halvorson, M., Porter, P. M., Le Gare, D. G., and Lamey, H. A. 2007. Impact of Sclerotinia stem rot on yield of canola. Plant Dis. 91:191-194.

del Rio Mendoza, L. E., Nepal, A., and Markell, S. 2012. Outbreak of blackleg in canola in North Dakota is caused by new pathogenicity groups. Online. Plant Health Prog. doi:10.1094/PHP-2012-0410-01-RS.

Derbyshire, M. C., and Denton-Giles, M. 2016. The control of Sclerotinia stem rot on oilseed rape (*Brassica napus*): current practices and future opportunities. Plant Pathol. 65:859-877.

Dixon, G. R. 2009. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. J. Plant Growth Regul. 28:194-202.

Fernando, W. G. D., Nakkeeran, S., and Zhang. Y. 2004. “Ecofriendly Methods in Combating *Sclerotinia Sclerotiorum* (Lib.) de Bary.” Recent Res. Devel. Environ. Bio. 661:329-47.

Food and Agriculture Organization of the United Nations. 2018. FAOSTAT Database. Rome, Italy: FAO. http://www.fao.org/faostat/en/#data/QC.

Garg, H., Sivasithamparam, K., Banga, S. S., and Barbetti, M. J. 2008. Cotyledon assay as a rapid and reliable method of screening for resistance against *Sclerotinia sclerotiorum* in *Brassica napus* genotypes. Australas. Plant Path. 37:106-111.

Garg, H., Atri, C., Sandhu, P. S., Kaur, B., Renton, M., Banga, S. K., Singh, H., Singh, C., Barbetti, M. J., and Banga, S. S. 2010. High level of resistance to *Sclerotinia sclerotiorum* in introgression lines derived from hybridization between wild crucifers and the crop Brassica species *B. napus* and *B. juncea*. Field Crops Res. 117:51-58.

Gyawali, S., Harrington, M., Durkin, J., Horner, K., Parkin, I. A., Hegedus, D. D., Bekkaoui, D., and Buchwaldt, L. 2016. Microsatellite markers used for genome-wide association mapping of partial resistance to *Sclerotinia sclerotiorum* in a world collection of *Brassica napus*. Mol. Breed. 36:72-84.

Harikrishnan, R., and del Río, L. E. 2008. A logistic regression model for predicting risk of white mold incidence on dry bean in North Dakota. Plant Dis. 92:42-46.

Hind-Lanoiselet, T. L., Lewington, F., Murray, G. M., Hamblin, P., and Castleman, L. 2005. Economics and timing of fungicide application to control Sclerotinia stem rot in canola. 14th Australian Research Assembly on Brassicas. SARDI, Primary Industries and Resources South Australia, Port Lincoln, South Australia.

Jain, A., Singh, S., Sarma, B. K., and Singh, H. B. 2012. Microbial consortium-mediated reprogramming of defense network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. J. Appl. Microbiol. 112:537-50.

Khot, S. D., Bilgi, V. N., del Río, L. E., and Bradley, C. A. 2011. Identification of *Brassica napus* lines with partial resistance to *Sclerotinia sclerotiorum*. Plant Health Prog. 12:15-24.

Lamey, H. A. 1995. Blackleg and Sclerotinia disease of canola in North Dakota in 1991 and 1994. Plant Dis. 79:322-324.

Lamey, H. A. 2003. The status of *Sclerotinia sclerotiorum* on canola in North America. Sclerotinia Initiative Annual Meeting, Bloomington, MN.

Lehner, M. S., Paula Júnior, T. J., Silva, R. A., Vieira, R. F., Carneiro, J. E. S., Schnabel, G., and Mizubuti, E. S. G. 2015. Fungicide sensitivity of *Sclerotinia sclerotiorum*: A thorough assessment using discriminatory dose, EC50, high-resolution melting analysis, and description of new point mutation associated with thiophanate-methyl resistance. Plant Dis. 99:1537-1543.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., and Jones, P. J. 2013. Evidence of health benefits of canola oil. Nutr. Rev. 71:370-385.

Mag, T. K. 1983. Canola oil processing in Canada. J. Am. Oil Chem. Soc. 60:380-384.

Mundt, C. C., Cowger, C., and Garrett, K. A. 2002. Relevance of integrated disease management to resistance durability. Euphytica 124:245-252.

Oladzad, A., Zitnick-Anderson, K., Jain, S., Simons, K., Osorno, J. M., McClean, P. E., and Pasche, J. 2019. Genotypes and genomic regions associated with *Rhizoctonia solani* resistance in common bean. Front. Plant Sci. 10:956-979.

Peltier, A. J., Bradley, C. A., Chilvers, M. I., Malvick, D. K., Mueller, D. S., Wise, K. A., and Esker, P. D. 2012. Biology, yield loss and control of Sclerotinia stem rot of soybean. J. Integr. Pest Manag. 3:1-7.

Qasim, M. U., Zhao, Q., Shahid, M., Samad, R. A., Ahmar, S., Wu, J., Fan, C., and Zhou, Y. 2020. Identification of QTLs containing resistance genes for Sclerotinia stem rot in *Brassica napus* using comparative transcriptomic studies. Front. Plant Sci. 11:776-889.

Rothmann, L. A., and McLaren, N. W. 2018. *Sclerotinia sclerotiorum* disease prediction: A review and potential applications in South Africa. S. Afr. J. Sci. 114:1-9.

Shahoveisi, F., and del Río Mendoza L. E. 2020. Effect of wetness duration and incubation temperature on development of ascosporic infections by *Sclerotinia sclerotiorum*. Plant Dis. 104:1817-1823.

Sharma, P., Verma, P. R., Meena, P. D., Kumar, V., and Singh, D. 2016. Research progress analysis of Sclerotinia rot (*Sclerotinia sclerotiorum*) of oilseed brassicas through bibliography. J. oilseed Brassica 1:45-125.

Taylor, A., Coventry, E., Jones, J. E., and Clarkson, J. P. 2015. Resistance to a highly aggressive isolate of *Sclerotinia sclerotiorum* in a *Brassica napus* diversity set. Plant Pathol. 64:932-940.

Uloth, M., You, M. P., Finnegan, P. M., Banga, S. S., Yi, H., and Barbetti, M. J. 2014. Seedling resistance to *Sclerotinia sclerotiorum* as expressed across diverse cruciferous species. Plant Dis. 98:184-190.

United States Department of Agriculture National Agricultural Statistics Service. 2019. Canola. https://www.nass.usda.gov/Data_and_Statistics/index.php.

Wei, D., Mei, J., Fu, Y., Disi, J. O., Li, J., and Qian, W. 2014. Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol. Breed. 34:1797-1804.

Workneh, F., and Yang, X. B. 2000. Prevalence of Sclerotinia stem rot of soybeans in the north-central United States in relation to tillage, climate, and latitudinal positions. Phytopathology 90:1375-1382.

Wu, J., Cai, G., Tu, J., Li, L., Liu, S., Luo, X., Zhou, L., Fan, C., and Zhou, Y. 2013. Identification of QTLs for resistance to Sclerotinia stem rot and BnaC. IGMT5. a as a candidate gene of the major resistant QTL SRC6 in *Brassica napus*. PLoS One 8:e67740.

Wu, J., Chen, P., Zhao, Q., Cai, G., Hu, Y., Xiang, Y., Yang, Q., Wang, Y., and Zhou, Y. 2019. Co-location of QTL for Sclerotinia stem rot resistance and flowering time in *Brassica napus*. Crop J. 7:227-237.

W, J., Zhao, Q., Liu, S., Shahid, M., Lan, L., Cai, G., Zhang, C., Fan, C., Wang, Y., and Zhou, Y. 2016. Genome-wide association study identifies new loci for resistance to Sclerotinia stem rot in *Brassica napus*. Front. Plant Sci. 7:1418-1430.

Yi, X., Yi, B., Chen, W., Zhang, W., Tu, J., Fernando, W. D., and Fu, T. 2010. Mapping of QTLs detected in a *Brassica napus* DH population for resistance to *Sclerotinia sclerotiorum* in multiple environments. Euphytica 173:25-35.

Yu F., Zhang, X., Peng, G., Falk, K. C., Strelkov, S. E., and Gossen, B. D. 2017. Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Sci. Rep. 7:1-11.

Zao, J., and Meng, J. 2003. Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 106:759-764.

Zho, J., Peltier, A. J., Meng, J., Osborn, T. C., and Grau, C. R. 2004. Evaluation of Sclerotinia stem rot resistance in oilseed *Brassica napus* using a petiole inoculation technique under greenhouse conditions. Plant Dis. 88:1033-1039.

Zhao, J., Udall, J. A., Quijada, P. A., Grau, C. R., Meng, J., and Osborn, T. C. 2006. Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. 112:509-516.

Zitnick-Anderson, K., Oladzadabbasabadi, A., Jain, S., Modderman, C., Osorno, J. M., McClean, P. E., and Pasche, J. S. 2020. Sources of resistance to *Fusarium solani* and associated genomic regions in common bean diversity panels. Front. Genet. 11:475-488.

CHAPTER 2. LITERATURE REVIEW

The Host: *Brassica napus*

Brassica napus is a member of the Brassicaceae family which contains 107 genera and total of 1208 accepted taxa (USDA NRSC). *B. napus* is the result of an interspecific hybridization between *B. rapa* L. *and B. oleracea* L. (Downey and Rimmer 1993; OECD 2012). Nagaharu (1935) proposed U triangle to describe the cytogenetic relationship between the species (Figure 2.1). The U triangle indicates three diploid species including *B. rapa* (AA genome, n=10), *B. nigra* (L.) W. D. J. Koch (BB genome, n=8), and *B. oleracea*e (CC genome, n=9) and three tetraploid species including *B. napus* (AACC genome, n=19), *B. carinata* A. Braun (BBCC genome, n=17), and *B. juncea* (L.) Czern. (AABB genome, n=18).

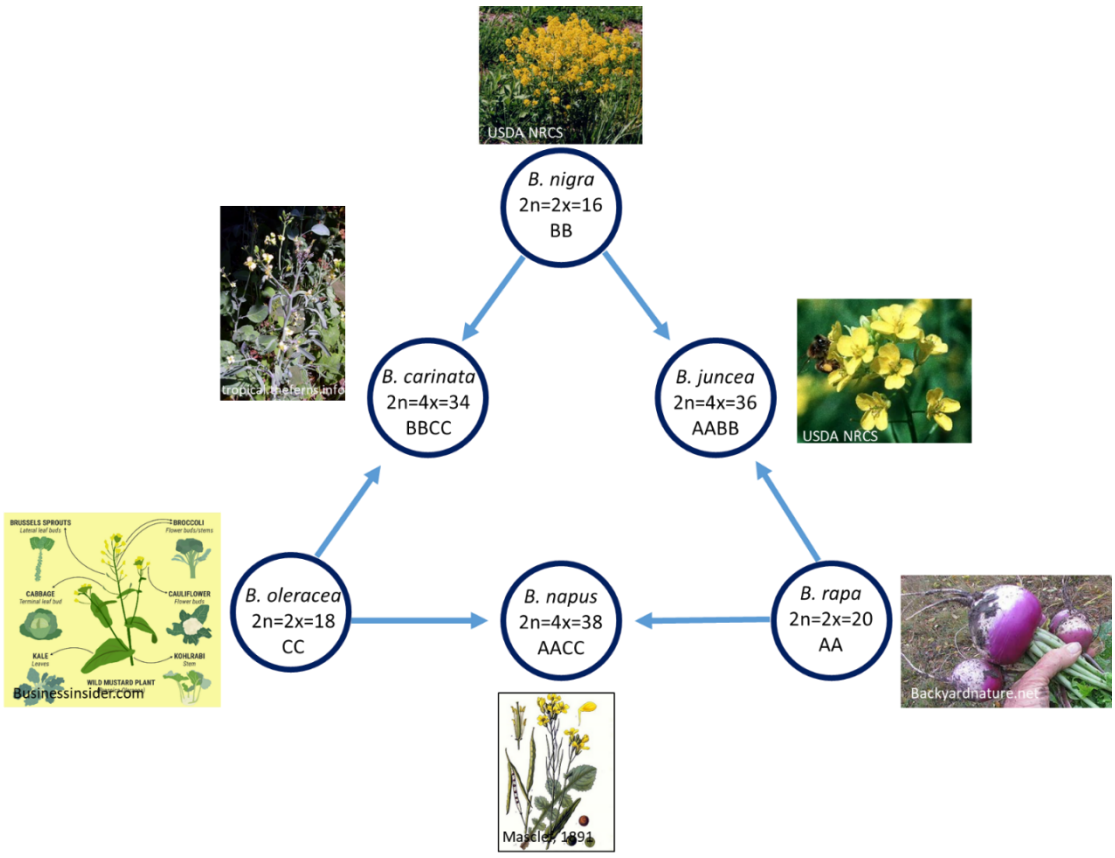


Figure 2.1. Cytogenetic relationship among *Brassica* spp. (Adapted from U 1935)

The origin of *B. napus* is debatable; while some believe that it could be traced back to the Mediterranean area and Northern Europe, others support the evidence of multiple centers of origin (Rakow 2004; OECD 2012; Lin et al. 2013). Similarly, for one of the parents, *B. rapa*, the suggested center of origin is the Mediterranean area from where its habitat extended to Scandinavia, Germany, Central Europe, and finally towards Asia (Mizushima and Tsunoda 1967). Several varieties and subspecies such as *B. rapa* subsp. *rapa* (turnip), *B. rapa* subsp. *chinensis* (L.) Hanelt (bok choy), *B. rapa* subsp. *pekinensis* (Lour.) Hanelt (napa cabbage), and *B. rapa* subsp. *chinensis* var. *parachinensis* L. H. Bailey (choy sum) have been defined in this species (Dixon 2006). The other parent, *B. oleracea*, was domesticated in ancient Greek-speaking area of Central and East Mediterranean (Maggioni 2010). *B. oleracea* contains several cultivated varieties including cabbage, leafy kale, broccoli, Brussels sprouts, cauliflower, and kohlrabi that are generally known as cole crops (Haynes et al. 2009; Maggioni 2010).

History of rapeseed/ canola

Compared to *B. rapa* and *B. oleracea*e, the domestication history of *B. napus* is relatively short. Winter type rapeseed was documented about 400 years ago in Europe. One hundred years later, spring type was domesticated. The difference between two types is the need for a vernalization period in the winter crop (Gómez-Campo and Prakash 1999). According to Wei et al. (2017), Semi-winter rapeseed was widely grown in China after adaptation of the crop to the local environment. The spring type rapeseed was introduced into Canada and Australia in the 1960s and 1970s (Chen et al. 2008).

Rapeseed plants cultivated in North America had mainly industrial use due to a high level of erucic acid (>40%) in the oil until 1976 when conventional breeding used by Canadian researchers resulted in production of consumable rapeseed (Mag 1983; Dupont et al. 1989;

Busch et al. 1994; Wittenberger 2012). In 1979, Canada registered "canola" as a new oil seed crop for human and animal consumption. The term "canola" stands for "Canadian oil low acid" and by definition, it contains less than 2% erucic acid and less than 30 umol/g glucosinolates (including: 2-hydroxy- 4-pentenyl glucosinolate, 2-hydroxy-3butenyl glucosinolate, 3-butenyl glucosinolate, and 4-entenyl glucosinolate) (Mag 1983; Brown et al. 2008). Three species of Brassica including *B. napus* (Argentine variety), *B. juncea* (mustard), and *B. rapa* (polish variety) that are double low (low erucic acid and glucosinolate) are known as canola (Thomas 2003). However, *B. napus* and *B. rapa* are the most predominant species grown worldwide. Canola seed contains 35-45% of its weight in oil and its meal contains 38-43% protein along with essential amino acids such as methionine, lysine, threonine, cysteine, and tryptophan (Larsen and Sorensen 1985; Snowdon et al. 2007). In this dissertation, *B. napus* plant introductions, and mapping populations lines derived from their crosses with modern canola breeding lines are referred to as canola instead of rape even though they may not meet the definition coined by Canadian researchers.

Canola production and importance

Most rapeseed oil is produced from *B. napus* and *B. rapa* while *B. juncea* and *Sinapis alba* L. contribute to the total production to a lesser extent (Raymer 2002). Rapeseed production in 2020 was 68.1 million metric tons which placed this crop as the second major oilseed crop after soybean in the world; this amount represents 11% of the world's total oilseed crop production. Rapeseed was the second leading crop after soybean in protein meals production and third place in vegetable oil ranking after palm and soybean. Increasing demand for rapeseed products has resulted in a significant growth of production from 49.2 million metric tons in 2007 to 68.1 million metric tons in 2020. Based on the most recent statistics by FAOSTAT, the top

rapeseed producer countries during 1994-2018 have been China, Canada, and India (Figure 2.2). The United States of America was ranked as the 11th rapeseed producer in 2018 (FAOSTAT 2020).

The US canola production in 2019 was estimated at 3.4 billion pounds which was approximately six percent less than the total production in 2018. The average yield for the former year was 1,781 pounds per acre, which was 80 pounds lower than the average yield of 2018. Similarly, the total value of the crop had a slight decrease in 2019 at $494 million, compared to 2018 which was $569 million. North Dakota is the leading canola producer nationwide and 85% of the total US canola is produced in this state. Other canola producing states are Idaho, Kansas, Minnesota, Montana, North Carolina, Oklahoma, Oregon, and Washington; with southern states producing winter type canola (USDA NASS 2019).

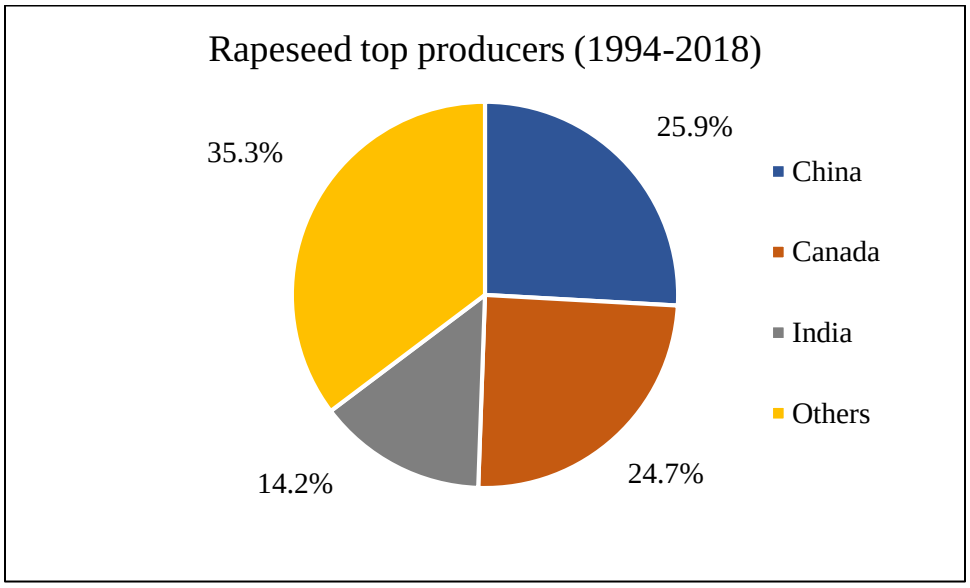


Figure 2.2. Top rapeseed producer countries from 1994 to 2018 (Adapted from FAOSTAT 2020).

B. napus genome

Natural hybridization of the A subgenome from *B. rapa* (314.2 Mb) and the C subgenome from *B. oleraceae* (525.8 Mb) and the following chromosome duplication around

7500 years ago resulted in the formation of *B. napus*. The total genome length of *B. napus* is estimated at 1130 Mb with 101,040 genes where the assembled A subgenome (314.2 Mb) is smaller than the C subgenome (525.8 Mb). A and C subgenomes of *B. napus* are mainly colinear to their diploid A and C genomes and 93% of the diploid genes could be found in orthologous blocks; 34,255 and 38,661 orthologous gene pairs were detected in A and C subgenomes, respectively. Different genes, such as lipid biosynthesis, glucosinolates catabolism, flowering time (FLOWERING LOCUS C), and nucleotide binding site leucine-rich repeat (NBS-LRR) sequences that are involved in various growth and defense mechanisms, have been identified in *B. napus* (Chalhoub et al. 2014).

Important diseases

Canola could be affected by several pathogens including *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Leptosphaeria maculans* (blackleg), *Plasmodiophora brassicae* (clubroot), *Fusarium oxysporum* f.sp. *conglutinans* (Wollenw.) Snyder & Hansen (Fusarium wilt), *Hyaloperonospora parasitica* (Pers.) Constant (downy mildew), *Alternaria brassicae* (Berk.) Sacc. and *A. japonica* Yoshii (Alternaria black spot), and *Candidatus phytoplasma asteris* Lee et al., Gundersen-Rindal, Davis, Bottner, Marcone & Seemüller (Aster yellows). Since this dissertation focuses on Sclerotinia stem rot, the remainder of this literature review also will be focused on this disease and its causal agent.

Sclerotinia stem rot is endemic to canola-producing areas of the state. Although significant yield losses have been reported from all canola-growing regions of North Dakota, SSR is commonly more prevalent and severe in the northcentral and northeastern regions of North Dakota. Incidence of the disease varies depending on the environmental conditions during flowering (Shahoveisi et al. 2020).

The Pathogen: *Sclerotinia sclerotiorum*

S. sclerotiorum is a soil-borne, omnivorous plant pathogen from ascomycetes class that can invade more than 500 plant species including 278 genera from 75 families; these hosts are primarily from dicotyledonous plants and a small number of monocotyledonous species including tulip and onion (Purdy 1979; Boland and Hall 1994; Saharan and Mehta 2008, Sharma et al. 2016a).

Taxonomy and nomenclature

S. sclerotiorum is one of the species in Sclerotiniaceae family, Helotiales order, and Ascomycota division. All species of this family produce inoperculate asci on stipitate apothecia that is formed on sclerotia within or on the host (Whetzel 1945; Holst-Jensen et al. 1997). Sclerotia production is one of the conserved features of the Sclerotiniaceae while the anamorphic characteristics of the genera are highly diverse (Kohn 1979).

Purdy et al. (1979) reviewed the history of taxonomic classification of *S. sclerotiorum* and indicated that the first description of *S. sclerotiorum* was recorded in 1837 as *Peziza sclerotiorum*. Later in 1870, the species was transferred to a newly described genus named Sclerotinia and the Latin binomial was changed to *S. libertiana* Fuckel (Purdy 1979). The species name was widely accepted until 1924 when Wakefield indicated that the name does not meet the criteria of International Code of Botanical Nomenclature (the species name should remain the same when a species is transferred to a new genus). Purdy (1979) showed that de Bary used the name "*S. sclerotiorum*" for the first time in 1884 and hence, the name and authority of the species was changed to *S. sclerotiorum* (Lib.) de Bary.

Symptoms and signs

S. sclerotiorum can infect all above ground parts of the plant, including flower petals, leaves, petioles, stems, and pods. Initial infection takes place during the flowering. Typically, pathogen spores first colonize flower petals and begin to produce mycelium. The mycelium then quickly begins to invade healthy green tissues; the first visible symptom is soft brown-white lesions formed on leaves and petioles that might be covered with decaying flower petals (Shahoveisi et al. 2020). Once established, the lesions grow in and on the plant. Lesions that reach the main stem are most damaging and as they develop, turn to a bleached or white-grayish color. Infected areas of the stem become brittle and their epidermis starts to shred. Under severe disease conditions, stem girdling resulting in wilting and lodging will occur. Light greyish to white fungal growth may appear on the lesions during periods of high humidity, which is why the disease is commonly called 'white mold'. At the end of the season, sclerotia are formed in or on the stem where lesions exist. Sclerotia serve as survival structures and can persist for more than four years in the soil (Lumsden and Dew 1973; Willetts and Wong 1980).

Disease cycle

The pathogen survives in the soil as sclerotia. While many sclerotia may be incorporated into the soil during tillage, only sclerotia in the top two inches (2.5 cm) will germinate. Continuous soil moisture for a minimum period of ten days, accompanied by soil temperatures of 60- 77° F (15-25 °C) are required for germination. Sclerotia can germinate myceliogenically or carpogenically which is determined by environmental conditions. Myceliogenic germination results in the production of hyphae that can infect plant tissues directly (Bardin and Huang 2001; Le Tourneau 1979). Carpogenic germination, on the other hand, is the process of apothecia (sexual fruiting bodies) and ascospore (sexual spores) production. Ascospores are capable of

infecting above ground parts of the host specially senescing and degrading tissues (Jamaux et al. 1995; Bardin and Huang 2001). Most infections caused by *S. sclerotiorum* are established following carpogenic germination (Abawi and Grogan 1979; Schwartz and Steadman 1978; Steadman 1979). Ascospores produced by apothecia are the primary inoculum for the disease; these spores can be carried by wind for long distances; however, most ascospores are deposited within 10-40 meters from the source depending on the crop (Wegulo et al. 2000; Qandah and del Rio Mendoza 2012). Ascospores are able to germinate on the healthy tissues such as leaves and stems; however, they need an exogenous nutrient source, such as senescing flower petals, and free water to initiate the infection (Abawi and Grogan 1979; Lumsden 1979; McLean 1958). The infection process begins when ascospores land on flower petals and subsequently germinate and colonize the tissue (Turkington and Morrall 1993). From flower petals, the fungus grows into leaves, leaf axils, branches, and stems. Once the pathogen penetrates the plant, it will colonize and feed on the plant tissues. Sclerotia will be formed inside or on the stem once the nutritional value of infected plant tissues has been exhausted or if environmental conditions become unfavorable for the pathogen (Figure 2.3).

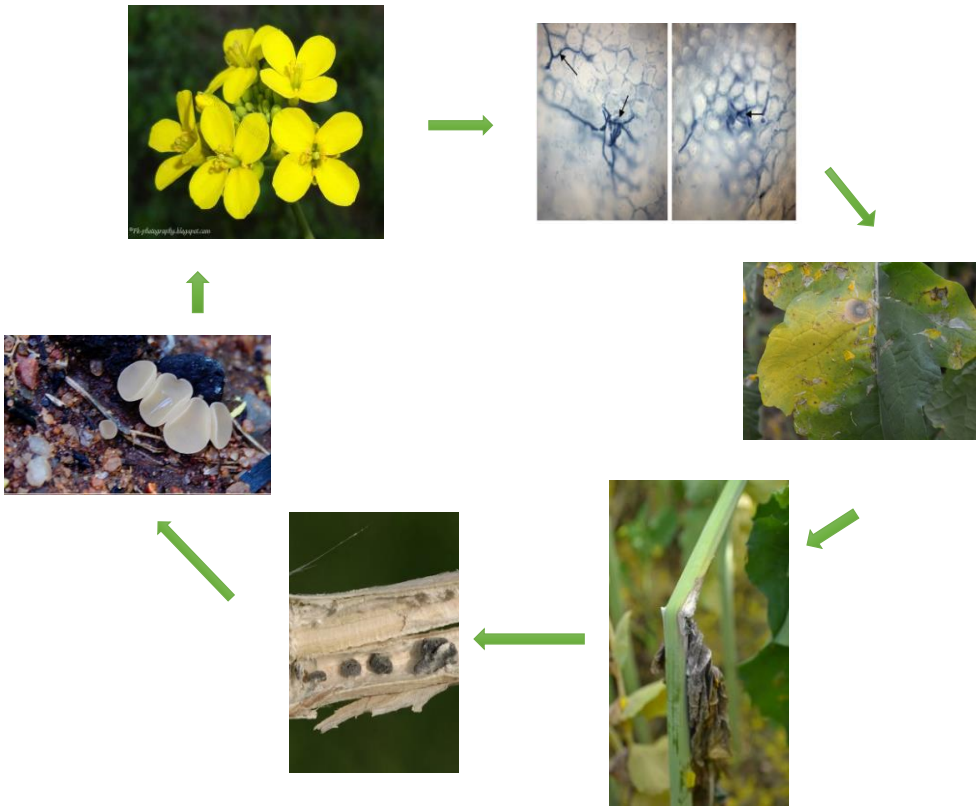


Figure 2.3. Disease cycle of Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (photo credits: Fernando et al. 2007; The Department of Primary Industries and Regional Development (DPIRD) (https://www.agric.wa.gov.au/); and Canola Watch (https://www.canolawatch.org/).

Management strategies

Management of diseases caused by *S. sclerotiorum* is challenging. Sclerotinia stem rot is one of the few diseases where scouting a field for the presence of disease is minimally beneficial because of the monocyclic nature of the disease. The longevity of sclerotia in the soil and the wide host range of the pathogen limit the efficiency of crop rotations, as well. However, planting canola or other susceptible crops (sunflower, dry bean, soybean, and pulse crops) no more than every two to three years is recommended. Small grains and corn are ideal rotational crops (Rousseau et al. 2007). Effectiveness of a few mycoparasites, such as *Sporidesmium sclerotivorum* and *Coniothyrium minitans* in degrading sclerotia has been proven (del Rio et al.

2002; Huang et al. 2000; Bolton et al. 2006); however, biological control of Sclerotinia diseases is known as a complementary method to other strategies.

High levels of resistance to Sclerotinia diseases are not available in major crops and most of the breeding programs rely on plant materials with partial resistance. Several genetic studies have been conducted to identify molecular markers associated to SSR resistance in different crops including canola (Zhao and Meng 2003; Zhao et al. 2006; Yin et al. 2010; Wei et al. 2014; Derbyshire and Denton-Giles 2016; Wu et al. 2013; Wu et al. 2019; Qasim et al. 2020). These genetic loci could be used in future breeding programs to integrate resistance into breeding lines.

Lack of complete host resistance has resulted in considering fungicides as the primary method of the disease management (Bolton et al. 2006). When conditions are favorable for disease, foliar fungicide application can reduce the severity of SSR and prevent significant yield losses (Spitzer et al. 2017). Timing of fungicide applications are very important. Foliar fungicides are recommended when 20 to 50% of the flowers are open. Canola typically reaches 10% flowering two to four days after the first flower appears on the main stem. From this point, we can expect another 10% increase in blooming every one or two days. The crop typically reaches the 20% bloom stage when 14 to 16 flowers are open on the main stem. At 30% bloom, petals from older flowers start to be casted off. This stage is typically reached between six and ten days from the day the first flowers opened. At 50% bloom, the field has its highest yellow intensity. The color starts to fade when flowering reaches 60%. Typically, fungicide applications made after the crop reaches the full bloom do not produce positive economic returns (Shahoveisi et al. 2020).

Overall, integrated pest management strategies that include crop rotation, use of resistant cultivars, and fungicide application are required to reduce the disease incidence and potential yield losses in presence of *S. sclerotiorum*.

Disease epidemiology and forecasting models

Disease caused by *S. sclerotiorum* are monocyclic meaning only one infection cycle during crop cycle is produced. Development of ascosporic epidemics caused by *S. sclerotiorum* occurs in three stages: apothecia formation, ascospore production and dispersal, and lesion development. Some of the factors that affect the likelihood of completion of each stage are displayed in Figure 2.4.

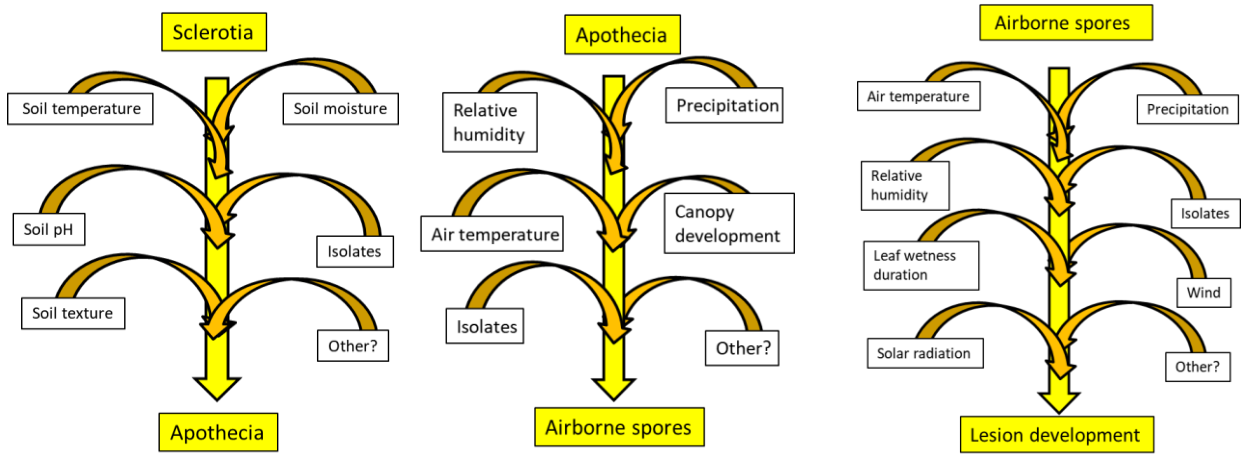


Figure 2.4. Three stages of Sclerotinia stem rot infections from the carpogenic germination of the sclerotia and factors affecting the development of each stage (figure credit: Luis del Río Mendoza).

Since a part of this dissertation focuses on development of a model for the latter stage, this section provides a review of what is known from each stage. The first stage is the production of apothecia from sclerotia. The apothecium is formed at or near the soil surface while specific environmental conditions are required. Soil temperature and moisture are the two most important factors for the carpogenic germination of sclerotia (Morrall 1977; Huang and Kozub 1989; Dillard et al. 1995; Sun and Yang 2000; Hao et al. 2003; Wu and Subbarao 2008). Soil moistures

above 30% are favorable for apothecia formation (Clarkson et al. 2004; Saharan and Mehta 2008). In another study conducted by Nepal and del Rio (2012), the relationship between moisture and carpogenic germination of seven isolates of *S. sclerotiorum* was studied. Results of the study indicated that sclerotial moisture content has a significant impact ($P = 0.05$) on the germination where maximum and no germinations were observed on fully saturated and below 70 to 80% of saturation, respectively. Optimum temperature for sclerotial germination varies depending on other environmental factors. Sun and Yang (2000) investigated the role of temperature, moisture, and light intensity on sclerotia germination. Results of the study indicated that optimal temperature for germination was different based on light intensity and the moisture level in the sand. At a low light intensity (80 to 90 mol m^{-2} s^{-1}), the optimal temperature range was 12 to 18 °C regardless of moisture; however, at high light intensity of 120 to 130 mol m^{-2} s^{-1}, the optimal temperature was increased to 20 °C when the soil moisture level was high. Further, apothecia were smaller at low light intensity compared to those produced at high light intensity regardless of temperature. In this study, the relationship between apothecia formation and degree days was modeled. Nonlinear regression equations were highly significant between the variables (Sun and Yang 2000). In another study, conducted by Clarkson et al. (2004), a prediction model for carpogenic germination of sclerotia was developed. In that study, different temperature regimes, isolates, and soil water potentials were used to model sclerotia germination. Results showed that carpogenic germination of sclerotia can occurred between 5 and 25 °C if the soil water potential was > -100 kPa. No apothecia production was observed at temperatures above 26 °C.

Conditioning is one of the requirements for carpogenic germination of sclerotia. In temperate climates, a combination of low temperatures, and wetting and drying periods are

required for sclerotia germination (Bardin and Huang 2001). Isolates from cool geographical regions (10 °C) can germinate easier than those originating from higher temperatures (25-30 °C); therefore, geographical origin of isolates has an important role of the first stage of the disease development (Huang and Kozub 1991).

The role of other environmental factors such as soil pH and type on germination of sclerotia has been investigated in other species of Sclerotinia, such as *S. minor* and *S. rolfsii* but not in *S. sclerotiorum.* Shim and Starr (1997) demonstrated that germination of *S. rolfsii* sclerotia was greater in acidic soils. Another factor that may affect apothecia production is soil texture. A study conducted by Hao et al. (2003) compared the effect of two soil types a sandy loam and a silty clay loam on carpogenic germination of *S. sclerotiorum* and mycelial germination of *S. minor*. Their results showed that soil type does not have a significant role on germination of sclerotia in these species.

The second stage of disease development is production of ascospores from apothecia. Concentration of released ascospores is positively correlated with infection and it could be used as a predictor in development of disease prediction models (McCartney and Lacey 1999; Twengström et al. 1998). Ascospore maturation is a complex process which is influenced by several factors including precipitation, relative humidity, and air temperature; other factors such as canopy development and fungal isolate could be determinant, as well (Figure 2.4). Studies in this area have shown that temperature is the most significant factor affecting asci maturation within the apothecia where the optimum temperature is 21 °C and that aggregated volatile substances and light do not affect (Wu et al. 2007). Ascospore release is also an important factor in the disease development. Clarkson et al. (2003) investigated the role of multiple environmental factors including temperature, light, and relative humidity on ascospore release

and survival. The results indicated that light and dark treatments, and relative humidity (90-95% or 65-75%) do not hamper the ascospore discharge; however, temperature has a significant effect on the ascospore release; in this study, ascospores were continuously discharged when incubated in saturated air (90–95% relative humidity) at 15 °C. Moreover, a sudden drop in atmospheric pressure or humidity is required for ascospore discharge where each apothecium can release approximately 2×10^6 ascospores over a period of 72-84 hours (Clarkson et al. 2003; Wu et al. 2007; Smolińska and Kowalska 2018). Apothecia discharge ascospores during the day; however, discharge peaks between 9 am and 1 pm (Bourdot et al. 2001; Qandah and del Río Mendoza 2011). Under dry conditions, however, the peaks of discharge occur much earlier, e.g. 2- 7 am (Qandah and del Río Mendoza 2011).

The third stage of the disease development, from airborne ascospores to lesion development, includes different steps such as ascospore survival, dispersal, and germination, lesion establishment, and expansion of the lesion to the main stem. This stage is affected by environmental factors including air temperature, precipitation, relative humidity, leaf wetness duration, wind, solar radiation, and fungal isolates (Figure 2.4).

High temperature and relative humidity regimes are detrimental to ascospore survival. Studies have shown that ascospore survival at temperatures below 15 °C is consistently high irrespective of the relative humidity. However, at temperatures above 15 °C and high humidity, ascospore survival was lower (Caesar and Pearson 1983; Clarkson et al. 2003). Reported ascospore survival length varies in different studies e.g. 2-8 days survival at 25-30 °C and humidity >60% compared to 2-3 weeks survival at 30 °C; Clarkson et al. (2007) explained that the origin of isolates could be the potential reason of this discrepancy. Another important factor affecting ascospore survival is canopy development. In a study conducted by Caesar and Pearson

(1983), it was shown that ascospore survival was 21.5% higher in dense canopies, approximately 12-15 cm above the ground, compared to upper portion's leaves. Further, ascospore survival is also affected by solar radiation and UV light. Caesar and Pearson (1983) showed that using plastic shelters that protect the plants from UV light significantly reduce ascospore mortality. While the effect of environmental factors on ascospore survival is adequately studied, less work has been done on development of stochastic models that explain this association. Clarkson et al. (2003) unsuccessfully attempted to model the relationship between ascospore survival, temperature, and humidity; and attributed their failure to inadequate levels of spore mortality in some of the temperature and humidity regimes used in the experiment.

Most ascospores land on the plants close to the source of production which is normally within the same field (Wegulo et al. 2000; Qandah and del Río Mendoza 2012); however, wind can carry some of the spore for long distances (Li et al. 1994). Ascospore dispersal is also affected by several environmental factors such as humidity and temperature. Studies conducted by Qandah and del Río Mendoza (2011) demonstrated that daytime and nighttime discharges initiated when relative humidity was greater than 90% and air temperature was 15-16 °C. Ascospore dispersal pattern can be used to predict the disease incidence. Qandah and del Río Mendoza (2012) studied ascospores dispersal in canola fields. They found that inoculum gradients and the disease incidence decreased with greater distances from the inoculum source. Inoculum gradients declined 30-75% within 40 meters from the source while the incidence dropped by 50% within 12-17 meter from the source. Lambert's model (pseudo-$R^2 \geq 0.98$) and a negative exponential model ($R^2 \geq 0.81$) provided the most accurate description of the disease gradients.

Sticky mucilage on the ascospores facilitate their adherence to the surface where they can survive up to two weeks under favorable weather conditions (Caesar and Pearson 1983; Clarkson et al. 2003). While ascospores are capable of germinating on all plant parts, they cannot penetrate healthy tissues directly and require senescent tissues as sources of nutrient to produce hyphae that could penetrate and initiate infection (Abawi and Grogan 1979; Lumsden 1979; McLean 1958). Leaf wetness and temperature are the two most significant factors affecting spore germination (Abawi and Grogan 1979; Tu 1989; Clarkson et al. 2014); where free water or relative humidity ≥ 98% is required and the optimum temperature is ranged between 15-25 °C (Young et al. 2004; Clarkson et al. 2014; Shahoveisi and del Río Mendoza 2020). At optimum temperatures, it only takes 2-4 hours for the ascospores to germinate in presence of free water while this period extends to 8-10 hours at 5-10 °C (Young et al. 2004). Different results have been reported about the ability of ascospores to germinate at 30 °C. While some studies have shown that no germination occurs at this temperature (Young et al. 2004; Clarkson et al. 2014), preliminary reports by other researchers suggests *S. sclerotiorum* is capable to germinate and infect plants at this temperature (Shahoveisi and del Río Mendoza 2019, 2020). Due to this conflicting results, additional research is needed in this subject. Further, alternating wet and dry periods for any length of time, if occurs after two hours of incubation, prevents germination of spores (Shahoveisi and del Río Mendoza 2020).

S. sclerotiorum hosts are mostly susceptible to the pathogen during the flowering period because senescent flower parts that fall on to the leaves, petioles or stems are the primary nutrient source for ascospores (Inglis and Boland 1990; Turkington and Morrall 1993). At flowering time, the canopy is fully developed and closed which provides favorable environmental conditions for the pathogen growth (Bolton et al. 2006). Fewer studies on

Sclerotinia disease establishment and its required environmental conditions have been conducted compared to the next step which is disease development on the stem. Koch et al. (2007) observed that presence of ascospore inoculated petals on the leaf axil at temperatures of 16-22 °C and saturated humidity results in 100% disease incidence while this value decreased to 23% in absence of the flower petals. While this study provides valuable information, two important factors have not been addressed: a) ascospore suspension was used for inoculation in this experiment which facilitates ascospore germination and disease establishment while using dry ascospore inoculated can mimic the natural infection in the field; b) leaf wetness duration is one of the determinant factors for the disease establishment on the leaf and axils. These factors have been included in a recent study that is a part of this dissertation.

The final step of the infection is development of the lesion to the main stem. The majority of epidemiological studies and developed prediction models are focused on this step where impact of several factors including petal infestation rate, weather variables, and history of the disease and crop in the field on disease incidence and severity is evaluated.

Petal infestation could be used as a predictor for SSR. Early observations suggested that as the rate of infested petal increased so did disease incidence (Morrall et al. 1990). However, the accuracy of models based on petal infestation is highly affected by disease pressure; accurate predictions are expected only under low levels of infestation and the model becomes inaccurate at moderate to high levels of infections (Turkington et al. 1991). Therefore, other environmental factors are required for the disease prediction. Turkington and Morrall (1993) investigated the role of petal infestation, precipitation, number of plants per square meter, crop density and height, leaf area index, and light penetration in canopy in SSR incidence. Using multiple regression analyses, these variables explained 55-98% of the disease incidence variation. More

recently, Ficke et al. (2018) investigated the importance of precipitation, and petal and leaf infections in SSR incidence. Interestingly, results of this work showed that accumulated precipitation before and during flowering was not a good predictor of the disease development in the field trials ($P = 0.24$) while total leaf and petal infestations could explain 45-57% of variation of the disease incidence. These findings verified the necessity of using other weather factors, besides precipitation, to improve the prediction accuracy (Ficke et al. 2018).

Using multiple weather variables as predictors results in development of accurate disease estimations. Twengström et al. (1998) generated a risk point table-forecasting model for rapeseed using field specific data including disease incidence in last susceptible crop, last rapeseed planting time, crop density and weather variables such as precipitation and weather forecast (Twengström et al. 1998). The model showed a high accuracy where the spraying recommendations were accurate in 75% of the fields that required applications and 16% of the fields that did not need fungicide spray. In another study conducted on spring and winter canola in Canada, effect of soil moisture, rainfall, plant height, canopy density, petal infection, and growth stage on SSR incidence before and during flowering periods was investigated. No strong correlation between the predictors and response variables were identified using linear and non-linear regression analyses while classification of disease severity, to low (<20%) and high (>20%), resulted in significant association between soil moisture, rainfall, crop height, the amount of apothecia, and percentage of petal infestation and SSR severity within and between years (Bom and Boland 2000). More recently, a higher number of weather factors were used in model development (Mila et al. 2004; Koch et al. 2007; Harikrishnan and del Río 2008; Clarkson et al. 2014). Koch et al. (2007) generated a forecasting model for oilseed rape in Germany where a crop loss-related model, SkleroPro, was developed using relative humidity, air temperature,

rainfall, and sunlight duration. Results of this experiment showed that 80 to 86% relative humidity and temperatures of 7 to 11 °C are the minimum requirements for the infection of stems; these predictors were expressed as an index to differentiate infection hours (Inh). Inh and disease incidence were significantly correlated ($P \leq 0.001$ and $r=0.42$) when Inh occurred at late bud stage. Using cumulative Inh values from 23 hours after the late bud stage increased the correlation with SSR incidence significantly. This model could correctly predict the requirement for fungicide application in 81% of the assessed fields. Koch et al. (2007) concluded that when high level of inoculum is present in rapeseed fields, SSR development could be accurately predicted using conditions of stem infection during flowering periods where information about apothecial development and ascospore dispersal is not required (Koch et al. 2007).

Disease resistance

Planting genetically resistant cultivars is an effective, environmentally friendly, and cost-effective method for SSR management. However, the complex quantitative host resistance in most of *S. sclerotiorum*'s hosts including canola, common bean, soybean, sunflower, and lettuce (Micic et al. 2004; Rönicke et al. 2005; Patzoldt et al. 2005; Zhao et al. 2006; Mei et al. 2013; Wu et al. 2013, 2019; McCaghey et al. 2017; Vasconcellos et al. 2017; Mamo et al. 2019; Qasim et al. 2020) and lacking host specificity in *S. sclerotiorum* make the breeding studies challenging (Sharma et al. 2016b). Partially resistant *B. napus*, *B. juncea*, and wild Brassica species have been identified in several studies (Li et al. 2006, 2009; Zhao et al. 2004; Khot et al. 2011; Mei et al. 2012; Denton-Giles et al. 2018). Some of these plant materials have been used in complementary mapping studies to identify genetic regions and quantitative trait loci (QTL) associated with resistance (Zhao et al. 2006; Mei et al. 2013).

Identification of sources of resistance

Screening Brassica germplasms to identify SSR resistant materials is the preliminary step required before genetic mapping studies which are used to identify molecular markers associated with the trait. Several studies have been conducted to identify resistant materials among Brassica species (Bradley et al. 2006; Garg et al. 2010; Khot et al. 2011; Mei et al. 2012; Uloth et al. 2014; Taylor et al. 2015). In field trials conducted in North Dakota between 2001-2004, reaction of canola cultivars to SSR was assessed using ascospore inoculation. The experiments also were conducted under controlled environments using detached leaf assay, petiole inoculation technique, and oxalic acid assay. While results of lab and greenhouse studies were consistent and significant differences ($P \leq 0.05$) were detected among cultivars for SSR, resistance responses from field trials were inconsistent among years and locations (Bradley et al. 2006). In another study, conducted by Khot et al. (2011), the reaction of 447 *B. napus* PI lines to SSR was assessed under greenhouse and field conditions. Eight PI lines were identified as having the highest level of resistance. Although five of them were not significantly different from the resistant check Hyola 357 Magnum. Mei et al. (2012) evaluated 17 Brassica spp. including three *B. rapa*, two *B. oleracea*, six *B. napus*, two *B. carinata*, and four *B. juncea* accessions using detached stem inoculation under controlled environments and toothpick method in the field. In general, *B. rapa*, and *B. oleracea* showed the most susceptible and most resistance reactions, respectively, while other species had intermediate resistance (Mei et al. 2012).

Identification of markers associated with disease resistance

The next step after identification of resistance sources is the construction of the mapping population. Different biparental segregating populations such as F2, recombinant inbred lines (RIL), double haploid (DH), near-isogenic (NIL), and backcross (BC) lines could be used in

QTL studies. DH populations are preferred due to several advantages such as proving replication, complete homozygosity, and rapid construction compared to RIL (Xu et al. 2017). Microspore embryogenesis is one the most efficient methods for DH production in Brassica species and several protocols have been developed for microspore culture and subsequent DH plant generation for U's triangle species (Custers 2003; da Silva Dias 2003; Ferrie 2003; Hansen 2003; Friedt and Zarhloul 2005; Rahman and de Jiménez 2016). Achieving extraordinary progress in microspore culture and DH plant production in Brassica species has facilitated genetic mapping studies, including QTL biparental mapping analysis, and breeding programs worldwide (Ferrie and Möllers 2011). Not all is rosy, however, as amenability for DH production seems genotype dependent (del Río Mendoza, personal communication, Sept 2020).

Molecular markers and QTL mapping analysis have been extensively used for identification of genomic loci associated to SSR resistance in *B. napus* (Zhao and Meng 2003; Zaho et al. 2006; Yin et al. 2010; Wu et al. 2013; Wei et al. 2014; Derbyshire and Denton-Giles 2016; Wu et al. 2019; Qasim et al. 2020). These studies have identified QTL specific to different life stages of the host including seedling and adult plants (Zhao and Meng 2003; Wu et al. 2013). However, QTL identified in mature plants provide more robust information for breeding programs. Yin et al. (2010) studied adult plants of a DH mapping population and a range of one to 10 QTL, depending on the inoculation techniques, was identified in this study. These QTL could explain 10.2 to 36.1% of the phenotypic variation detected in the population. In another study conducted by Wei et al. (2014), 261 DH plants were used for QTL analysis at flowering stage. The result of this work was the identification of five and six QTL under controlled and filed conditions, respectively. These genomic regions were located on chromosomes A02, A03, A09, C01, and C02 and were responsible for 4.65 to 17.43% of the variation in the trait. In a

more recent study, the relationship between SSR resistance and flowering time in DH and RIL populations was investigated. Results showed that SSR resistance and flowering time are negatively correlated and four genomic regions on A02, C02, A06, and C08 chromosomes were involved in both traits (Wu et al. 2019). Similarly, Qasim et al. (2020) reported the indirect association (correlation coefficient of -0.53) between response to SSR and flowering time where early flowering plants were more susceptible to the disease. A DH population with 181 individuals was used for this QTL analysis; 17 QTL associated with SSR response on chromosomes A02, A09, C02, C03, C04, and C06 were identified over three years of field trials. These QTL had a R^2 (percentage of the phenotypic variations explained by the QTL) of 5.94-14.75%. These extensive genetic mapping studies have resulted in identification of numerous QTL associated with reaction to SSR in *B. napus*; however, there are no reports of integration of these genetic loci into breeding lines or their application in marker assisted selection process. Potential reasons for this gap are: a) quantitative nature of the host resistance which makes transferring the genetic loci to the breeding lines difficult, and b) less priority of breeding for disease resistant lines compared to other agronomic traits such as yield, oil content, abiotic stress resistance, and seed shattering in canola.

Another approach to identify genomic regions associated with resistance to SSR is the use of genome-wide association study (GWAS) where genetically diverse individuals are used as the mapping population. While GWAS benefit from a wide genetic diversity in natural populations and high level of recombination events and they have been used in canola-*S. sclerotiorum* pathosystems (Wei et al. 2016; Wu et al. 2016), it is considered complementary to QTL biparental analysis where they are useful in identification of resistant and susceptible parents required for biparental studies (Korte and Farlow 2013).

Disease severity measurement

One of the essential components of a successful QTL mapping study is using an accurate phenotyping dataset. Phenotyping data is highly affected by the inoculation method, the plant organ that is used for the inoculation, and the severity measurement method. Excised leaf and stem, petiole, and stem are the most common parts of the canola plants used for inoculation with *S. sclerotiorum* (Derbyshire and Denton-Giles 2016). Using stem inoculation provides the most reliable results since stem infection is the final step of the disease development and plants showing small stem lesion size contain some level of resistance. In terms of inoculation techniques, agar plug to the stem, and colonized toothpick are the most frequently used methods for QTL analysis (Arahana et al. 2001; Zhao and Meng 2003; Zhao et al. 2006; Garg et al. 2010; Wu et al. 2013, 2019; Qasim et al. 2020). The advantage of agar plug inoculation is that the inoculum is deposited on the surface of the plant while in toothpick method, pathogen is placed inside the host and it basically escapes one of the basal resistance layers. Dry ascospore inoculated petals could also be used for inoculation of plants. While this technique resembles the natural infection in the field, its application on large mapping populations, however, is challenging and therefore it is not commonly used.

Measuring average lesion size and days to wilt (mortality) are the most common methods of disease severity measurement in QTL mapping studies of canola-*S. sclerotiorum* pathosystem (Derbyshire and Denton-Giles 2016). While the area under the disease progress curve (AUDPC) has been used for disease screening and epidemiological studies in canola (Aghajani et al. 2010; Denton-Giles et al. 2018), its use in detection of QTL is rather limited (Guo et al. 2008; Gyawali et al. 2016). Similarly, the use of qualitative phenotyping scoring systems, e.g. binomial and polynomial datasets, have been successfully used in other pathosystems (Oladzad et al. 2019;

Zitnick-Anderson et al. 2020) where they increased power of detecting genomic regions associated with major resistance QTL. In these studies, the phenotypic response of the lines in each population was evaluated using severity scales containing up to nine categories but for the analysis, the number of categories were reduced to two or three depending on the breeder's perceived reaction to disease. This approach has not been used in QTL mapping studies of SSR in canola. Using extra disease quantification methods such as percentage of plant mortality, AUDPC or qualitative scoring systems in QTL studies could increase the power of studies and probability of identifying more genomic regions associated with the trait in *B. napus*.

Defense genes

Using QTL biparental and GWAS analyses, several genomic regions associated with response to *S. sclerotiorum* have been identified in canola (Zhao and Meng 2003; Zhao 2006; Yin et al. 2010; Wu et al. 2013; Wei et al. 2014, 2016; Derbyshire and Denton-Giles 2016; Wu et al. 2016, 2019; Qasim et al. 2020). However, the pattern of transcriptomic changes of the genes involved in defense responses to *S. sclerotiorum* in *B. napus* has been investigated to a lesser extent (Zhao et al. 2007; Zhao et al. 2009; Wei et al. 2016; Wu et al. 2016; Qasim et al. 2020). These studies have shown that differential gene expression could be observed in both resistant and susceptible cultivars; however, transcript levels of genes that encode chitinases, glucanases, osmotins and lectins, and transcription factors (zinc finger, WRKY, APETALA2 (AP2) and MYB classes) are higher and increase faster in resistant plants (Zhao et al. 2007; Zhao et al. 2009). In a qRT-PCR analysis conducted on two resistant and susceptible lines, cv. ZY821 and cv. Westar, four genes coding a lipoxygenase *BnLOX1* (BN22526), a legume lectin family protein *BnLectin* (BN25790), a WRKY-like protein *BnWRKY40* (BN23912), and a WRKY-like transcription factor *BnWRKY33* (BN17285) showed early increased transcription level in cv.

ZY821 compared to cv. Westar. These findings suggest that resistant plants respond more quickly to the pathogen (Zhao et al. 2009). Wei et al. (2016) demonstrated that glutathione and glucosinolates transcription levels increased in both resistant and susceptible genotypes upon interaction with the pathogen; however, genes associated to jasmonic acid pathway, lignin biosynthesis, signal transduction and encoding transcription factors, and defense response were activated only in the resistant line. Similarly, a study conducted by Wu et al. (2016) verified a higher expression magnitude of genes involved in pathogen recognition, WRKY transcription regulation, jasmonic acid/ethylene signaling pathways, MAPK signaling cascade, and biosynthesis of defense-related protein and indolic glucosinolate in the resistant plant. In a most recent study, the role of a few well-known resistance class genes in reaction to *S. sclerotiorum* in *B. napus* has been verified. A TIR-NBS-LRR gene (BnaC03g05380D) and two zinc-induced facilitator 1 genes (BnaC03g06010D and BnaC03g06020D), all located on chromosome C03, were differently expressed in the resistant genotype; while the former one was only expressed and the latter ones were highly upregulated in the resistant plant. Further, several other genes including a nitrilase 4 gene (BnaC02g09450D), an EXORDIUM-like 2 gene (BnaC02g48820D), an ATAF2 subfamily of NAC proteins (BnaC02g00990D), two UDP-glucosyl transferase genes (BnaA09g33440D and BnaA09g29790D) were highly upregulated at different time points in the resistant genotype (Qasim et al. 2020).

Overall, understanding epidemiology of SSR and genetic of resistance in *Brassica napus* provide insights into effective management strategies of the disease. The next chapters of this dissertation focus on the progress made in the epidemiology and host resistance of canola-*S. sclerotiorum* pathosystem.

References

Abawi, G. S., and Grogan, R. G. 1979. Epidemiology of diseases caused by Sclerotinia species. Phytopathology 69:899-904.

Aghajani, M. A., Safaei, N., and Alizadeh, A. 2010. Disease progress curves of Sclerotinia stem rot of canola epidemics in Golestan Province, Iran. J. Agr. Sci. Tech. 12: 471-478

Bardin, S. D., and Huang, H. C. 2001. Research on biology and control of Sclerotinia diseases in Canada. Can. J. Plant Pathol. 23:88-98.

Boland, G. J., and Hall, R. 1994. Index of plant hosts of Sclerotinia. Canadian J. Plant Pathol. 16: 93-108.

Bolton, M. D., Thomma, B. P., and Nelson, B. D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol. Plant Pathol. 7:1-16.

Bom, M., and Boland, G. J. 2000. Evaluation of disease forecasting variables for sclerotinia stem rot (Sclerotinia sclerotiorum) of canola. Can. J. Plant Sci. 80:889-898.

Bourdot, G. W., Hurrell, G. A., Saville, D. J., and De Jong, M. D. 2001. Risk analysis of *Sclerotinia sclerotiorum* for biological control of *Cirsium arvense* in pasture: ascospore dispersal. Biocontrol Sci. Technol. 11:119-139.

Bradley, C. A., Henson, R. A., Porter, P. M., LeGare, D. G., del Rio, L. E., and Khot, S. D. 2006. Response of canola cultivars to *Sclerotinia sclerotiorum* in controlled and field environments. Plant Dis. 90:215-219.

Brown, J., Davis, J. B., Lauver, M., and Wysocki, D. 2008. US canola association canola growers' manual. US Canola Association. http://www.uscanola.

Busch, L., Gunter, V., Mentele, T., Tachikawa, M., and Tanaka, K. 1994. Socializing nature: technoscience and the transformation of rapeseed into canola. Crop Sci. 34:607-614.

Caesar, A. J., and Pearson, R. C. 1983. Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. Phytopathology 73:1024-1030.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. A., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., and Corréa, M. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950-953.

Chen, S., Nelson, M. N., Ghamkhar, K., Fu, T. D., and Cowling, W. A. 2008. Divergent patterns of allelic diversity from similar origins: the case of oilseed rape (*Brassica napus* L.) in China and Australia. Genome 51:1-10.

Clarkson, J. P., Fawcett, L., Anthony, S. G., and Young, C. 2014. A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. PLoS One 9:e94049.

Clarkson, J. P., Phelps, K., Whipps, J. M., Young, C. S., Smith, J. A., and Watling, M. 2004. Forecasting Sclerotinia disease on lettuce: toward developing a prediction model for carpogenic germination of sclerotia. Phytopathology 94:268-279.

Clarkson, J. P., Staveley, J., Phelps, K., Young, C. S., and Whipps, J. M. 2003. Ascospores release and survival in *Sclerotinia sclerotiorum*. Mycol. Res. 107:213-222.

Custers, J. B. M. 2003. Microspore culture in rapeseed (*Brassica napus* L.). In: Maluszynski, M., Kasha K. J., Forster, B. P., Szarejko, I. (eds) Doubled haploid production in crop plants. Kluwer, Dordrecht, pp 185-193.

Da Silva Dias, J. C. 2003. Protocol for broccoli microspore culture. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds) Doubled haploid production in crop plants. Kluwer, Dordrecht, pp 195-204.

Del Rio, L. E., Martinson, C. A., and Yang, X. B. 2002. Biological control of Sclerotinia stem rot of soybean with *Sporidesmium sclerotivorum*. Plant Dis. 86:999-1004.

Denton-Giles, M., Derbyshire, M. C., Khentry, Y., Buchwaldt, L., and Kamphuis, L. G. 2018. Partial stem resistance in *Brassica napus* to highly aggressive and genetically diverse *Sclerotinia sclerotiorum* isolates from Australia. Can. J. Plant Pathol. 40:551-561.

Derbyshire, M. C., and Denton-Giles, M. 2016. The control of Sclerotinia stem rot on oilseed rape (*Brassica napus*): current practices and future opportunities. Plant Pathol. 65:859-877.

Dillard, H. R., Ludwig, J. W., and Hunter, J. E. 1995. Conditioning sclerotia of *Sclerotinia sclerotiorum* for carpogenic germination. Plant Dis. 79:411-415.

Dixon, G. R. 2006. Origins and diversity of Brassica and its relatives. Vegetable brassicas and related crucifers 1-33.

Downey, R. K., and Rimmer, S. R. 1993. Agronomic improvement in oil seed. Adv. Agron. 50:1-66.

Dupont, J., White, P. J., Johnston, K. M., Heggtveit, H. A., McDonald, B. E., Grundy, S. M., and Bonanome, A. 1989. Food safety and health effects of canola oil. J. Am. Coll. Nutr. 8:360-375.

Fernando, W. G. D., Nakkeeran, S., and Zhang, Y. 2004. Ecofriendly Methods in Combating *Sclerotinia Sclerotiorum* (Lib.) de Bary. Recent Res. Devel. Environ. Bio. 661:329-47.

Fernando, W. G. D., Nakkeeran, S., Zhang, Y., and Savchuk, S. 2007. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by Pseudomonas and Bacillus species on canola petals. Crop Prot. 26:100-107.

Ferrie, A. M. R. 2003. Microspore culture of Brassica species. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds) Doubled haploid production in crop plants. Kluwer, Dordrecht, pp 195-204.

Ferrie, A. M., and Möllers, C. 2011. Haploids and doubled haploids in Brassica spp. for genetic and genomic research. Plant Cell Tiss. Org. 104:375-386.

Ficke, A., Grieu, C., Brurberg, M. B., and Brodal, G. 2018. The role of precipitation, and petal and leaf infections in Sclerotinia stem rot of spring oilseed Brassica crops in Norway. Eur. J. Plant Pathol. 152:885-900.

Food and Agriculture Organization of the United Nations. 2020. FAOSTAT Database. http://www.fao.org/faostat/en/#data/QC/visualize.

Friedt, W., and Zarhloul, M. K. 2005. Haploids in the improvement of crucifers. In: Palmer, C. E., Keller, W. A., Kasha, K. J. (eds) Haploids in crop improvement II, biotechnology in agriculture and forestry, vol 56. pp 191-213.

Garg, H., Atri, C., Sandhu, P. S., Kaur, B., Renton, M., Banga, S. K., Singh, H., Singh, C., Barbetti, M. J., and Banga, S. S. 2010. High level of resistance to *Sclerotinia sclerotiorum* in introgression lines derived from hybridization between wild crucifers and the crop Brassica species *B. napus* and *B. juncea*. Field Crops Res. 117:51-58.

Gomez-Campo C., and Prakash, S. 1999. Origin and domestication. In: Gomez-Campo C. (ed.) Developments in Plant Genetics and Breeding. Elsevier, Amsterdam, pp 33-58.

Guo, X., Wang, D., Gordon, S. G., Helliwell, E., Smith, T., Berry, S. A., St. Martin, S. K., and Dorrance, A. E. 2008. Genetic mapping of QTLs underlying partial resistance to *Sclerotinia sclerotiorum* in soybean PI 391589A and PI 391589B. Crop Sci. 48:1129-1139.

Hansen, M. 2003. Protocol for microspore culture in Brassica. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds) Doubled haploid production in crop plants. Kluwer, Dordrecht, pp 217-222.

Hao, J. J., Subbarao, K. V., and Duniway, J. M. 2003. Germination of *Sclerotinia minor* and *S. sclerotiorum* sclerotia under various soil moisture and temperature combinations. Phytopathology 93:443-450.

Harikrishnan, R., and del Río, L. E. 2008. A logistic regression model for predicting risk of white mold incidence on dry bean in North Dakota. Plant Dis. 92:42-46.

Haynes, C., Everhart, E., Jauron, R., Nelson, D., and Lenahan, J. 2009. Cole Crops. Iowa State University Extension.

Holst-Jensen, A., Kohn, L. M., and Schumacher, T. 1997. Nuclear rDNA phylogeny of the Sclerotiniaceae. Mycologia 89:885-899.

Huang, H. C., and Kozub, G. C. 1991. Temperature requirements for carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* isolates of different geographic origin. Bot. Bull. Aca. Sin. 32:279-286.

Inglis, G. D., and Boland, G. J. 1990. The microflora of bean and rapeseed petals and the influence of the microflora of bean petals on white mold. Can. J. Plant Pathol. 12:129-134.

Jamaux, I., Gélie, B., and Lamarque, C. 1995. Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by scanning electron microscopy. Plant Pathol. 44:22-30.

Khot, S. D., Bilgi, V. N., del Río, L. E., and Bradley, C. A. 2011. Identification of *Brassica napus* lines with partial resistance to *Sclerotinia sclerotiorum*. Plant Health Prog. 12:15-24.

Koch, S., Dunker, S., Kleinhenz, B., Röhrig, M., and Tiedemann, A.V. 2007. A crop loss-related forecasting model for Sclerotinia stem rot in winter oilseed rape. Phytopathology 97:1186-1194.

Kohn, L. M. 1979. A monographic revision of the genus Sclerotinia. Mycotaxon 9:365-444.

Korte, A., and Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:1-9.

Larsen, L. M., and Sorensen, H. 1985. The value of oilseed rape production in Denmark and the EEC. Pages: 1-18 in: Advances in production and utilization of cruciferous crops; Sorensen. H., Ed., Martinus Nijhof/Dr. Junk Publishers, Denmark.

Le Tourneau, D. 1979. Morphology, cytology and physiology of Sclerotinia species in culture. Phytopathology 69:887-890.

Li, C. X., Li, H., Sivasithamparam, K., Fu, T. D., Li, Y. C., Liu, S. Y., and Barbetti, M. J. 2006. Expression of field resistance under Western Australian conditions to *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *Brassica juncea* germplasm and its relation with stem diameter. Aust. J. Agric. Res. 57:1131-1135.

Li, C. X., Liu, S. Y., Sivasithamparam, K., and Barbetti, M. J. 2009. New sources of resistance to Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *B. juncea* germplasm screened under Western Australian conditions. Australas. Plant Path. 38(2), pp.149-152.

Li, Y. B., Yongli, Z., and Nian, L. B. 1994. Study on the dissemination distance of sunflower stem rot fungus. Plant Prot. 20:12-13.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., and Jones, P. J. 2013. Evidence of health benefits of canola oil. Nutr. Rev. 71:370-385.

Lumsden, R. D. 1979. Histology and physiology of pathogenesis in plant diseases caused by Sclerotinia species. Phytopathology 69:890-896.

Lumsden, R. D., and Dew R. L. 1973. Histopathology of *Sclerotinia sclerotiorum* infection of bean. Phytopathology 63:708-715.

Mag, T. 1983. Canola oil processing in Canada. J. Am. Oil Chem. Soc. 60:380-384.

Maggioni, L., von Bothmer, R., Poulsen, G., and Branca, F. 2010. Origin and domestication of cole crops (*Brassica oleracea* L.): linguistic and literary considerations. Econ. Bot. 64:109-123.

Mamo, B. E., Hayes, R. J., Truco, M. J., Puri, K. D., Michelmore, R. W., Subbarao, K. V., and Simko, I. 2019. The genetics of resistance to lettuce drop (Sclerotinia spp.) in lettuce in a recombinant inbred line population from Reine des Glaces× Eruption. Theor. Appl. Genet. 132:2439-2460.

McCaghey, M., Willbur, J., Ranjan, A., Grau, C. R., Chapman, S., Diers, B., Groves, C., Kabbage, M., and Smith, D. L. 2017. Development and evaluation of Glycine max germplasm lines with quantitative resistance to *Sclerotinia sclerotiorum*. Front. Plant Sci. 8:1495-1508.

McCartney, H. A., and Lacey, M. E. 1999. Timing and infection of sunflowers by *Sclerotinia sclerotiorum* and disease development. Aspect. Appl. Biol. 56:151-156.

Mclean, D. M. 1958. Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) de Bary. Plant Dis. Report 42:663-666.

Mei, J., Ding, Y., Lu, K., Wei, D., Liu, Y., Disi, J. O., Li, J., Liu, L., Liu, S., McKay, J., and Qian, W. 2013. Identification of genomic regions involved in resistance against *Sclerotinia sclerotiorum* from wild *Brassica oleracea*. Theor. Appl. Genet. 126:549-556.

Mei, J., Wei, D., Disi, J. O., Ding, Y., Liu, Y., and Qian, W. 2012. Screening resistance against *Sclerotinia sclerotiorum* in Brassica crops with use of detached stem assay under controlled environment. Eur. J. plant pathol.134:599-604.

Mila, A. L., Carriquiry, A. L., and Yang, X. B. 2004. Logistic regression modeling of prevalence of soybean Sclerotinia stem rot in the north-central region of the United States. Phytopathology 94:102-110.

Micic, Z., Hahn, V., Bauer, E., Schön, C., Knapp, S. J., Tang, S., and Melchinger, E. 2004. QTL mapping of Sclerotinia midstalk rot resistance in sunflower. Theor. Appl. Genet. 109:1474-1484

Mizushima, U., and Tsunoda, S. 1967. A plant exploration in Brassica and allied genera. Tohoku J. Agric. Res. 17:249-277.

Morrall, R. A. A. 1977. A preliminary study of the influence of water potential on sclerotium germination in *Sclerotinia sclerotiorum*. Can. J. Bot. 55:8-11.

Morrall, R. A. A., Rude, S. V., Turkington, R. K., and Kaminski, D. A. 1990. Sclerotinina stem rot of canola: Integrating chemical control with disease forecasting based on petal infestation. Can. J. Plant Pathol. 11:195 (Abstract).

Nagaharu, U. 1935. Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. 7:389-452.

Nepal, A., and del Río Mendoza, L. E. 2012. Effect of sclerotial water content on carpogenic germination of *Sclerotinia sclerotiorum*. Plant Dis. 96:1315-1322.

Nordin, K., Sigvald, R., and Svensson, C. 1992. Forecasting the incidence of Sclerotinia stem rot on springsown rapeseed/Die Vorhersage von Rapskrebs auf Sommerraps. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. J. Plant Dis. Protect. 245-255.

OECD. 2012. Organization for Economic Co-operation and Development. Consensus document on the biology of the *brassica* crops (*Brassica* spp.). Series on Harmonisation of Regulatory oversight of Biotechnology, No 54, OECD, Paris, pp 142.

Oladzad, A., Zitnick-Anderson, K., Jain, S., Simons, K., Osorno, J. M., McClean, P. E., and Pasche, J. 2019. Genotypes and genomic regions associated with *Rhizoctonia solani* resistance in common bean. Front. Plant Sci. 10:956-979.

Patzoldt, M. E., Grau, C. R., Stephens, P. A., Kurtzweil, N. C., Carlson, S. R., and Diers, B. W. 2005. Localization of a quantitative trait locus providing brown stem rot resistance in the soybean cultivar Bell. Crop Sci. 45:1241-1248.

Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathology 69: 875-880.

Qandah, I. S., and del Río Mendoza, L. E. 2011. Temporal dispersal patterns of *Sclerotinia sclerotiorum* ascospores during canola flowering. Can. J. Plant Pathol. 33:159-167.

Qandah, I. S., and del Río Mendoza, L. E. 2012. Modelling inoculum dispersal and Sclerotinia stem rot gradients in canola fields. Can. J. Plant Pathol. 34:390-400.

Qasim, M. U., Zhao, Q., Shahid, M., Samad, R. A., Ahmar, S., Wu, J., Fan, C., and Zhou, Y. 2020. Identification of QTLs containing resistance genes for Sclerotinia stem rot in *Brassica napus* using comparative transcriptomic studies. Front. Plant Sci. 11:776-790.

Rahman, M., and de Jiménez, M. M. 2016. Behind the scenes of microspore-based double haploid development in *Brassica napus*: A review. J. Plant Sci. Mol. Breed. 5:1-9.

Raymer, P. L. 2002. Canola: an emerging oilseed crop. Trends in new crops and new uses 1:122-126.

Rakow, G. 2004. Species Origin and Economic Importance of *Brassica*. Springer-Verlag Berlin, Heidelberg.

Rönicke, S., Hahn, V., Vogler, A., and Friedt, W. 2005. Quantitative trait loci analysis of resistance to *Sclerotinia sclerotiorum* in sunflower. Phytopathology 95:834-839.

Rousseau, G., Rioux, S., and Dostaler, D. 2007. Effect of crop rotation and soil amendments on Sclerotinia stem rot on soybean in two soils. Can. J. Plant Sci. 87:605-614.

Saharan, G. S., and Mehta, N. 2008. Sclerotinia diseases of crop plants: Biology, ecology and disease management. Springer Science+Busines Media B.V. The Netherlands.

Schwartz, H. F., and Steadman, J. R. 1978. Factors affecting sclerotium populations of, and apothecium production by, *Sclerotinia sclerotiorum*. Phytopathology 68:383-388.

Shahoveisi, F., and del Río Mendoza, L. E. 2019. Effect of temperature and wetness duration on temporal rate of Sclerotinia stem rot disease development in canola. American Phytopathological Society, North Central meeting, Cleveland, Ohio, USA.

Shahoveisi, F., and del Río Mendoza, L. E. 2020. Effect of Wetness Duration and Incubation Temperature on Development of Ascosporic Infections by Sclerotinia sclerotiorum. Plant Dis. 104:1817-1823.

Shahoveisi, F., Markell, S., del Río, L., and Kandel, H. 2020. Sclerotinia stem rot of canola. North Dakota State University Extension.

Sharma, P., Meena, P. D., Verma, P. R., Saharan, G. S., Mehta, N., Singh, D., and Kumar, A. 2016a. *Sclerotinia sclerotiorum* (Lib) de Bary causing Sclerotinia rot in oilseed Brassicas: a review. J. Oilseed Brassica 1:1-44.

Sharma, P., Verma, P. R., Meena, P. D., Kumar, V., and Singh, D. 2016b. Research progress analysis of Sclerotinia rot (*Sclerotinia sclerotiorum*) of oilseed brassicas through bibliography. J. Oilseed Brassica 1:45-125.

Shim, M. Y., and Starr, J. L. 1997. Effect of soil pH on sclerotial germination and pathogenicity of *Sclerotium rolfsii*. Peanut Sci. 24:17-19.

Smolińska, U., and Kowalska, B. 2018. Biological control of the soil-borne fungal pathogen Sclerotinia sclerotiorum–a review. J. Plant Pathol. 100:1-12.

Snowdon, R., Lühs, W., and Friedt, W. 2007. Chapter 2: Oilseed rape. In: Genome Mapping and Molecular Breeding in Plants, Volume 2 Oilseeds, Kole, C., ed . Springer-Verlag Berlin Heidelberg, pp 55-114.

Spitzer, T., Bílovský, J., and Kazda, J. 2017. Effect of fungicide application timing on *Sclerotinia sclerotiorum* infection rate and yield in winter oilseed rape. Gesunde Pflanz. 69:67-72.

Steadman, J. R. 1979. Control of plant diseases caused by Sclerotinia species. Phytopathology 69: 904-907.

Sun, P., and Yang, X. B. 2000. Light, temperature, and moisture effects on apothecium production of *Sclerotinia sclerotiorum*. Plant Dis. 84:1287-1293.

Taylor, A., Coventry, E., Jones, J. E., and Clarkson, J. P. 2015. Resistance to a highly aggressive isolate of *Sclerotinia sclerotiorum* in a *Brassica napus* diversity set. Plant Pathol. 64:932-940.

Thomas, P. 2003. Canola growers manual. The canola council of Canada, Canada Available from http://www.canolacouncil.org/contents2.aspx.

Tu, J. C. 1989. Modes of primary infection caused by *Sclerotinia sclerotiorum* in navy bean. Microbios 57:85-91.

Turkington, T. K., and Morrall, R. A. A. 1993. Use of petal infestation to forecast Sclerotinia stem rot of canola: the influence of inoculum variation over the flowering period and canopy density. Phytopathology 83:682-689.

Turkington, T. K., Morrall, R. A. A., and Gugel, R. A. 1991. Use of petal infestation to forecast Sclerotinia stem rot of canola. Evaluation of early bloom sampling 1985-90. Can. J. Plant Pathol. 13:50-59.

Twengström, E., Sigvald, R., Svensson, C., and Yuen, J. 1998. Forecasting Sclerotinia stem rot in spring sown oilseed rape. Crop Prot. 17:405-411.

Uloth, M., You, M. P., Finnegan, P. M., Banga, S. S., Yi, H., and Barbetti, M. J. 2014. Seedling resistance to *Sclerotinia sclerotiorum* as expressed across diverse cruciferous species. Plant Dis. 98:184-190.

United States Department of Agriculture Natural Recourses Conservation Service. 2019. https://plants.usda.gov/java/ClassificationServlet?source=continue&sort=sciname.

Vasconcellos, R. C., Oraguzie, O. B., Soler, A., Arkwazee, H., Myers, J. R., Ferreira, J. J., Song, Q., McClean, P., and Miklas, P. N. 2017. Meta-QTL for resistance to white mold in common bean. PLoS One 12:p.e0171685.

Wei, D., Cui, Y., He, Y., Xiong, Q., Qian, L., Tong, C., Lu, G., Ding, Y., Li, J., Jung, C., and Qian, W. 2017. A genome-wide survey with different rapeseed ecotypes uncovers footprints of domestication and breeding. J. Exp. Bot. 68:4791-4801.

Wei, L., Jian, H., Lu, K., Filardo, F., Yin, N., Liu, L., Qu, C., Li, W., Du, H., and Li, J. 2016. Genome-wide association analysis and differential expression analysis of resistance to Sclerotinia stem rot in *Brassica napus*. Plant Biotechnol. J. 14:1368-1380.

Wei, D., Mei, J., Fu, Y., Disi, J. O., Li, J., and Qian, W. 2014. Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol. Breed. 34:1797-1804.

Wegulo, S. N., Sun, P., Martinson, C. A., and Yang, X. B. 2000. Spread of Sclerotinia stem rot of soybean from area and point sources of apothecial inoculum. Can. J. Plant Sci. 80:389-402.

Whetzel, H. H. 1945. Synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia 37:648-714.

Willetts, H. J., and Wong, J. A. L. 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. Bot. Rev. 46:101-165.

Wittenberger, K. 2012. USDA Economic Research Service. ERS/USDA Briefing Room–Soybeans and Oil Crops: Canola Seed, Oil, and Meal.

Wu, J., Cai, G., Tu, J., Li, L., Liu, S., Luo, X., Zhou, L., Fan, C., and Zhou, Y. 2013. Identification of QTLs for resistance to Sclerotinia stem rot and BnaC. IGMT5. a as a candidate gene of the major resistant QTL SRC6 in *Brassica napus*. PLoS One 8:e67740.

Wu, J., Chen, P., Zhao, Q., Cai, G., Hu, Y., Xiang, Y., Yang, Q., Wang, Y., and Zhou, Y. 2019. Co-location of QTL for Sclerotinia stem rot resistance and flowering time in *Brassica napus*. Crop J. 7:227-237.

Wu, B. M., Peng, Y. L., Qin, Q. M., and Subbarao, K. V. 2007. Incubation of excised apothecia enhances ascus maturation of *Sclerotinia sclerotiorum*. Mycologia 99:33-41.

Wu, B. M., and Subbarao, K. V. 2008. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathology 98:1144-1152.

Wu, J., Zhao, Q., Liu, S., Shahid, M., Lan, L., Cai, G., Zhang, C., Fan, C., Wang, Y., and Zhou, Y. 2016. Genome-wide association study identifies new loci for resistance to Sclerotinia stem rot in *Brassica napus*. Front. Plant Sci. 7:1418-1430.

Xu, Y., Li, P., Yang, Z., and Xu, C. 2017. Genetic mapping of quantitative trait loci in crops. Crop J. 5:175-184.

Yin, X., Yi, B., Chen, W., Zhang, W., Tu, J., Fernando, W. D., and Fu, T. 2010. Mapping of QTLs detected in a *Brassica napus* DH population for resistance to *Sclerotinia sclerotiorum* in multiple environments. Euphytica 173:25-35.

Young, C. S., Clarkson, J. P., Smith, J. A., Watling, M., Phelps, K., and Whipps, J. M. 2004. Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. Plant Pathol. 53:387-397.

Zhao, J., Buchwaldt, L., Rimmer, S. R., Sharpe, A., McGregor, L., Bekkaoui, D., and Hegedus, D. 2009. Patterns of differential gene expression in *Brassica napus* cultivars infected with *Sclerotinia sclerotiorum*. Mol. Plant Pathol. 10:635-649.

Zhao, J., and Meng, J. 2003. Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 106:759-764.

Zhao, J., Peltier, A. J., Meng, J., Osborn, T. C., and Grau, C. R. 2004. Evaluation of Sclerotinia stem rot resistance in oilseed *Brassica napus* using a petiole inoculation technique under greenhouse conditions. Plant Dis. 88:1033-1039.

Zhao, J., Udall, J. A., Quijada, P. A., Grau, C. R., Meng, J., and Osborn, T. C. 2006. Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. 112:509-516.

Zhao, J., Wang, J., A., L., Doerge, R. W., Chen, Z. J., Grau, C. R., Meng, J., and Osborn, T. C. 2007. Analysis of gene expression profiles in response to *Sclerotinia sclerotiorum* in *Brassica napus*. Planta 227:13-24.

Zitnick-Anderson, K., Oladzadabbasabadi, A., Jain, S., Modderman, C., Osorno, J. M., McClean, P. E., and Pasche, J. S. 2020. Sources of resistance to *Fusarium solani* and associated genomic regions in common bean diversity panels. Front. Genet. 11:475-488.

CHAPTER 3. ASSESSING THE EFFECT OF PHENOTYPING SCORE SYSTEMS AND SNP CALLING AND FILTERING METHODS ON DETECTION OF QTL ASSOCIATED WITH REACTION TO *SCLEROTINIA SCLEROTIORUM* ON CANOLA (*BRASSICA NAPUS* L.)

Abstract

The polyploid nature of canola (*Brassica napus*) represents a challenge for the accurate identification of single nucleotide polymorphisms (SNPs) and the detection of quantitative trait loci (QTL). In this study, combinations of eight phenotyping scoring systems and nine SNP calling and filtering methods were evaluated for their efficiency in detection of QTL associated with response to Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, in two doubled haploid (DH) canola mapping populations. Most QTL were detected in lesion length, relative areas under the disease progress curve for lesion length, and binomial-plant mortality datasets. Binomial data derived from lesion size were less efficient in QTL detection. Inclusion of additional phenotypic sets to the analysis increased QTL detection by 2.4-fold. Imputation of genotypic data increased detection of QTL in one population with a high level of missing data but not in the other. Using unique mapping reads did not improve QTL detection or QTL's R^2 values in either populations. Inclusion of segregation-distorted SNPs increased QTL detection but did not impact their R^2 values significantly. Fifteen of the 19 detected QTL were on chromosomes A02 and C01, and the rest were on A07, A09, and C03. Marker A02-7594120, associated with a QTL on chromosome A02 was detected in both populations. Results of this study suggest the impact of genotypic variant calling and filtering methods may be population dependent while deriving binomial and polynomial sets from quantitative data may improve QTL detection efficiency.

Introduction

Canola or oilseed rape (*Brassica napus* L., AACC/ 2n = 4x = 38) is an important allopolyploid oilseed crop characterized for producing oil with less than 2% erucic acid (Snowdon et al. 2007). The economic value of the crop in the United States was estimated at $494 million in 2019 (USDA NASS 2019). The species was formed from spontaneous hybridization events between *B. oleracea* (2n = 2x = 18, CC) and *B. rapa* (2n = 2x = 20, AA) approximately 6700 years ago (Howell et al. 2008; Sun et al. 2017). Breeding programs have had a significant role in shaping the agronomic traits including disease resistance exhibited by modern cultivars, but in doing so they also have reduced their genetic diversity. One of the effective strategies to increase the variation in the genetic basis of the species including agronomic traits and disease resistance is introduction of new genetic sources such as wild relatives and plant introduction (PI) lines into the breeding programs (Amosova et al. 2019; Delourme et al. 2013; Girke et al. 2012).

Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is one of the major fungal diseases of canola (*Brassica napus* L.) worldwide (Saharan and Mehta 2008). In North Dakota, every unit increase in SSR incidence reduces canola yield by 0.5% (del Río et al. 2007). Several strategies such as rotation with non-host crops, using forecasting models as a decision tool, fungicide applications, and planting resistant varieties are recommended for SSR management (Derbyshire and Denton-Giles 2016; Rothmann and McLaren 2018; Shahoveisi and del Río 2020).

Breeding for resistant cultivars is a nature-friendly, economic, and sustainable approach to reduce the severity of SSR. However, breeding for quantitative traits, like resistance to *S. sclerotiorum* (Disi et al. 2014; Wu et al. 2019) is challenging and limited progress has been made

in canola. For example, within the past 10 years almost twice as many QTL have been identified in dry bean (*Phaseolus vulgaris* L.) than in canola (Arkwazee et al. 2018; Lara et al. 2014; Miklas et al. 2013; Mkwaila et al. 2011; Pérez-Vega et al. 2012; Qasim et al. 2020; Soule et al. 2011; Vasconcellos et al. 2017; Wei et al. 2014; Wu et al. 2013, 2019; Yin et al. 2010; Zhao and Meng 2003; Zhao et al. 2006). Nevertheless, QTL associated with SSR in canola have been identified on 16 of its 19 chromosomes, e.g. A01, A02, A03, A06, A07, A08, A09, A10, C01, C02, C03, C04, C05, C06, C07, and C08 (Gyawali et al. 2016; Qasim et al. 2020; Wei et al. 2014; Wu et al. 2019; Zhao and Meng 2003; Zhao et al. 2006). Despite these efforts, however, most cultivars remain susceptible to the disease; therefore, additional work is required to identify QTL that could facilitate transfer of resistance to SSR into modern canola cultivars.

Detection of QTL requires the development of phenotypic and genotypic data from individuals in a mapping population. To generate phenotypic data, *S. sclerotiorum* is commonly inoculated on canola plants using agar plug to the stem and leaf axis, toothpick, and detached leaf methods (Ashtari Mahini et al. 2020; Arahana et al. 2001; Garg et al. 2013; Mahini 2018; Wu et al. 2013, 2019; Zhao and Meng 2003; Zhao et al. 2006). Among these methods, agar plug provides the closest semblance to natural infection in the field since the inoculum is deposited on the surface of the plant rather than inside (toothpick method) and the plant parts are not excised (detached leaf). In terms of plant response quantification, average lesion length and days to wilt at single points in time are the most reported methods in QTL mapping studies (Derbyshire and Denton-Giles 2016). These two variables offer a snapshot of the reaction of plants to infection that in some instances may not be accurate enough. In contrast, the area under the disease progress curve (AUDPC) provides a more complete picture of that reaction; however, its use in QTL studies has been limited and not thoroughly investigated. More recently, Oladzad et al.

(2019) and Zitnick-Anderson et al. (2020), working with diploid crops, showed that reducing disease severity data collected using categorical scales with up to nine classes down to three classes, e.g. resistant, moderately resistant, and susceptible (polynomial distribution) or just two, e.g. resistant and susceptible (binomial distribution), may increase the power of detecting QTL associated with reaction to root pathogens. Given the importance of phenotyping data in detecting and mapping disease resistance QTL, a comprehensive study in which a variety of phenotyping systems are investigated, will provide valuable insights into plant pathology genome studies.

The methods used to generate genotypic data have improved markedly in recent years. Genotyping by sequencing (GBS) and SNP arrays are the most common and efficient genotyping methods used in recent QTL mapping studies for *B. napus* (ArifUzZaman et al. 2016; Wu et al. 2019; Yu et al. 2017). The data produced by these platforms, however, need to be processed using variant calling and filtering methods before being used for QTL analyses. Many of these methods, however, were developed while working with diploid crops and may not work well in polyploid crops. For example, the presence of highly homoeologous chromosomes, e.g. A and C genomes in *B. napus*, would result in detection of false positive polymorphisms among the subgenomes instead of true SNPs (Clevenger et al. 2015; Clevenger and Ozias-Akins 2015). The use of uniquely mapped reads (UMR) has been proposed to overcome this issue (Clevenger et al. 2015). Using this technique, homoeologous loci are filtered out. Imputation of missing-data is a common process in SNPs pipeline development for genome-wide association studies (GWAS) (Lu et al. 2019; Oladzad et al. 2019; Porcu et al. 2013). This procedure can be beneficial when low coverage GBS data is used to genotype the mapping population (Zheng et al. 2018) because it may increase the power of the QTL mapping and marker density (Porcu et al. 2013). Data

imputation is used less frequently in bi-parental QTL mapping studies where it could be beneficial for populations with high number of missing data and heterozygote calls. Despite the availability of several promising imputation algorithms developed for recombinant inbred line (Xie et al. 2010); full-sib (Swarts et al. 2014); and F_2 and F_1BC (Fragoso et al. 2016) mapping populations, researchers have yet to answer the question whether imputation increases the QTL detection power in bi-parental population studies. Distortion from Mendelian segregation law is a common phenomenon in bi-parental mapping populations (Xu 2008). Deviation from Mendelian segregation could be determined using Chi-Square test (Dai et al. 2017; Nixon 2006). The benefits of discarding markers with distorted segregation, however, are still debatable and researchers may discard these types of markers in the QTL analysis (ArifUzZaman et al. 2016; da Silva et al. 2019; Huang and Yan 2019; Pascual et al. 2016) or keep them (Tyrka et al. 2018). Retaining markers with distorted segregation, may result in better coverage of linkage groups and improve the output of the QTL mapping (Zuo et al. 2019). One of the goals of the current study was to compare the results of the QTL analyses of DH populations in the presence and absence of segregation distortion loci.

Identification of QTL associated with disease resistance is needed for implementation of marker assisted selection (MAS) in breeding programs. Despite its importance, however, research that compares the efficacy of different phenotyping scoring system and/or sequencing analysis approach regarding development of variant pipelines are lacking. Revealing the effect of scoring systems on detecting QTL associated with disease resistance could assist researchers to avoid excessive phenotyping work and to speed up the accurate evaluation of a phenotype; further, using multiple phenotyping datasets might result in detection of additional QTL. In this study, some challenges and gaps in phenotyping and genotyping methods used in QTL analysis

of *B. napus- S. sclerotiorum* pathosystem were addressed. The aim of the study was to evaluate the efficacy of multiple combinations of phenotyping scoring systems and SNP calling and filtering methods (presence/ absence of UMR, missing data imputation, and segregation distorted markers) on detection of QTL associated with response to *S. sclerotiorum* in two *B. napus* DH populations.

Materials and methods

Doubled haploid population development

The first DH population, T63DH, with 127 individuals was constructed from a cross between Topas and Nep63. Topas is an open pollinated spring cultivar with a very low level of SSR resistance which is amenable for microspore culture process; therefore, it was used as the female in the crosses. Nep63 is a semi-winter DH line produced from a cross between plant introductions (PI) 458940 and 649136 of the U.S. National Plant Germplasm System that were originally collected from Japan and Germany, respectively. Nep63 is highly resistant to SSR and it was used as the male in the crosses. The second mapping population, T54DH, with 201 lines was generated from a cross between Topas and PI 436554 that is a spring type line originated from China.

For DH production, a modified microspore culture process reported by Ferrie et al. (2005) was used. Approximately 150 unopened floral buds, 2-3 mm long, were collected from F_1 plants of each cross. Buds were surface disinfested in an aqueous solution of NaOCl at 0.525% v/v for 10 minutes and rinsed with cold distilled autoclaved water three times. Microspores were released from the buds by grinding them in 5 ml of half-strength B5 medium (Gamborg et al. 1968) with 13% sucrose (½ B5-13). The microspores were filtered and suspended in 15 ml of clean ½ B5-13 medium and centrifuged for 3 minutes at 800 rpm after which the supernatant was

carefully discarded. The pelleted microspores were resuspended in 5 ml of clean ½B5-13 medium. This process was repeated twice and after the third centrifuge, the pellet was suspended in 5 ml of NLN-13 medium (PhytoTechnologies Labs, Lenexa, KS) and the concentration of the microspores was adjusted to 10^5 microspores per ml. The suspension was poured into deep Petri dishes (20 ml per dish) and incubated in the following conditions: at 32 °C and dark conditions for three days, at 24 °C for three weeks, and finally on a shaker set at 70 rpm with 24 h light daily and 22-24 °C until embryos reached 2-3 mm long. Then, they were immersed in 50 µM colchicine (MilliporeSigma, Burlington, MA) solution for 72 h at 24 °C and then transferred into solid B5 medium (MilliporeSigma). Germinated plantlets were transferred to 5-inch pots in greenhouse. The size of leaf stomata from DH plantlets were compared with that of haploid check to verify the production of DH plants (Mohammadi et al. 2012). At flowering, plants were covered with pollination bags to avoid cross pollination. Fertile DH plants in each population along with their parental lines were used for phenotyping and genotyping.

Phenotyping

Greenhouse trials were conducted to assess the reaction of both populations against *S. sclerotiorum*. All the DH lines and parental lines were inoculated 45 to 55 days after planting using agar stem method. The experiment was conducted twice in randomized complete blocks with five replications in each trial. Agar plugs, 6 mm in diameter, containing hyphal tips of isolate WM031 were made from two-day old *S. sclerotiorum* colonies growing on potato dextrose agar medium. Isolate WM031 was originally collected from Nebraska, USA, and is considered highly virulent on canola. Single plugs were placed with the mycelium side in contact with the main stem of plants between the third and fourth leaves. The plugs were secured in position by wrapping them to the stems with a piece of Parafilm. The length of the lesions and

plant mortality were measured starting 3 days post inoculation (dpi) and then at two-day intervals until the 11th dpi. Plants were considered dead when irreversible wilting symptoms were observed on plant parts above the inoculated area of the stem. To characterize the response of each population to the disease, their average rate of stem lesion expansion and mortality were calculated using linear and non-linear regressions.

The eight phenotyping scoring systems used in this study were:

I) Lesion length, binomial-, and polynomial-lesion lengths: The average stem lesion length data collected on the 9th dpi was used to derive binomial and polynomial sets. For the binomial dataset, the lines were given a value of 0 if their average lesion length was $\leq$ 3 cm. These lines were considered resistant. The remaining lines were considered susceptible and were assigned a value of 1. For the polynomial dataset, resistant lines from the binomial set remain as resistant and were assigned a value of 1 while the susceptible lines were further split into intermediate and susceptible categories with the latter group having lesions > 6 cm in length. Intermediate lines were assigned a value of 2 and susceptible lines a value of 3 (Appendix A).

II) Relative area under the disease progress curve (rAUDPC): Stem lesion length was used to calculate three rAUDPC datasets for each line. For rAUDPC1 data from all reading times were used; for rAUDPC2, data from the 5th to 11th dpi were used; and for rAUDPC3, data from the 3rd, 7th, and 11th dpi were used. Since lesion length was not measured once a plant was declared dead, the largest lesion length at each reading time in every replication was identified and ascribed to dead plants. In this way, we ensured that each rAUDPC would be larger on susceptible lines than on resistant lines. At the same time, each susceptible line with greater plant mortality would have larger rAUDPC than susceptible plants with lower mortality. The rAUDPC was calculated as follows: $rAUDPC = \frac{\sum\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)}{n}$ where y_i and y_{i+1} represent lesion

lengths at two consecutive reading times, t_i and t_{i+1} represent the time in dpi for the two readings, and n represents the number of days between the first and last reading times.

III) Binomial- and polynomial-plant mortality: Data from plant mortality calculated on the 11th dpi was used to create binomial and polynomial datasets. For the binomial set, lines with plant mortality $\leq$ 20% were classified as resistant and assigned a value of 0; the remainder lines were considered susceptible and assigned a value of 1. For the polynomial dataset, lines considered resistant in the binomial dataset were classified as resistant and assigned a value of 1 while susceptible lines were split into moderately resistant and susceptible with the latter group having plant mortality > 60%. Intermediate lines were assigned a value of 2 and susceptible lines a value of 3 (Appendix A).

Datasets for lesion length and rAUDPC were tested for normality using the Univariate procedure of SAS version 9.4 (SAS Institute, Cary, NC). Homogeneity of variances ($P \leq 0.05$) for each population were tested by using Levene's test. When permitted, trials were combined, and average value of each line was calculated across trials and replications.

Genotyping

DNA extraction and sequencing

Leaf samples were collected from 20-days-old plants and DNA was extracted with Qiagene DNeasy 96 Plant kit (Qiagene, Hilden, Germany) for T54DH population and with MagMax Plant DNA Isolation kit (Thermo Fisher Scientific, Walthman, MA) for T63DH population. The decision to switch DNA extraction kits was based on the simplicity of working with the MagMax kit. A total of 332 DNA samples, 127 progeny lines from T63DH and 201 from T54DH population and the four parents, were sent to University of Minnesota (UMN) Genomics center (Minneapolis, MN) for sequencing analyses. GBS dual-index libraries were

created using ApeK1 enzyme (Elshire et al. 2011) and were combined into a single pool and sequenced across two lanes of a NovaSeq SP. Subsequently, sequencing barcodes and adaptors were removed.

SNP calling

Sequencing reads were trimmed for minimum quality score of 20 and minimum sequence length of 80bp using Sickle (Joshi and Fass. 2011). The data (FASTQ) files for each population were compared to the *Brassica napus* reference genome (http://www.genoscope.cns.fr/brassicanapus/data/) using Burrows-Wheeler Alignment Tool's Maximal Exact Match algorithm (BWA-MEM, Li 2013) to generate binary alignment map (BAM) files. SNP calling was conducted through three different pipelines:

No imputation, no UMR

The BAM files were sorted and indexed using SAMtools (Li et al. 2009) for each population. Using VarScan v2.3.7 (Koboldt et al. 2012), SNPs were called using SAMtools mpileup output files. The minimum coverage and minimum read numbers were set at three and two, respectively. The SNPs and genotypes were filtered if 1) the reference allele was N; 2) 20% of the SNPs were not called at the given position; 3) a genotype had more than 40% missing SNPs; and 4) a genotype had more than 30% heterozygosity. Only markers that were polymorphic between parents and had homozygous alleles were used for the construction of the linkage groups.

No imputation, with UMR

Similar filtering and SNP calling criteria were used for this pipeline, but only mapping reads to a unique position were extracted from the BAM files using SAMtools. A mapping quality (Q) of 10 or less indicates there is at least a 0.1 chance that the read originated elsewhere

(Ewing and Green 1998; Greenfield et al. 2016). Therefore, the Q>10 option was used to generate BAM files with reads mapped to a unique position.

With imputation and UMR

The SNP dataset from the second pipeline (no imputation, with UMR) was manipulated further for data imputation. Here, the SNPs with less than 20% missing data were imputed using fastPHASE (Scheet and Stephens 2006). After imputation, the genotypes were tested for heterozygosity level at threshold of 30%. Polymorphic markers with homozygous alleles were used for construction of the genetic maps.

Linkage map construction and QTL analyses

MapDisto software (Lorieux 2012) was used to construct genetic linkage maps. Two additional versions of each map were prepared by filtering out SNPs that showed significant departures from the expected Mendelian segregation using a Chi-Square test with α of 0.001 and 0.0001. Recombination frequency was measured using Kosambi mapping function (Kosambi 1943) and sum of adjacent recombination frequencies (SARF) criteria was used for ordering the loci for all nine genotyping datasets. Initially, linkage groups were generated at recombination frequency of 0.3 and a stringent logarithm of odds (LOD) value of 10 for datasets with the Chi-Square test and 20 for those datasets that this test was not conducted. The final framework map for each linkage group was created at LOD of 4 for all the datasets (Chi-Square tests were conducted at this step). Reversed groups were flipped using "flip group" option. QTL associated with SSR response were identified using QTL IciMapping 4.2 (Meng et al. 2015) using its inclusive composite interval mapping (ICIM) algorithm that increases the detection power and reduces false detection. The walking step was set to 1 cM and 1000 permutation test at significant level of $P < 0.05$; the percentage of phenotypic variation explained by each QTL (R^2)

was also determined. Mapchart software version 2.23 was used for the graphical presentation of linkage groups (Voorrips 2002).

Markers' selection efficiency

Selection efficiency of markers linked to the detected QTL in multiple genotyping and phenotyping analyses were calculated. The selection efficiency was calculated as the percentages of correct association of the A allele (from resistant parent) to the resistant phenotypic reaction and B allele (from susceptible parent) to the susceptible reaction (Oladzad-Abbasabadi et al. 2018). The efficiency of the markers was evaluated using lesion length data with imputed or non-imputed genotypic datasets, depending on the population. Lines with lesion lengths smaller than 3 cm were considered resistant.

Identification of potential candidate genes

Gene models between the flanking markers of the QTL with the highest selection efficiency were identified based on the whole genome sequence published by Chalhoub et al. (2014) using Genoscope website (https://www.genoscope.cns.fr/brassicanapus/). Subsequently, protein sequences of the identified gene models were searched using Basic Local Alignment Search Tool (BLAST). Proteins with > 98% identity were selected and their functions were identified using Uniprot (https://www.uniprot.org/uniprot/A0A1S2Y793) and TAIR website (www. arabidopsis.org). Proteins known to be involved in plant defense mechanisms were selected and their functions were verified using literature search.

Results

Phenotyping

A range of susceptible to resistance reactions was observed in both populations (Figure 3.1). Lesion length and rAUDPC phenotyping datasets were normally distributed ($P > 0.11$) for

T63DH and T54DH populations and variances of the trials within each population were homogeneous ($P > 0.3$). Therefore, average values across all trials and replications of each population were calculated for these scoring systems. Length of stem lesions increased almost linearly with time in both populations at an average rate of 0.5 and 0.6 cm per day in T63DH and T54DH, respectively (Appendix B). In contrast, plant mortality behaved markedly different. In T54DH, it increased linearly over time at a rate of 0.6% per day to an average maximum of 6%, while in T63DH it followed a quadratic function and reached a maximum of 41% (Appendix B). Since lesion length was not recorded once a plant was declared dead, data on lesion length measured at 9 dpi was used to derive binomial and polynomial datasets. Many lines had a very reduced number of plants alive at 11 dpi; however, it provided the most representative dataset for mortality analysis. This indicates that the optimum time for recoding the disease reaction varies depending on the scoring system.

T63DH population: Nine dpi, the mapping population showed a range of resistant to susceptible reactions to the disease with approximately 18% of lines having average lesions ≤ 3 cm, 65% of its members having lesions between 3 and 6 cm, and the remaining 17% of lines having lesions > 6 cm. The resistant and susceptible parents had average lesion lengths of 1.5 and 8.2 cm, respectively (Figure 3.2A, Table 3.1). The most susceptible line of this population had on average 9 cm long lesions while the most resistant had 1.2 cm long lesions. In the lesion-binomial dataset, 23 DH lines of the population and the resistant parental line, Nep63, were categorized as resistant and 104 DH lines along with the susceptible parent, Topas, as susceptible. In the lesion-polynomial dataset, 23 DH lines and Nep63 were considered resistant, 82 lines showed intermediate reactions, and the remaining 22 lines and Topas were susceptible.

Figure 3.1. Examples of resistant (A) and susceptible (B, C) reactions of *Brassica napus* doubled haploid lines to inoculation with *Sclerotinia sclerotiorum* 11 days post inoculation.

Table 3.1. Phenotypic variation in the response of T54DH and T63DH populations and their parental lines to stem inoculation with *Sclerotinia sclerotiorum* measured nine and 11 days after inoculation for lesion size and plant mortality, respectively.

		Parental lines		DH population		
Population	**Scoring system[a]**	**Resistant**	**Susceptible**	**Mean**	**Min**	**Max**
T63DH	Lesion (cm)	1.5	8.2	4.7	1.2	9
	rAUDPC1 (cm)	1.4	6.1	3.6	1.2	7.8
	rAUDPC2 (cm)	1.5	7	4.1	1.2	8.7
	rAUDPC3 (cm)	1.5	5.9	3.6	1.1	7.6
	Mortality (%)	10	90	40	0	100
T54DH	Lesion (cm)	2.5	8.5	5.4	1.1	11.8
	rAUDPC1 (cm)	3.1	6.2	4.7	2.1	8.7
	rAUDPC2 (cm)	2.3	6.1	4.2	1.0	8.9
	rAUDPC3 (cm)	4.2	7.1	5.6	3.2	9
	Mortality (%)	10	88	41.8	0	100

[a]cm: centimeter; rAUDPC1-3: relative area under the disease progress curve for lesion length with readings between 3 and 11 dpi (1), 5 and 11 dpi (2), at 3, 7, and 11 dpi (3).

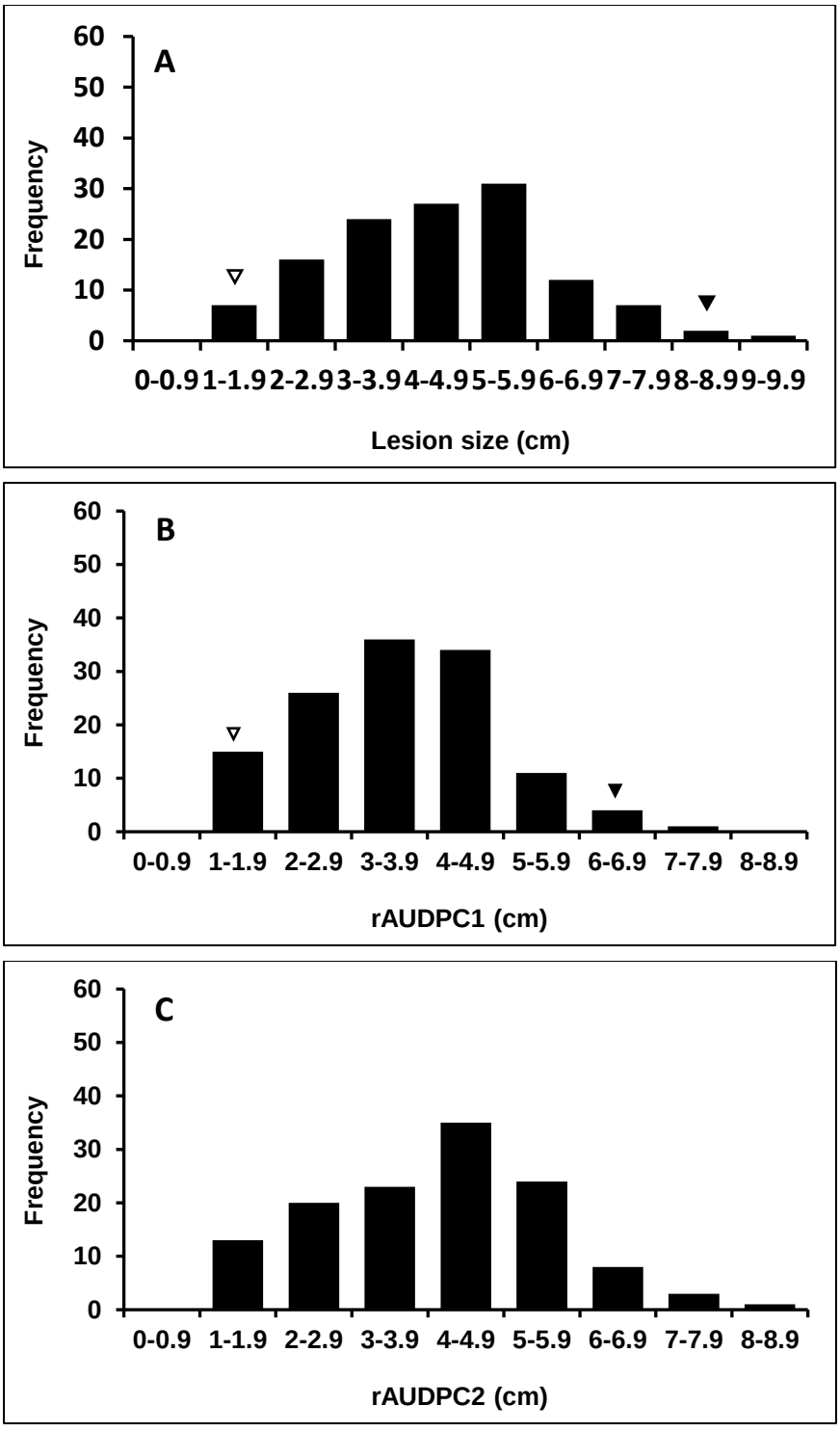


Figure 3.2. Frequency distribution of reaction of 127 doubled haploid lines from population T63DH to inoculation with *Sclerotinia sclerotiorum*. The location of the resistant (Nep63) and susceptible (Topas) parents are shown by white and black triangles, respectively. A) Lesion length dataset, 9 days post inoculation (dpi) B) relative area under the disease progress curve (rAUDPC) 1, calculated using data from 3, 5, 7, 9, and 11 dpi; C) rAUDPC2, calculated using data from 5, 7, 9, and 11 dpi; D) rAUDPC3, calculated using data from 3, 7, and 11 dpi.

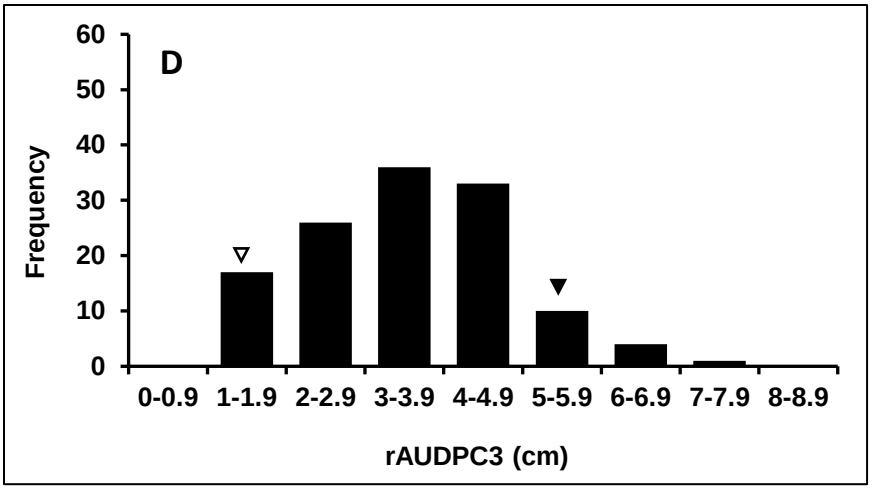


Figure 3.2. Frequency distribution of reaction of 127 doubled haploid lines from population T63DH to inoculation with *Sclerotinia sclerotiorum* (continued). The location of the resistant (Nep63) and susceptible (Topas) parents are shown by white and black triangles, respectively. A) Lesion length dataset, 9 days post inoculation (dpi) B) relative area under the disease progress curve (rAUDPC) 1, calculated using data from 3, 5, 7, 9, and 11 dpi; C) rAUDPC2, calculated using data from 5, 7, 9, and 11 dpi; D) rAUDPC3, calculated using data from 3, 7, and 11 dpi.

Using rAUDPC datasets, distribution of the populations was similar in rAUDPC1 and rAUDPC3. Cutoff value to separate resistant and susceptible reactions in rAUDPC datasets was set at 2.5 cm because that value was associated with a line showing a 3 cm lesion in T63DH. In total, 29, 21, and 32 lines showed resistant reaction in rAUDPC sets 1, 2, and 3, respectively (Figure 3.2B-D). These values represented 22, 17, and 25% of that population, respectively. The range of rAUDPC values for the resistant parent, Nep63, was 1.4 to 1.5 cm and for the susceptible parent, Topas, 5.9 to 7 cm. Plant mortality in the population was observed starting five days post inoculation (Appendix B). In the mortality-binomial dataset, 39 lines and the resistant parent had less than 20% plant mortality and 88 lines along with the susceptible parent showed a susceptible reaction with more than 20% plant mortality. In the polynomial dataset, 39 lines were considered resistant, 63 intermediate, and 25 susceptible.

The five most resistant lines in this population had lesion lengths ranging between 1.2 and 1.9 cm and rAUDPC values between 1.2 and 1.6, 1.2 and 1.8, and 1.1 and 1.6 cm, for rAUDPC1, 2, and 3, respectively. These lines did not have dead plants.

T54DH population: This mapping population was in general more susceptible to *S. sclerotiorum* than the one derived from Nep63. At nine dpi, approximately 15% of lines had average lesion lengths < 3 cm, 46% of its members had lesions between 3 and 6 cm, and the remaining 39% of lines had lesions > 6 cm nine dpi (Figure 3.3A, Table 3.1). At that time, the resistant and susceptible parents had average lesion lengths of 2.5 and 8.5 cm, respectively (Figure 3.3A, Table 3.1). The most susceptible line of this population had on average 11.8 cm long lesions while the most resistant had 1.1 cm long lesions. In the lesion-binomial dataset, 31 DH lines of the population and the resistant parental line, PI 436554, were categorized as resistant and 170 DH lines along with the parental line Topas, as susceptible. In the lesion-polynomial dataset, 31 DH lines and Nep63 were considered resistant, 92 lines showed intermediate reactions, and the remaining 78 lines and parental line Topas were susceptible. The rAUDPC cutoff values for resistant and susceptible reactions in T54DH were 3.1, 2.6, and 4.2 for rAUDPC1, 2, and 3, respectively. The three datasets contained 13%, 15%, and 12% resistant lines. The range of rAUDPC values for the resistant parent, Nep63, was 2.3 to 4.2 cm and for the susceptible parent, Topas, 6.1 to 7.1 cm (Figure 3.3B-D). Like T63DH population, plant mortality was initially observed on the 5th dpi. For the mortality-binomial dataset, 56 lines and the resistant parent had less than 20% plant mortality and 145 lines along with the susceptible parent showed a susceptible reaction with more than 20% mortality. In the polynomial dataset, 56 lines were considered resistant, 107 intermediate, and 38 susceptible.

The five most resistant lines in this population had lesion lengths ranging between 1.1 and 1.4 cm, and rAUDPC values ranging between 2.1 and 2.6, 1.0 and 1.4, and 3.2 and 3.8 cm, for rAUDPC1, 2, and 3, respectively. All five genotypes had survival rates of 100%.

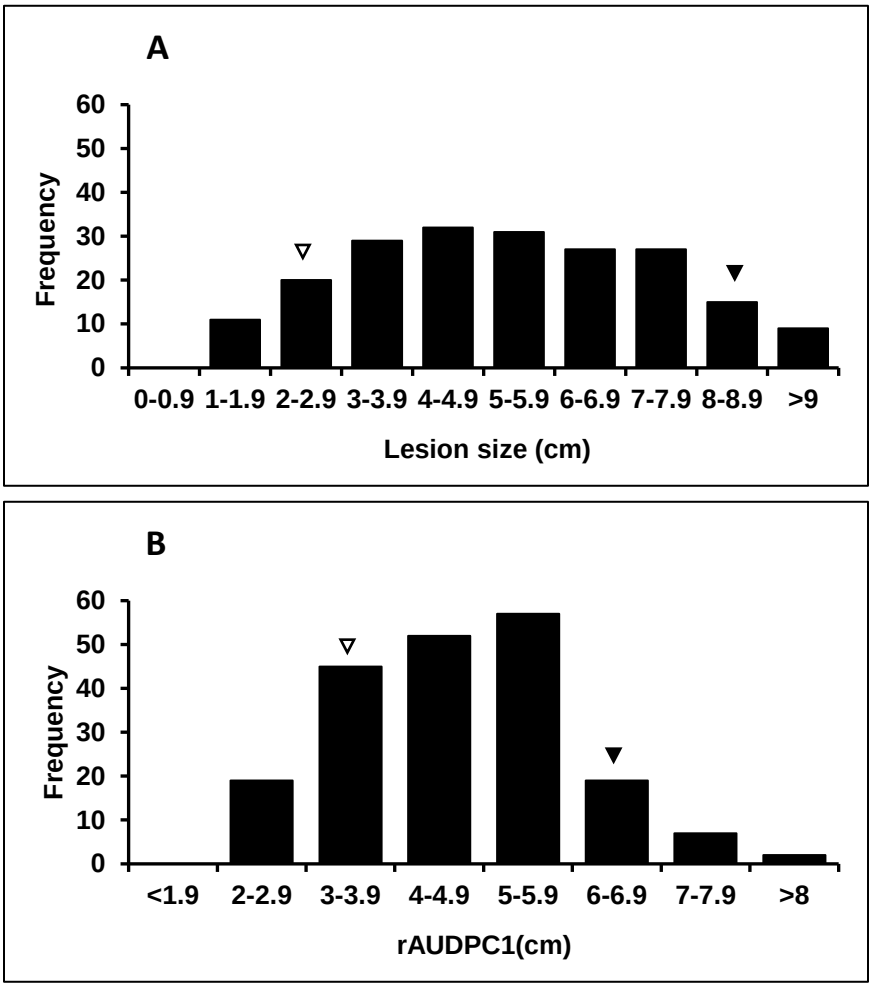


Figure 3.3. Frequency distribution of reaction of 201 doubled haploid lines from population T54DH to inoculation with *Sclerotinia sclerotiorum.* The mean values of resistant parent (PI436554) and susceptible parent (Topas) are shown by white and black triangles, respectively. A) Lesion length dataset, 9 days post inoculation (dpi); B) rAUDPC1, calculated using data from 3, 5, 7, 9, and 11 dpi; C) rAUDPC2, calculated using data from 5, 7, 9, and 11 dpi; D) rAUDPC3, calculated using data from 3, 7, and 11 dpi.

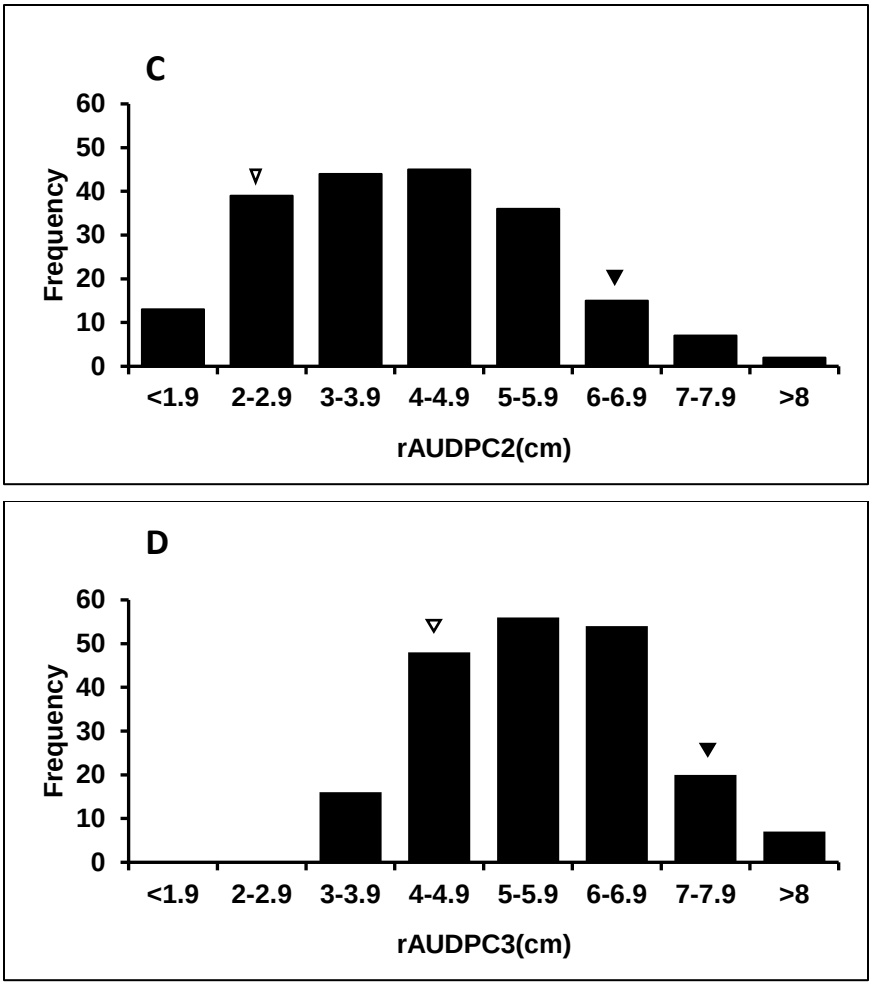


Figure 3.3. Frequency distribution of reaction of 201 doubled haploid lines from population T54DH to inoculation with *Sclerotinia sclerotiorum* (continued). The mean values of resistant parent (PI436554) and susceptible parent (Topas) are shown by white and black triangles, respectively. A) Lesion length dataset, 9 days post inoculation (dpi); B) rAUDPC1, calculated using data from 3, 5, 7, 9, and 11 dpi; C) rAUDPC2, calculated using data from 5, 7, 9, and 11 dpi; D) rAUDPC3, calculated using data from 3, 7, and 11 dpi.

Genotyping

SNP calling

More than 750 million reads were generated for the whole sequencing run and the average yield was approximately 2.4 million reads per library (332 libraries). The mean quality score was greater than Q30 for all libraries. For T63DH a total of 312,641,414 and for T54DH a

total of 493,048,370 Single-end 101bp raw reads were generated from Illumina NovaSeq 6000 system and used for further processing for SNP calling.

The impact of the different SNP calling and filtering procedures on the number of markers ascribed to linkage groups vary between populations (Table 3.2). Imputing the missing data increased the number of markers available for mapping in both populations but with different magnitudes. The number of markers available for mapping in the set of imputed data with unique mapping reads was 964 for the T63DH population and 1107 for the T54DH population. The sets with imputed data and unique mapping reads had 1.5 and 2.4 times more SNPs available for mapping than the non-imputed data with unique mapping reads in T63DH and T54DH, respectively (Table 3.2). Similarly, the percentage of available markers ascribed to linkage groups in the imputed data with unique mapping reads were greater in both populations compared to the percentages in non-imputed set with unique mapping reads, e.g. 86% vs. 71% in T63DH and 31% vs. 20% in T54DH.

Screening potential SNPs for departures from the 1:1 ratio using the Chi-Square test for segregation distortion in general reduced the percentage of available markers that were mapped to linkage groups, but the magnitude of the impact varied with population. On T63DH, the percentage of available markers mapped to linkage groups was 85, 79, and 95 for sets with no imputation and no UMR, no imputation with UMR, and with imputation and UMR, respectively. When the test was conducted, the percentage of available markers mapped to linkage groups was reduced between 10 and 20%. On T54DH, the percentage of available markers mapped to linkage groups was 17, 26, and 54 for sets with no imputation and no UMR, no imputation with UMR, and with imputation and UMR, respectively. When the test was conducted, the average

percentage of available markers mapped to linkage groups were reduced by 35, 35 and 61%, respectively.

The stringency of the Chi-Square test for segregation distortion, whether using α values of 0.001 or 0.0001, had a reduced effect on the percentage of available markers mapped to linkage groups from any dataset within both populations (Table 3.2). On T63DH, conducting the Chi-Square test using $\alpha = 0.001$ resulted in sets with 63, 62, and 79% of available markers being mapped to linkage groups on sets with no imputation and multiple mapping reads, no imputation and unique mapping reads, and with imputation and unique mapping reads, respectively. When the test was conducted using $\alpha = 0.0001$, the percentage of markers in T63DH increased between 7 and 15% in all sets whereas on T54DH it increased between 9 and 17%.

QTL mapping

The impact of the SNP calling processes on the number of QTL associated with reaction to disease varied with the population. On T63DH, the use of imputation and unique mapping reads with or without test for segregation distortion resulted in the lowest numbers of QTL while in T54DH this approach yielded the highest number (Table 3.2). In the latter population no QTL were detected using non-imputed data. In general, the use of segregation distortion test did not have a consistent influence on the number and effect (R^2) of QTL detected independent of the population evaluated.

Table 3.2. Effect of variant calling methods and filtering procedures on the number of single nucleotide polymorphisms (SNP) available for mapping in two doubled haploid *Brassica napus* mapping populations.

Population[a]	SNP calling and χ^2 tests	Markers on LGs / # of LGs	Map length (cM)	DH lines	QTL
T63DH	No imputation, no UMR (with 658 markers available for LG construction)				
	χ^2 α=0.001	413/15	766.87		4
	χ^2 α=0.0001	494/15	822.06	126	5
	No χ^2	562/18	951.82		4
	No imputation with UMR (635 markers available for LG construction)				
	χ^2 α=0.001	394/19	913.47		2
	χ^2 α=0.0001	460/19	948.75	126	3
	No χ^2	500/20	1007.14		3
	With imputation and UMR (964 markers available for LG construction)				
	χ^2 α=0.001	759/19	1720.71		1
	χ^2 α=0.0001	818/20	1780.49	127	2
	No χ^2	915/29	2228.62		2
T54DH	No imputation, no UMR (741 markers available for LG construction)				
	χ^2 α=0.001	77/8	337		0
	χ^2 α=0.0001	87/8	388.95	200	0
	No χ^2	129/17	494.68		0
	No imputation with UMR (467 markers available for LG construction)				
	χ^2 α=0.001	75/11	282.88		0
	χ^2 α=0.0001	84/11	337.80	200	0
	No χ^2	123/14	406.43		0
	With imputation and UMR (1107 markers available for LG construction)				
	α=0.001	216/16	1220.90		2
	α=0.0001	244/17	1383.74	201	3
	No χ^2	595/27	3315.47		3

The choice of phenotypic scoring system had a strong impact on the number of QTL detected but that effect varied with population. The original lesion length set and the rAUDPCs derived from it were the most consistent in allowing detection of significant QTL in both populations. In T63DH, using the polynomial set derived from lesion length, four QTL were detected while a single QTL was detected using the derived binomial set; In contrast, the binomial dataset derived from plant mortality yielded seven QTL and mortality-polynomial identified only two QTL. Of the 14 QTL detected in this population, some showed overlaps in physical positions; however, we identified them as different QTL to enable the comparison of the methods. Six of the 14 QTL were detected in each of the three rAUDPC sets and the original lesion length set while the remaining seven were identified only in the mortality-binomial and

polynomial datasets (Table 3.3). In contrast, in T54DH, four of the five detected QTL were identified using either rAUDPC sets 1, 2 or 3, and the original set for lesion length (Table 3.4) and a single QTL was detected in the lesion-binomial set. Only one QTL was detected using polynomial datasets. The type of phenotypic data in which QTL were detected did not influence the effect of the QTL as expressed by their R^2 value. The average R^2 value for QTL from lesion length and rAUDPC sets was 13% in T63DH and 10% in T54DH whereas the average R^2 for QTL detected in binomial or polynomial sets derived from lesion length or plant mortality were 12% and 10%, for the respective populations.

To distinguish the QTL identified in different methods, specific nomenclature to each QTL was devoted (Figure 3.4). QTL SR63.A2.1 was associated with stem resistance to *S. sclerotiorum* in population T63DH and it was located on chromosome A02; sequential numbers were placed if more than one QTL were identified on the same chromosome. QTL SR63.A2.1 had negative additive effect ranging between -0.18 and -0.58; it was detected using lesion length, lesion-polynomial, and the three rAUDPC sets; this QTL contributed to explain between 11 and 15% of the phenotypic variation (R^2) with higher R^2 values where lesion size and rAUDPC datasets were used (Table 3.3). QTL SR63.A2.2 was detected using lesion length, the three rAUDPC derived from it, and the lesion-binary sets; it had negative additive effect ranging from -0.12 to -0.65 and contributed to explain between 10 and 17% of the phenotypic variation. QTL SR63.A2.4 was detected using lesion length, the three rAUDPC sets derived from it, and the lesion-polynomial set. This QTL had negative additive effect ranging from -0.18 to -0.56 and contributed to explain between 11 and 16% of the phenotypic variation. QTL SR63.A2.2 and SR63.A2.4 shared flanking marker chrA02-7458423. All QTL detected in the mortality-binary set had negative additive values and explained between 10 and 13% of the phenotypic variation

of lines, except SR63.C1.4 which explained 16%. This QTL shared a flanking marker with QTL SR63.C1.1 (Table 3.3) and the 95% confidence interval for its position on the chromosome overlapped with that of QTL SR63.C1.5. The latter QTL was not detected in the mortality-binary dataset, had a negative additive effect, and explained approximately 10% of the phenotypic variation.

A total of five QTL were detected in population T54DH. QTL SR54.A2.1 was detected in all eight phenotypic datasets and had the strongest negative additive effect when present in the lesion length dataset (Table 4.4). In that set, its presence explained between 15 and 16% of the phenotypic variation. However, its R^2 value in the mortality-binomial and polynomial sets were on average 7 and 11%, respectively. This QTL shared chrA02-7594120 as flanking marker with SR54.A2.2 in T54DH and with QTL SR63.A2.1 in population T63DH, suggesting it may be significantly close to the locus associated with response to *S. sclerotiorum*. Its other flanking marker, chrA02-10934422, was shared with QTL SR54.A2.3 which was detected in rAUDPC1 dataset (Figure 3.5, Table 3.4). All remaining QTL in T54DH, explained less than 10% of phenotypic variation except SR54.A2.3 which explained 14% (Table 3.4).

Table 3.3. Quantitative trait loci (QTL) identified for T63DH populations using different phenotyping and genotyping analyzing methods.

QTL name	Chr.	Physical Interval	Analysis[a]	Scoring systems[b]	LOD[c]	R^2 (%) [d]	Additive effect
SR63.A2.1	A02	6194984-7594120	1	Lesion	3.76	14	-0.57
			4	Lesion	3.82	14	-0.58
			1	Lesion-polynomial	3.11	11	-0.19
			2	Lesion-polynomial	3.02	11	-0.18
			3	Lesion-polynomial	3.08	11	-0.19
			4	Lesion-polynomial	3.02	11	-0.18
			6	Lesion-polynomial	3.15	13	-0.19
			1	rAUDPC1	4.09	15	-0.45
			4	rAUDPC1	4.39	13	-0.48
			1	rAUDPC2	4.11	15	-0.53
			4	rAUDPC2	4.19	15	-0.54
			1	rAUDPC3	4.26	15	-0.46
			4	rAUDPC3	4.36	15	-0.47
SR63.A2.2	A02	7315579-7458423	2	Lesion	4.44	14	-0.63
			3	Lesion	4.7	15	-0.65
			6	Lesion	4.33	14	-0.62
			1	Lesion-binomial	2.74	10	-0.12
			2	Lesion-binomial	2.84	10	-0.12
			3	Lesion-binomial	2.85	10	-0.12
			6	Lesion-binomial	2.84	10	-0.12
			2	rAUDPC1	4.91	15	-0.51
			3	rAUDPC1	4.97	15	-0.51
			6	rAUDPC1	4.76	16	-0.50
			2	rAUDPC2	4.95	16	-0.59
			3	rAUDPC2	5.10	16	-0.60
			6	rAUDPC2	4.81	16	-0.58
			2	rAUDPC3	5.21	17	-0.52
			3	rAUDPC3	5.27	16	-0.53
			6	rAUDPC3	5.17	17	-0.54

Table 3.3. Quantitative trait loci (QTL) identified for T63DH populations using different phenotyping and genotyping analyzing methods (continued).

QTL name	Chr.	Physical Interval	Analysis[a]	Scoring systems[b]	LOD[c]	R^2 (%) [d]	Additive effect
SR63.A2.3	A02	7315579-8650073	2	Mortality-binomial	3.11	10	-0.14
SR63.A2.4	A02	7458423-8650073	5	Lesion	3.54	16	-0.56
			5	Lesion-polynomial	2.94	11	-0.18
			5	rAUDPC1	4.08	14	-0.46
			5	rAUDPC2	4.10	14	-0.53
			5	rAUDPC3	4.23	15	-0.47
SR63.A7.1	A07	5117104-6001704	1	Mortality-binomial	3.08	10	-0.14
			2	Mortality-binomial	3.45	11	-0.14
			6	Mortality-binomial	2.87	11	-0.13
SR63.A7.2	A07	5082112-5840027	3	Mortality-binomial	2.93	10	-0.13
SR63.C1.1	C01	18520875-26829272	1	Mortality-binomial	3.88	13	-0.16
			3	Mortality-binomial	3.66	13	-0.15
			1	Mortality-polynomial	3.44	13	-0.25
			3	Mortality-polynomial	3.43	13	-0.25
SR63.C1.2	C01	22057760-24895526	2	Mortality-binomial	3.45	11	-0.15
SR63.C1.3	C01	21846849-31347615	3	Lesion	2.94	9	-0.52
			2	Lesion-polynomial	3.14	11	-0.19
			3	Lesion-polynomial	3.14	12	-0.19
			2	rAUDPC1	2.85	9	-0.39
			3	rAUDPC1	3.1	9	-0.4
			2	rAUDPC2	2.79	8	-0.45
			3	rAUDPC2	3.04	9	-0.46
			3	rAUDPC3	2.96	8	-0.39
SR63.C1.4	C01	11871303-26829272	4	Mortality-binomial	3.71	16	-0.15
			5	Mortality-binomial	3.79	16	-0.16
			6	Mortality-binomial	3.48	13	-0.15
			4	Mortality-polynomial	3.50	15	-0.24

Table 3.3. Quantitative trait loci (QTL) identified for T63DH populations using different phenotyping and genotyping analyzing methods (continued).

QTL name	Chr.	Physical Interval	Analysis[a]	Scoring systems[b]	LOD[c]	R^2 (%) [d]	Additive effect
SR63.C1.4	C01		5	Mortality-polynomial	3.47	15	-0.25
			6	Mortality-polynomial	3.48	14	-0.25
SR63.C1.5	C01	2059483-31347615 [e]	5	Lesion-polynomial	3.14	12	-0.19
			5	rAUDPC1	2.97	11	-0.4
			5	rAUDPC2	2.92	10	-0.47
			5	rAUDPC3	2.85	10	-0.4
SR63.C1.6	C01	32371151-33230435	7	Lesion	3.09	11	0.56
SR63.C1.7	C01	21580914-3377685[e]	8	Lesion	3.13	11	0.57
			9	Lesion	3.13	11	0.57
			8	rAUDPC1	3.07	11	0.41
			9	rAUDPC1	3.07	11	0.41
SR63.C1.7			8	rAUDPC2	3.01	11	0.48
			9	rAUDPC2	3.08	11	0.48
			8	rAUDPC3	3.04	11	0.41
			9	rAUDPC3	3.04	11	0.41
SR63.C1.8	C01	3563014-33230500[e]	8	Mortality-binomial	3.12	11	0.16
			9	Mortality-binomial	3.12	11	0.16

[a] Genotypic data processes: 1) no imputation, no unique mapping reads (UMR), χ^2 test at α=0.001; 2) like 1 with α=0.0001; 3) like 1 with no χ^2 test; 4) no imputation, with UMR, χ^2 test at α=0.001; 5) like 4 with α=0.0001; 6) like 4 with no χ^2 test; 7) with imputation, UMR, and χ^2 test at α=0.001; 8) like 7 with α=0.0001; 9) like 7 with no χ^2 test.

[b] Lesion: lesion length measured 3, 5, 7, 9, and 11 days post inoculation (dpi); Lesion-binomial: 0= resistant (lesions $\leq$ 3 cm, nine dpi), 1= susceptible (lesions > 3 cm); Lesion-polynomial: 0= resistant (lesions $\leq$ 3 cm, nine dpi), 1= susceptible (lesions > 6 cm), 2= intermediate; rAUDPC1-3: relative area under the disease progress curve for lesion length with readings between 3 and 11 dpi (1), 5 and 11 dpi (2), at 3, 7, and 11 dpi (3); mortality: percentage dead plants at 11 dpi; mortality-binomial: 0= resistant (mortality $\leq$ 20%, 11 dpi), 1= susceptible (mortality > 20%); mortality-polynomial: 0= resistant (mortality $\leq$ 20%, 11 dpi), 1 susceptible (mortality > 60%), 2= intermediate.

[c] Final LOD of 4 were used to construct the linkage groups

[d] Percentage of phenotypic variation explained by the QTL.

[e] Random position on the chromosome.

Table 3.4. Quantitative trait loci (QTL) identified for T54DH populations using different phenotyping and genotyping analyzing methods.

QTL name	Chr.	Physical Interval	Analysis[a]	Scoring systems[b]	LOD[c]	R^2 [d] (%)	Additive effect
SR54.A2.1	A02	7594120-10934422	7	Lesion	8.33	16	-0.93
			8	Lesion	8.33	16	-0.93
			9	Lesion	8.04	15	-0.88
			8	Lesion-binomial	3.19	7	-0.10
			7	Lesion-polynomial	5.83	13	-0.26
			8	Lesion-polynomial	5.83	13	-0.26
			9	Lesion-polynomial	5.54	13	-0.25
			7	Mortality-binomial	2.73	6	-0.12
			8	Mortality-binomial	3.57	8	-0.15
			8	Mortality-polynomial	4.96	11	-0.24
			9	Mortality-polynomial	4.47	11	-0.22
			7	rAUDPC1	7.19	14	-0.52
			9	rAUDPC1	8.13	14	-0.52
			9	rAUDPC1	8.13	14	-0.52
			7	rAUDPC2	7.42	14	-0.63
			8	rAUDPC2	7.42	14	-0.63
			9	rAUDPC2	8.39	14	-0.64
			7	rAUDPC3	6.94	13	-0.48
			8	rAUDPC3	6.94	13	-0.48
			9	rAUDPC3	7.82	13	-0.48
SR54.A2.2	A02	6194984-7594120	7	Lesion-binomial	3.21	7	-0.10
SR54.A2.3	A02	7315716-10934422	8	rAUDPC1	7.21	14	-0.54
SR54.A9.1	A09	2813928-26006097 [e]	9	rAUDPC1	3.71	6	0.34
			9	rAUDPC2	3.79	6	0.41
SR54.C3.1	C03	23485864-31673721	7	Lesion	3.60	6	-0.59
			8	Lesion	3.56	6	-0.58
			7	rAUDPC1	3.90	7	-0.36
			8	rAUDPC1	3.88	6	-0.36
			9	rAUDPC1	4.58	7	-0.38
			7	rAUDPC2	3.94	7	-0.44
			8	rAUDPC2	3.91	7	-0.44
			9	rAUDPC2	4.63	7	-0.46
			7	rAUDPC3	3.71	6	-0.34
			8	rAUDPC3	3.68	6	-0.33
			9	rAUDPC3	4.34	7	-0.35

[a] Genotypic data processes: 1) no imputation, no unique mapping reads (UMR), χ^2 test at α=0.001; 2) like 1 with α=0.0001; 3) like 1 with no χ^2 test; 4) no imputation, with UMR, χ^2 test at α=0.001; 5) like 4 with α=0.0001; 6) like 4 with no χ^2 test; 7) with imputation, UMR, and χ^2 test at α=0.001; 8) like 7 with α=0.0001; 9) like 7 with no χ^2 test.

[b] Lesion: lesion length measured 3, 5, 7, 9, and 11 days post inoculation (dpi); Lesion-binomial: 0= resistant (lesions $\leq$ 3 cm, nine dpi), 1= susceptible (lesions > 3 cm); Lesion-polynomial: 0= resistant (lesions $\leq$ 3 cm, nine dpi), 1= susceptible (lesions > 6 cm), 2= intermediate; rAUDPC1-3: relative area under the disease progress curve for lesion length with readings between 3 and 11 dpi (1), 5 and 11 dpi (2), at 3, 7, and 11 dpi (3); mortality: percentage dead plants at 11 dpi; mortality-binomial: 0= resistant (mortality $\leq$ 20%, 11 dpi), 1= susceptible (mortality > 20%); mortality-polynomial: 0= resistant (mortality $\leq$ 20%, 11 dpi), 1 susceptible (mortality > 60%), 2= intermediate.

[c] Final LOD of 4 were used to construct the linkage groups

[d] Percentage of phenotypic variation explained by the QTL.

[e] Random position on the chromosome.

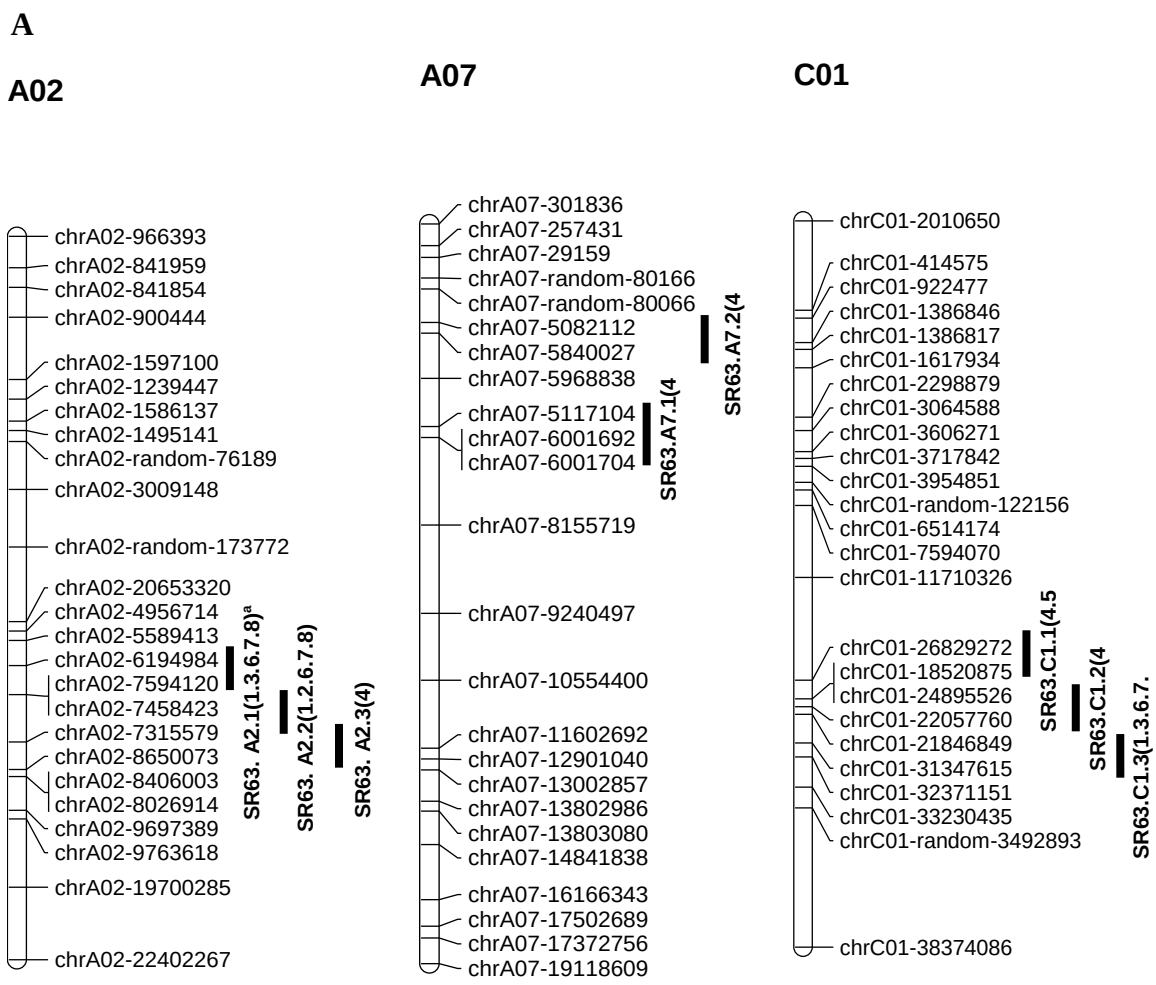


Figure 3.4. Partial genetic linkage map and significant quantitative trait loci (QTL) identified in T63DH population using different phenotyping and genotyping analyzing methods. A) no imputation, no unique mapping reads (UMR), B) no imputation, with UMR, C) with imputation and UMR. The linkage groups from "no Chi-Square" sub-analysis was used to represent QTL identified in all sub-analyses of each analysis. The vertical bars show the flanking markers of the QTL.

[a] Name of the QTL consists of the initials of the trait, name of the resistant parent in the mapping population, chromosome number, and a number was added if more than one QTL were identified on the same chromosome. For each QTL, numbers in parenthesis represent phenotyping scoring systems used in QTL detection: 1. Lesion length, 2) Lesion length-binomial, 3) Lesion length polynomial, 4) Mortality-binomial, 5) Mortality-polynomial, 6) relative area under the disease progress curve 1 (rAUDPC1) calculated using reading between 3 and 11 days post inoculation (dpi), 7) rAUDPC2, calculated using readings between 5 and 11 dpi, 8) rAUDPC3, calculated using readings from 3, 7, and 11 dpi.

[b] only one flanking marker is shown for QTL SR63.C1.5(3.6.7.8) since one of the markers was not mapped on the linkage group in " no Chi-Square" sub-analysis.

B

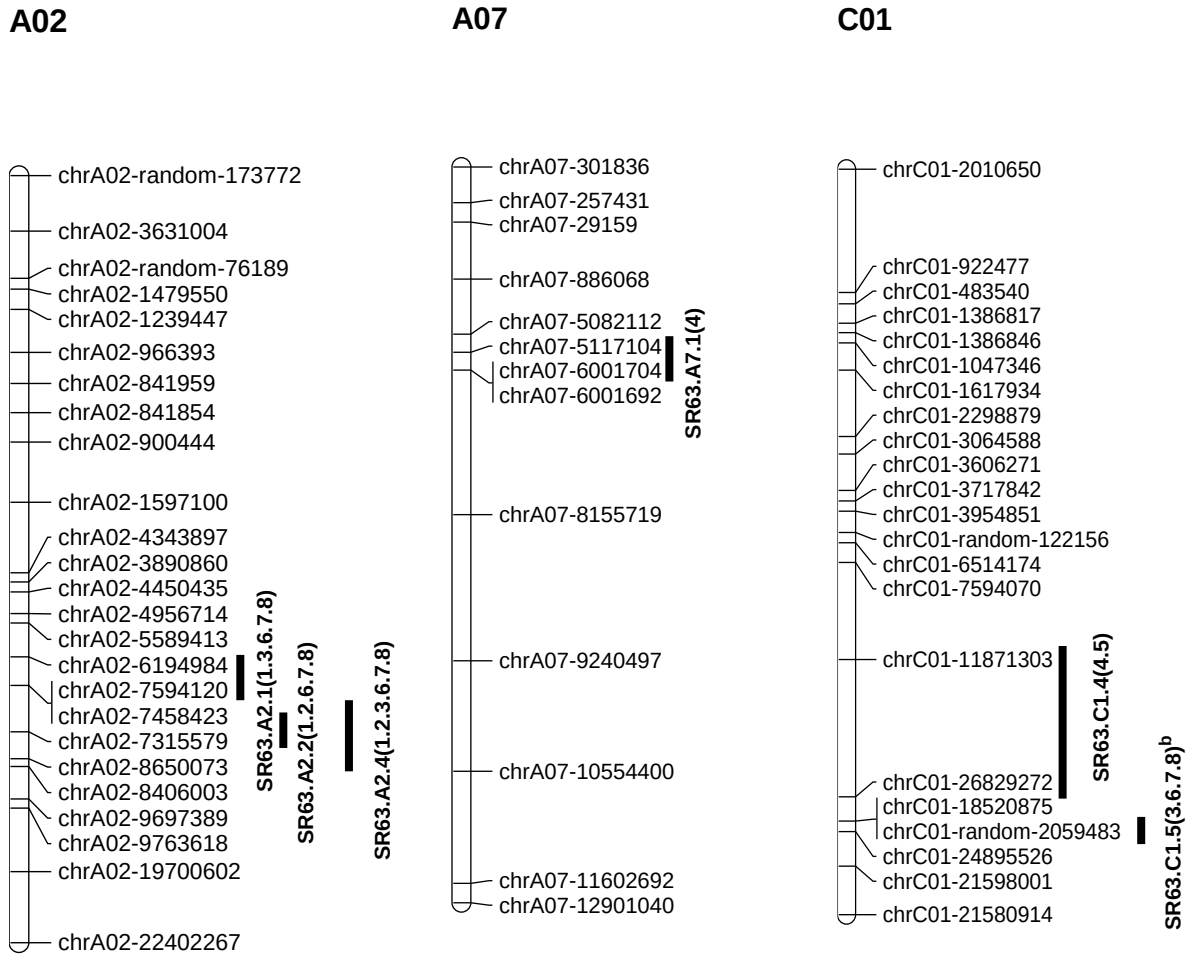


Figure 3.4. Partial genetic linkage map and significant quantitative trait loci (QTL) identified in T63DH population using different phenotyping and genotyping analyzing methods(continued). A) no imputation, no unique mapping reads (UMR), B) no imputation, with UMR, C) with imputation and UMR. The linkage groups from "no Chi-Square" sub-analysis was used to represent QTL identified in all sub-analyses of each analysis. The vertical bars show the flanking markers of the QTL.

[a] Name of the QTL consists of the initials of the trait, name of the resistant parent in the mapping population, chromosome number, and a number was added if more than one QTL were identified on the same chromosome. For each QTL, numbers in parenthesis represent phenotyping scoring systems used in QTL detection: 1. Lesion length, 2) Lesion length-binomial, 3) Lesion length polynomial, 4) Mortality-binomial, 5) Mortality-polynomial, 6) relative area under the disease progress curve 1 (rAUDPC1) calculated using reading between 3 and 11 days post inoculation (dpi), 7) rAUDPC2, calculated using readings between 5 and 11 dpi, 8) rAUDPC3, calculated using readings from 3, 7, and 11 dpi.

[b] only one flanking marker is shown for QTL SR63.C1.5(3.6.7.8) since one of the markers was not mapped on the linkage group in " no Chi-Square" sub-analysis.

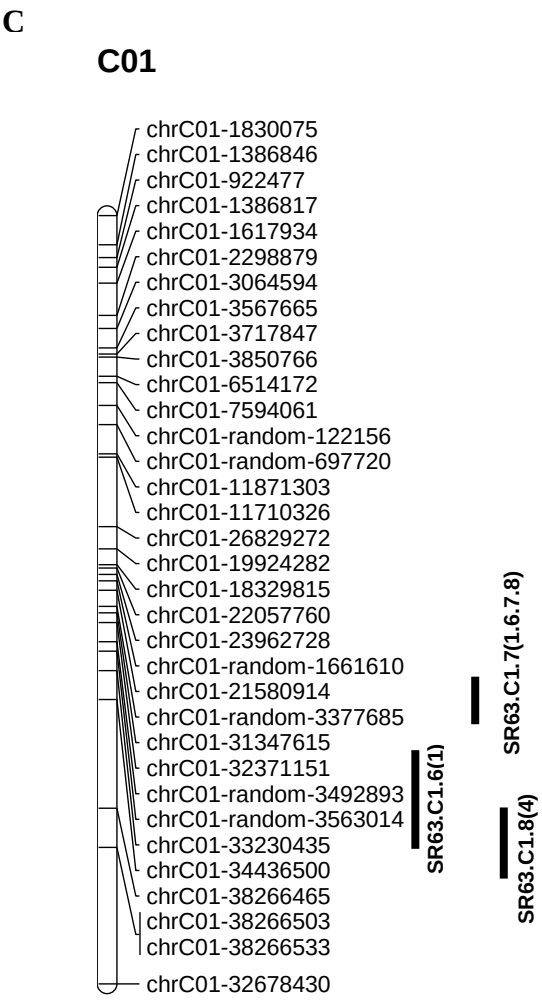


Figure 3.4. Partial genetic linkage map and significant quantitative trait loci (QTL) identified in T63DH population using different phenotyping and genotyping analyzing methods(continued). A) no imputation, no unique mapping reads (UMR), B) no imputation, with UMR, C) with imputation and UMR. The linkage groups from "no Chi-Square" sub-analysis was used to represent QTL identified in all sub-analyses of each analysis. The vertical bars show the flanking markers of the QTL.

[a] Name of the QTL consists of the initials of the trait, name of the resistant parent in the mapping population, chromosome number, and a number was added if more than one QTL were identified on the same chromosome. For each QTL, numbers in parenthesis represent phenotyping scoring systems used in QTL detection: 1. Lesion length, 2) Lesion length-binomial, 3) Lesion length polynomial, 4) Mortality-binomial, 5) Mortality-polynomial, 6) relative area under the disease progress curve 1 (rAUDPC1) calculated using reading between 3 and 11 days post inoculation (dpi), 7) rAUDPC2, calculated using readings between 5 and 11 dpi, 8) rAUDPC3, calculated using readings from 3, 7, and 11 dpi.

[b] only one flanking marker is shown for QTL SR63.C1.5(3.6.7.8) since one of the markers was not mapped on the linkage group in " no Chi-Square" sub-analysis.

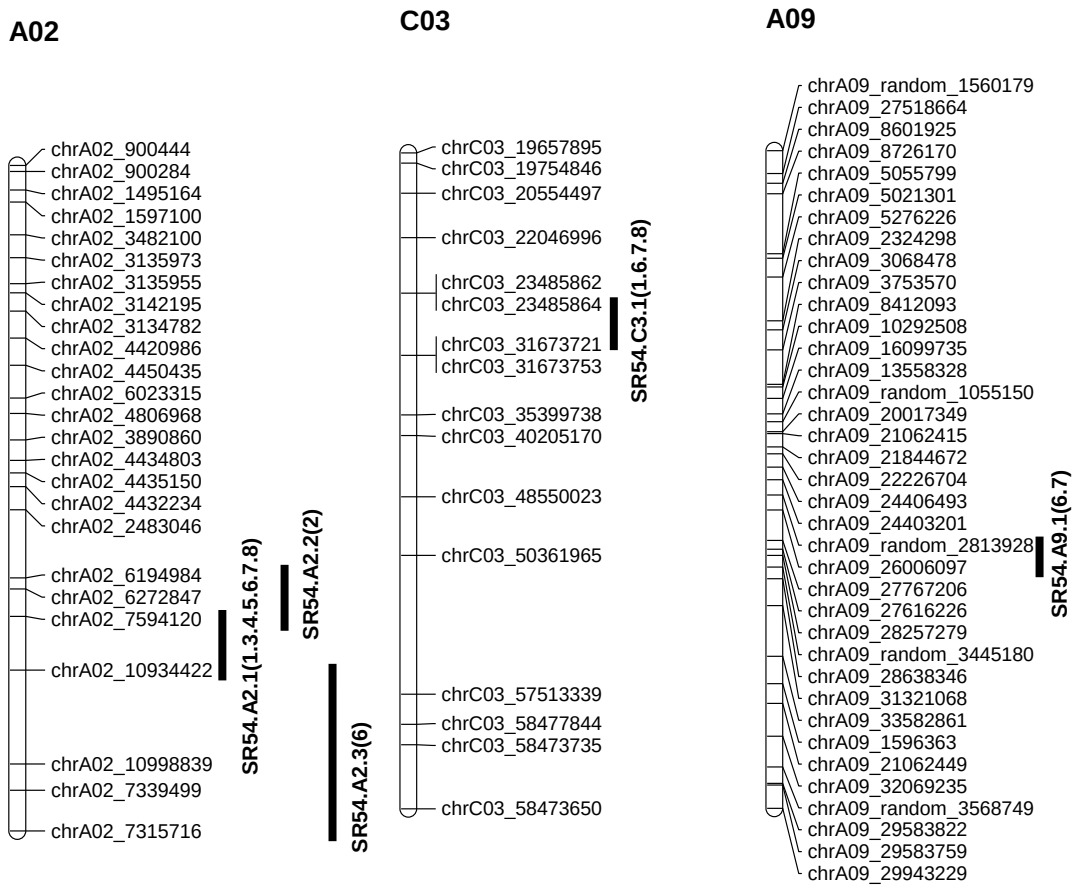


Figure 3.5. Partial genetic linkage maps and significant quantitative trait loci (QTL) identified in T54DH population using different phenotyping and genotyping analyses methods. The figure shows QTL identified in "with imputation and UMR", all three Chi-Square sub-analyses. The linkage groups from "no Chi-Square" sub-analysis was used to represent QTL identified in all sub-analyses of each analysis. The vertical bars show the flanking markers of the QTL.
[a] Name of the QTL consists of the initials of the trait, name of the resistant parent in the mapping population, chromosome number, and a number was added if more than one QTL were identified on the same chromosome. For each QTL, number in the parenthesis represent phenotyping scoring systems that detected the QTL. 1. Lesion, 2) Lesion-binomial, 3) Lesion polynomial, 4) Mortality-binomial, 5) Mortality-polynomial, 6) relative area under the disease progress curve 1 (rAUDPC1) calculated using reading between 3 and 11 days post inoculation (dpi), 7) rAUDPC2, calculated using readings between 5 and 11 dpi, 8) rAUDPC3, calculated using readings from 3, 7, and 11 dpi.

Markers' selection efficiency

The most efficient flanking marker was chrA02-7594120 which was linked to QTL SR63.A2.1 in T63DH, and to SR54.A2.1 and SR54.A2.2 in T54DH. This marker had an average

selection efficiency ranging between 60 and 66% in both populations and was linked to the QTL identified in imputed and non-imputed data and all eight phenotypic datasets. Two other markers, chrA02-7458423 linked to QTL SR63.A2.2 in T63DH and chrA02-10934422 linked to QTL SR54.A2.1 had selection efficiencies ranging between 59 and 60% (Table 3.5).

Table 3.5. Selection efficiency of single nucleotide polymorphism flanking markers linked to quantitative trait loci associated with reaction of *Brassica napus* lines in two doubled haploid mapping populations to inoculations with *Sclerotinia sclerotiorum*.

Population	QTL	Flanking markers	Selection efficiency (%)
T63DH	SR63.A2.1	chrA02-6194984	56
		chrA02-7594120	60
	SR63.A2.2	chrA02-7458423	60
		chrA02-7315579	45
	SR63.A7.1	chrA07-5117104	52
		chrA07-6001704	51
	SR63.C1.1	chrC01-26829272	42
		chrC01-18520875	41
	SR63.C1.3	chrC01-21846849	39
		chrC01-31347615	45
	SR63.C1.4	chrC01-11871303	40
		chrC01-26829272	42
	SR63.C1.7	chrC01-21580914	-[a]
		chrC01-random-3377685	30
T54DH	SR54.A2.1	chrA02-7594120	66
		chrA02-10934422	58
	SR54.A9.1	chrA09-random-2813928	55
		chrA09-26006097	49
	SR54.C3.1	chrC03-23485864	46
T54DH	SR54.C3.1	chrC03-31673721	50

[a] Marker was removed from the genotyping analysis during filtering step

Identification of potential candidate genes

Seventeen gene models associated with response to plant diseases were identified between the flanking markers of QTL SR63.A2.1 and SR63.A2.2 in T63DH, and SR54.A2.1 in T54DH. More information about the potential genes is presented in Appendix C.

Discussion

This study evaluated the impact of multiple arrangements of the phenotypic data in combination with different SNP calling and filtering procedures on the number of genomic

regions associated with reaction of *B. napus* DH plants to infection by *S. sclerotiorum*. A total of 19 QTL, located on chromosomes A02, A07, A09, C01, and C03 were identified. Multiple QTL detected on A02 were located between the physical positions of 6023309 to 10934422 base pair (bp). This region seems to be rich in QTL associated with reaction to *S. sclerotiorum* since an additional four QTL have been reported recently by other researchers in the same physical interval, e.g. SRA2a, SRA2b, and SRA2c by Qasim et al. (2020) and qSR10-1 by Wei et al. (2014). Similarly, two QTL detected on A09 (26006097-31321068 bp) also was detected in other studies; for example, QTL SRA9a, SRA9b (Qasim et al. 2020), qSR11-1 (Wei et al. 2014), and SRA9-1 (Wu et al. 2013). Qasim et al. (2020) reported QTL SRC3a, and SRC3b in the same physical region of QTL SR54.C.3.1 which was located on C03 chromosome (23485864-31673721 bp). Further, in a GWAS study conducted by Gyawali et al. (2016), a SSR marker located on C01 chromosome (C01- 29218479) was associated with SRR resistance; this locus is located within the C01 range as QTL SR63.C1.3 (21846849-31347615 bp) identified in our study. To our knowledge, QTL SR.63.A7.1 and SR63.A7.2, located on chromosome A07 (5117104-6001704 bp) have not been reported previously.

The choice of phenotypic dataset had a large effect on the ability to detect those genomic regions but not on their R^2 values. Further, additive effects of the QTL were generally greater when they were identified with lesion size and rAUDPC datasets. QTL were more consistently detected in sets carrying lesion length or rAUDPC data derived from lesion length than any other sets; mortality binomial data was useful in T63DH, which had an average mortality of 41% but not in T54DH, which had a mortality below 10%. Polynomial datasets allowed for the detection of only a few QTL. Some QTL mapping studies have been conducted in canola (Derbyshire and Denton-Giles 2016) to identify genomic regions associated with response to *S. sclerotiorum* but

in most of them a single observation of lesion length, usually recorded between three and nine dpi, was used (Qasim et al. 2020; Wu et al. 2013, 2019; Yin et al. 2010; Zhao et al. 2006). In this study, lesion length data collected on the ninth dpi was selected for analysis because it showed the clearest separation between lines before plant mortality significantly increased the number of missing data points. rAUPDC sets did not have that limitation and therefore provided a more reliable assessment of the reaction of lines to disease compared to a single reading of lesion length. This translated into a better ability to detect QTL as lesion length data allowed for the detection of a total of eight QTL while rAUDPC1, calculated using five lesion length reading times, detected a total of 10 including seven of the lesion length QTL. Reducing reading times to four and three (rAUDPC2 and 3) resulted in identification of nine and eight QTL, respectively. Deriving binomial and polynomial datasets from lesion length contributed to identification of three and five QTL, respectively, while their effects were reduced. These results contrast with those reported by researchers conducting GWAS on other pathosystems (Oladzad et al. 2019; Zitnick-Anderson et al. 2020). It is possible this discrepancy was due in part to the type of transformation required to derive the new sets, e.g. going from categorical to binomial/polynomial for the former two and from continuous to categorical in the work presented here; but also, to the fact that biparental mapping populations are genetically less diverse than those used in GWAS. Reducing the number of phenotypic categories in the former population contributes to the reduced probability of detecting QTL.

The role of plant mortality as (percentage of the dead plants) scoring system also was evaluated in this study. Distribution of this dataset was skewed to the right and showed that at 11 dpi, many lines had alive plants with large lesions (susceptible reaction). Recording mortality

percentage later, e.g. 15 dpi, could solve this issue since distribution of mortality was shifting towards normality as the distance between inoculation and mortality measurement increased.

SNP calling and filtering procedures influenced the results of the study. Detection of SNP markers in polyploid crops could be challenging due to a high rate of false positive SNPs (Clevenger and Ozias-Akins 2015). Using UMR, i.e. reading a single copy, (Clevenger et al. 2015) is an effective strategy to overcome this limitation in *B. napus*. Nevertheless, and while filtering based on UMR have been used in several mapping studies of *B. napus* agronomic traits such as pod number, (Ye et al. 2017), fatty acid composition, flowering time, and growth traits (Li et al. 2018), and seed yield, silique length, and oil content (Lu et al. 2019), detailed evaluations of the impact of this practice on detection of QTL associated with reaction to plant diseases had not been made. In this study, filtering based on UMR decreased the number of markers on the linkage groups and the number of the identified QTL decreased or remained unchanged in both populations. Further, UMR did not influence the R^2 values significantly; suggesting that this step might not be required for DH populations with a high level of homozygosity.

GBS is a cost effective and efficient method in development of high-density genetic maps; however, it normally generates a high level of missing data (Elshire et al. 2011; Rutkoski et al. 2013). Genomic imputation can recover a high percentage of these missing information (Huang et al. 2014). Although, imputation of the genotyping data is not a common step in QTL bi-parental mapping studies due to the categorical nature of the data (Demetrashvili et al. 2013), application of genotyping data imputation has been reported in some bi-parental QTL analyses studies. In a study conducted by Spindel et al. (2013) imputation of the genetic data expanded the genetic map of a recombinant inbred lines (RIL) population of rice. Our results agree with their

findings as data imputation increased the number of markers available for mapping and the total map length of *B. napus* by 34 and 45%, respectively, in T63DH population and by 33 and 79%, respectively, in T54DH population. However, the effect of data imputation on the number of QTL detected was population dependent. Fewer genomic regions associate to SSR were identified in T63DH where data was imputed; this could be due to inserting extra markers between the flaking markers of the detected QTL and therefore, mask those QTL. In T54DH, application of data imputation was essential since the high level of missing data hindered identification of significant QTL in non-imputed sets. Both imputed and non-imputed data could identify QTL that are located in the genomic regions known to be involved in reactions to SSR (Gyawali et al. 2016; Qasim et al. 2020; Wei et al. 2014; Wu et al. 2013) which verifies the accuracy of these methods in the QTL analysis of biparental populations.

Presence of segregation-distorted regions in DH populations, specifically in microspore culture-derived populations, has been verified (Alheit et al. 2011). While some researchers have found the exclusion of distorted markers useful (ArifUzZaman et al. 2016; Huang and Yan 2019; Pascual et al. 2016), others considered it excluded too many unique markers (Tyrka et al. 2018). Further, it has been theorized that segregation distortion has an adverse effect on the power of QTL with dominance effect while it is not necessarily detrimental to those with additive effects (Xu 2008). In doubled haploids and recombinant inbred lines, the QTL effects are purely additive (Liu 1997); therefore, including distorted markers in the QTL mapping could be beneficial. Results of our study showed the number of markers on linkage groups and total genetic map coverage increased when less stringent Chi-Square test or no test was used; this agrees with finding of the study conducted by Tyrka et al. (2018). However, the effect of the test on the number of detected QTL and R^2 values was minimal.

Deriving multiple phenotypic datasets from lesion length and plant mortality increased the number of QTL and therefore the return on investment. The standard approach, using lesion length data from a single point in time, e.g. 9 dpi in this study, yielded eight QTL. The combined use of binomial and polynomial datasets derived from lesion length and plant mortality and the use of rAUDPC sets increased that output by 2.4-fold. In terms of QTL effect, in general, QTL detected in lesion length and rAUDPC datasets had an average R^2 value of 13% and 10% in T63DH and T54DH, respectively, while binomial and polynomial sets had averages of 12% and 10%, respectively for the same populations.

A total of 17 candidate genes were identified in this study; QTL SR63.A2.1, SR63.A2.2, and SR54.A2.1, were associated to five, two, and twelve of these genes, respectively. Five defensin-like protein coding genes were identified between the flanking markers of the QTL SR54.A2.1. Defensin proteins are well known for their role in plant defense mechanisms (Lacerda et al. 2014; Sels et al. 2008; Seo et al. 2014). Proteins coded by other genes were classified in different classes including NDR1/HIN1 proteins, isocitrate dehydrogenase, farnesoic acid carboxyl-O-methyltransferase, clathrin assembly protein, peroxidase, WAT1-related protein, MLP-like protein, NAC domain-containing protein, and CLAVATA 3. Appendix C shows the details about the potential candidate genes. Three genes, BnaA02g12230D, BnaA02g14050D, and BnaA02g15660D, have been previously associated with resistance to *S. sclerotiorum*. The roles of two other genes, BnaA02g15190D and BnaA02g15240D, identified in proximity to QTL SR54.A2.1, could not be verified through literature search.

Overall, findings of this study provided insights into the role of phenotyping scoring systems and genotyping analyses in the result of the QTL mapping of a complicated pathosystem where the crop is a polyploid and the nature of the resistance to the disease is quantitative. Using

imputed and non-imputed genotyping data and different phenotyping systems resulted in identification of QTL located on regions known to be involved in SSR resistance and in new genomic regions. Markers associated with these regions could be useful in breeding programs after complementary experiments for evaluation of markers' efficiency under the field conditions, and functional analysis of potential candidate genes. Further, deriving phenotypic datasets may contribute to increase the output of the analysis providing unique QTL and a better return on the investment made.

References

Alheit, K. V., Reif, J. C., Maurer, H. P., Hahn, V., Weissmann, E. A., Miedaner, T., and Würschum, T. 2011. Detection of segregation distortion loci in triticale (*x Triticosecale Wittmack*) based on a high-density DArT marker consensus genetic linkage map. BMC genomics 12: 380-392.

Amosova, A. V., Zoshchuk, S. A., Volovik, V. T., Shirokova, A. V., Horuzhiy, N. E., Mozgova, G. V., Yurkevich, O. Y., Artyukhova, M. A., Lemesh, V. A., Samatadze, T. E., and Muravenko, O. V. 2019. Phenotypic, biochemical and genomic variability in generations of the rapeseed (*Brassica napus* L.) mutant lines obtained via chemical mutagenesis. PLoS One 14:e0221699.

Arahana, V. S., Graef, G. L., Specht, J. E., Steadman, J. R., and Eskridge, K. M. 2001. Identification of QTLs for resistance to *Sclerotinia sclerotiorum* in soybean. Crop Sci. 41:180-188.

ArifUzZaman, M., Mamidi, S., McClean, P., and Rahman, M. 2016. QTL mapping for root vigor and days to flowering in *Brassica napus* L. Can. J. Plant Sci. 97:99-109.

Arkwazee, H. A. 2018. Quantitative trait loci and genome wide association study for resistance to white mold in common (snap) bean. Dissertation, Oregano State University.

Ashtari Mahini, R., Kumar, A., Elias, E. M., Fiedler, J. D., Porter, L. D., and McPhee, K. E. 2020. Analysis and identification of QTL for resistance to *Sclerotinia sclerotiorum* in Pea (*Pisum sativum* L.). Front. Genet. 11:587968.

Bindschedler, L. V., Dewdney, J., Blee, K. A., Stone, J. M., Asai, T., Plotnikov, J., Denoux, C., Hayes, T., Gerrish, C., Davies, D. R., and Ausubel, F. M. 2006. Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. Plant J. 47:851-863.

Century, K. S., Shapiro, A. D., Repetti, P. P., Dahlbeck, D., Holub, E., and Staskawicz, B. J. 1997. NDR1, a pathogen-induced component required for Arabidopsis disease resistance. Science 278:1963-1965.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. A., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., and Corréa, M. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950-953.

Chatukuta, P., Dikobe, T. B., Kawadza, D. T., Sehlabane, K. S., Takundwa, M. M., Wong, A., Gehring, C., and Ruzvidzo, O. 2018. An Arabidopsis clathrin assembly protein with a predicted role in plant defense can function as an adenylate cyclase. Biomolecules 8:1-15.

Clevenger, J., Chavarro, C., Pearl, S. A., Ozias-Akins, P., and Jackson, S. A. 2015. Single nucleotide polymorphism identification in polyploids: a review, example, and recommendations. Mol. Plant 8:831-846.

Clevenger, J., and Ozias-Akins, P. 2015. SWEEP: a tool for filtering high-quality SNPs in polyploid crops. G3-Genes Genom. Genet. 5:1797-1803.

da Silva, C. L., Fritz, A., Clinesmith, M., Poland, J., Dowell, F., and Peiris, K. 2019. QTL mapping of Fusarium head blight resistance and deoxynivalenol accumulation in the Kansas wheat variety 'Everest'. Mol. Breed. 39:35-56.

Dai, B., Guo, H., Huang, C., Ahmed, M. M., and Lin, Z. 2017. Identification and characterization of segregation distortion loci on cotton chromosome 18. Front. Plant Sci. 7:2037-2047.

del Río, L. E., Bradley, C. A., Henson, R. A., Endres, G. J., Hanson, B. K., McKay, K., Halvorson, M., Porter, P. M., Le Gare, D. G., and Lamey, H. A. 2007. Impact of Sclerotinia stem rot on yield of canola. Plant Dis. 91:191-194.

Delourme, R., Falentin, C., Fomeju, B. F., Boillot, M., Lassalle, G., André, I., Duarte, J., Gauthier, V., Lucante, N., Marty, A., and Pauchon, M. 2013. High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. BMC genomics 14:120-137.

Demetrashvili, N., Van den Heuvel, E. R., and Wit, E. C. 2013. Probability genotype imputation method and integrated weighted lasso for QTL identification. BMC Genet. 14:125-143.

Denancé, N., Ranocha, P., Oria, N., Barlet, X., Rivière, M. P., Yadeta, K. A., Hoffmann, L., Perreau, F., Clément, G., Maia-Grondard, A., and Van den Berg, G. C. 2013. Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, *Ralstonia solanacearum*, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. Plant J. 73:225-239.

Derbyshire, M. C., and Denton-Giles, M. 2016. The control of Sclerotinia stem rot on oilseed rape (*Brassica napus*): current practices and future opportunities. Plant Pathol. 65:859-877.

Disi, J. O., Mei, J., Wei, D., Ding, Y., and Qian, W. 2014. Inheritance of leaf and stem resistance to *Sclerotinia sclerotiorum* in a cross between *Brassica incana* and *Brassica oleracea* var. *alboglabra*. J. Agric. Sci. 152:146-152.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379.

Ewing, B., and Green, P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8:186-194.

Ferrie, A. M. R., Dirpaul, J., Krishna, P., and Keller, W. A. 2005. Effects of brassinosteroids on microspore embryogenesis in Brassica species. In Vitro Cell. Dev. Biol. 41:742-745.

Fragoso, C. A., Heffelfinger, C., Zhao, H., and Dellaporta, S. L. 2016. Imputing genotypes in biallelic populations from low-coverage sequence data. Genetics 202:487-495.

Gamborg, O. L., Miller, R., and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.

Garg, H., Li, H., Sivasithamparam, K., and Barbetti, M. J. 2013. Differentially expressed proteins and associated histological and disease progression changes in cotyledon tissue of a resistant and susceptible genotype of *Brassica napus* infected with *Sclerotinia sclerotiorum*. PLoS One 8:e65205.

Girke, A., Schierholt, A., and Becker, H. C. 2012. Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theor. Appl. Genet. 124:1017-1026.

Greenfield, D. L., Stegle, O., and Rrustemi, A. 2016. GeneCodeq: quality score compression and improved genotyping using a Bayesian framework. Bioinformatics 32:3124-3132.

Gyawali, S., Harrington, M., Durkin, J., Horner, K., Parkin, I. A., Hegedus, D. D., Bekkaoui, D., and Buchwaldt, L. 2016. Microsatellite markers used for genome-wide association mapping of partial resistance to *Sclerotinia sclerotiorum* in a world collection of *Brassica napus*. Mol. Breed. 36:72-84.

Howell, E. C., Kearsey, M. J., Jones, G. H., King, G. J., and Armstrong, S. J. 2008. A and C genome distinction and chromosome identification in *Brassica napus* by sequential fluorescence in situ hybridization and genomic in situ hybridization. Genetics 180:1849-1857.

Huang, B. E., Raghavan, C., Mauleon, R., Broman, K. W., and Leung, H. 2014. Efficient imputation of missing markers in low-coverage genotyping-by-sequencing data from multiparental crosses. Genetics 197:401-404.

Huang, L., and Yan, X. 2019. Construction of a genetic linkage map in *Pyropia yezoensis* (Bangiales, Rhodophyta) and QTL analysis of several economic traits of blades. PLoS One 14:e0209128.

Joshi, N. A., and Fass J. N. 2011. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [software]. Available at https://github.com/najoshi/sickle.

Koboldt, D. C., Zhang, Q., Larson, D. E., Shen, D., McLellan, M. D., Lin, L., Miller, C. A., Mardis, E. R., Ding, L., and Wilson, R. K. 2012. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 22:568-576.

Kosambi, D. D. 1943. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175. Available: http://doi.wiley.com/10.1111/j.1469-1809.1943.tb02321.x.

Lacerda, A., Vasconcelos, É. A. R., Pelegrini, P. B., and Grossi-de-Sa, M. F. 2014. Antifungal defensins and their role in plant defense. Front. Microbial. 5:116-225.

Lara, L. A .D. C., Santos, J. B. D., Veloso, J. S., Balestre, M., Alves, F. C., and Leite, M. E. 2014. Identification of QTLs for resistance to *Sclerotinia sclerotiorum* in carioca common bean by the moving away method. Int. Sch. Res. Notices 1-7.

Lee, H., Chah, O. K., and Sheen, J. 2011. Stem-cell-triggered immunity through CLV3p–FLS2 signaling. Nature 473:376-379.

Leite, M. E., Santos, J. B., Ribeiro, P. M., Souza, D. A., Castro Lara, L. A., and Resende, M. L. V. 2014. Biochemical responses associated with common bean defense against *Sclerotinia sclerotiorum*. Eur. J. Plant. Pathol. 138:391-404.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Li, R., Jeong, K., Davis, J. T., Kim, S., Lee, S., Michelmore, R. W., Kim, S., and Maloof, J. N. 2018. Integrated QTL and eQTL mapping provides insights and candidate genes for fatty acid composition, flowering time, and growth traits in a F2 population of a novel synthetic allopolyploid *Brassica napus*. Front. Plant Sci. 9:1632-1650.

Lin, R., Zhao, W., Meng, X., Wang, M., and Peng, Y. 2007. Rice gene OsNAC19 encodes a novel NAC-domain transcription factor and responds to infection by *Magnaporthe grisea*. Plant Sci. 172:120-130.

Liu, B. H. 1997. Statistical genomics: linkage, mapping, and QTL analysis. CRC press, Boca Raton, Florida.

Lorieux, M. 2012. MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breed. 30:1231-1235.

Lu, K., Wei, L., Li, X., Wang, Y., Wu, J., Liu, M., Zhang, C., Chen, Z., Xiao, Z., Jian, H., and Cheng, F. 2019. Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. Nat. Commun.10:1-12.

Mahini, R. A. 2018. Analysis and Identification of QTL for Resistance to *Sclerotinia sclerotiorum* in Pea *(Pisum sativum* L.). Dissertation, North Dakota State University.

Meng, L., Li, H., Zhang, L., and Wang, J. 2015. QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J. 3:269-283.

Mhamdi, A., Mauve, C., Gouia, H., Saindrenan, P., Hodges, M., and Noctor, G. 2010. Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in Arabidopsis leaves. Plant Cell environ. 33:1112-1123.

Miklas, P. N., Porter, L. D., Kelly, J. D., and Myers, J. R. 2013. Characterization of white mold disease avoidance in common bean. Eur. J. Plant Pathol. 135:525-543.

Mkwaila, W., Terpstra, K. A., Ender, M., and Kelly, J. D. 2011. Identification of QTL for agronomic traits and resistance to white mold in wild and landrace germplasm of common bean. Plant Breed. 130:665-672.

Mohammadi, P. P., Moieni, A., Ebrahimi, A., and Javidfar, F. 2012. Doubled haploid plants following colchicine treatment of microspore-derived embryos of oilseed rape (*Brassica napus* L.). Plant Cell Tiss. Org. 108:251-256.

Nakashima, K., Tran, L. S. P., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2007. Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. Plant J. 51:617-630.

Nixon, J. 2006. Testing for segregation distortion in genetic scoring data from backcross or doubled haploid populations. Heredity 96:290-297.

Nuruzzaman, M., Sharoni, A. M., and Kikuchi, S. 2013. Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. Front. Microbial. 4:248-263.

O'Brien, J. A., Daudi, A., Finch, P., Butt, V. S., Whitelegge, J. P., Souda, P., Ausubel, F. M., and Bolwell, G. P. 2012. A peroxidase-dependent apoplastic oxidative burst in cultured Arabidopsis cells functions in MAMP-elicited defense. Plant Physiol. 158:2013-2027.

Oladzad-Abbasabadi, A., Kumar, A., Pirseyedi, S., Salsman, E., Dobrydina, M., Poudel, R. S., AbuHammad, W. A., Chao, S., Faris, J. D., and Elias, E. M. 2018. Identification and validation of a new source of low grain cadmium accumulation in durum wheat. G3-Genes Genom. Genet. 8:923-932.

Oladzad, A., Zitnick-Anderson, K., Jain, S., Simons, K., Osorno, J. M., McClean, P. E., and Pasche, J. 2019. Genotypes and genomic regions associated with *Rhizoctonia solani* resistance in common bean. Front. Plant Sci. 10:956-969.

Pan, Q., Cui, B., Deng, F., Quan, J., Loake, G. J., and Shan, W. 2016. RTP 1 encodes a novel endoplasmic reticulum (ER)-localized protein in Arabidopsis and negatively regulates resistance against biotrophic pathogens. New Phytol. 209:1641-1654.

Pascual, L., Albert, E., Sauvage, C., Duangjit, J., Bouchet, J. P., Bitton, F., Desplat, N., Brunel, D., Le Paslier, M. C., Ranc, N., and Bruguier, L. 2016. Dissecting quantitative trait variation in the resequencing era: complementarity of bi-parental, multi-parental and association panels. Plant Sci. 242:120-130.

Pérez-Vega, E., Pascual, A., Campa, A., Giraldez, R., Miklas, P. N., and Ferreira, J. J. 2012. Mapping quantitative trait loci conferring partial physiological resistance to white mold in the common bean RIL population Xana× Cornell 49242. Mol. Breed. 29:31-41.

Porcu, E., Sanna, S., Fuchsberger, C., and Fritsche, L. G. 2013. Genotype imputation in genome-wide association studies. Curr. Protoc. Hum. Genet. 78:1-25.

Qasim, M. U., Zhao, Q., Shahid, M., Samad, R. A., Ahmar, S., Wu, J., Fan, C., and Zhou, Y. 2020. Identification of QTLs containing resistance genes for Sclerotinia stem rot in *Brassica napus* using comparative transcriptomic studies. Front. Plant Sci. 11:776-789.

Rothmann, L. A., and McLaren, N. W. 2018. *Sclerotinia sclerotiorum* disease prediction: A review and potential applications in South Africa. S. Afr. J. Sci. 114:1-9.

Rutkoski, J. E., Poland, J., Jannink, J. L., and Sorrells, M. E. 2013. Imputation of unordered markers and the impact on genomic selection accuracy. G3-Genes Genom. Genet. 3:427-439.

Saharan, G. S., and Mehta, N. 2008. Sclerotinia diseases of crop plants: biology, ecology and disease management. Springer Science & Business Media, Netherlands.

Scheet, P., and Stephens, M. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. Am. J. Hum. Genet. 78:629-644.

Sels, J., Mathys, J., De Coninck, B. M., Cammue, B. P., and De Bolle, M. F. 2008. Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol. Biochem. 46:941-950.

Seo, H. H., Park, S., Park, S., Oh, B. J., Back, K., Han, O., Kim, J. I., and Kim, Y. S. 2014. Overexpression of a defensin enhances resistance to a fruit-specific anthracnose fungus in pepper. PLoS One 9:e97936.

Shahoveisi, F., and del Río Mendoza, L. E. 2020. Effect of wetness duration and incubation temperature on development of ascosporic infections by *Sclerotinia sclerotiorum*. Plant Dis. 104:1817-1823.

Snowdon, R., Lühs, W., and Friedt, W. 2007. Oilseed rape. Springer, Berlin, Heidelberg.

Soule, M., Porter, L., Medina, J., Santana, G. P., Blair, M. W., and Miklas, P. N. 2011. Comparative QTL map for white mold resistance in common bean, and characterization of partial resistance in dry bean lines VA19 and I9365-3. Crop Sci. 51:123-139.

Spindel, J., Wright, M., Chen, C., Cobb, J., Gage, J., Harrington, S., Lorieux, M., Ahmadi, N., and McCouch, S. 2013. Bridging the genotyping gap: using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. Theor. Appl. Genet. 126:2699-2716.

Sun, F., Fan, G., Hu, Q., Zhou, Y., Guan, M., Tong, C., Li, J., Du, D., Qi, C., Jiang, L., and Liu, W. 2017. The high-quality genome of *Brassica napus* cultivar 'ZS 11'reveals the introgression history in semi-winter morphotype. Plant J. 92:452-468.

Swarts, K., Li, H., Romero Navarro, J. A., An, D., Romay, M. C., Hearne, S., Acharya, C., Glaubitz, J. C., Mitchell, S., Elshire, R. J., and Buckler, E. S. 2014. Novel methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. Plant Genome 7:1-12.

Tena, G., Boudsocq, M., and Sheen, J. 2011. Protein kinase signaling networks in plant innate immunity. Curr. Opin. Plant Boil. 14:519-529.

Tyrka, M., Oleszczuk, S., Rabiza-Swider, J., Wos, H., Wedzony, M., Zimny, J., Ponitka, A., Ślusarkiewicz-Jarzina, A., Metzger, R. J., Baenziger, P. S., and Lukaszewski, A. J. 2018. Populations of doubled haploids for genetic mapping in hexaploid winter triticale. Mol. Breed. 38:46-61.

United States Department of Agriculture National agricultural Statistics Service. 2019. https://www.nass.usda.gov.

Varet, A., Parker, J., Tornero, P., Nass, N., Nürnberger, T., Dangl, J. L., Scheel, D., and Lee, J. 2002. NHL25 and NHL3, two NDR1/HIN1-like genes in *Arabidopsis thaliana* with potential role (s) in plant defense. Mol. Plant Microbe Interact. 15:608-616.

Vasconcellos, R. C., Oraguzie, O. B., Soler, A., Arkwazee, H., Myers, J. R., Ferreira, J. J., Song, Q., McClean, P., and Miklas, P. N. 2017. Meta-QTL for resistance to white mold in common bean. PLoS One 12:e0171685.

Vogel, J. P., Raab, T. K., Schiff, C., and Somerville, S. C. 2002. PMR6, a pectate lyase–like gene required for powdery mildew susceptibility in Arabidopsis. Plant Cell 14:2095-2106.

Voorrips, R. E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77-78.

Wang, X. E., Basnayake, B. V. S., Zhang, H., Li, G., Li, W., Virk, N., Mengiste, T., and Song, F. 2009. The Arabidopsis ATAF1, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens. Mol. Plant-Microbe Interact. 22:1227-1238.

Wei, D., Mei, J., Fu, Y., Disi, J. O., Li, J., and Qian, W. 2014. Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol. Breed. 34:1797-1804.

Wu, J., Cai, G., Tu, J., Li, L., Liu, S., Luo, X., Zhou, L., Fan, C., and Zhou, Y. 2013. Identification of QTLs for resistance to Sclerotinia stem rot and BnaC. IGMT5. a as a candidate gene of the major resistant QTL SRC6 in *Brassica napus*. PLoS One 8:e67740.

Wu, J., Chen, P., Zhao, Q., Cai, G., Hu, Y., Xiang, Y., Yang, Q., Wang, Y., and Zhou, Y. 2019. Co-location of QTL for Sclerotinia stem rot resistance and flowering time in *Brassica napus*. Crop J. 7:227-237.

Xie, W., Feng, Q., Yu, H., Huang, X., Zhao, Q., Xing, Y., Yu, S., Han, B., and Zhang, Q. 2010. Parent-independent genotyping for constructing an ultrahigh-density linkage map based on population sequencing. Proc. Natl. Acad. Sci. 107:10578-10583.

Xu, S. 2008. Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201-2208.

Yang, Y., Yuan, J. S., Ross, J., Noel, J. P., Pichersky, E., and Chen, F. 2006. An *Arabidopsis thaliana* methyltransferase capable of methylating farnesoic acid. Arch. Biochem. Biophys. 448:123-132.

Yang, C. L., Liang, S., Wang, H. Y., Han, L. B., Wang, F. X., Cheng, H. Q., Wu, X. M., Qu, Z. L., Wu, J. H., and Xia, G. X. 2015. Cotton major latex protein 28 functions as a positive

regulator of the ethylene responsive factor 6 in defense against *Verticillium dahliae*. Mole. Plant 8:399-411.

Ye, J., Yang, Y., Chen, B., Shi, J., Luo, M., Zhan, J., Wang, X., Liu, G., and Wang, H. 2017. An integrated analysis of QTL mapping and RNA sequencing provides further insights and promising candidates for pod number variation in rapeseed (*Brassica napus* L.). BMC genomics 18:71-84.

Yin, X., Yi, B., Chen, W., Zhang, W., Tu, J., Fernando, W. D., and Fu, T. 2010. Mapping of QTLs detected in a *Brassica napus* DH population for resistance to *Sclerotinia sclerotiorum* in multiple environments. Euphytica 173:25-35.

Yu, F., Zhang, X., Peng, G., Falk, K. C., Strelkov, S. E., and Gossen, B. D. 2017. Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Sci. Rep. 7:1-11.

Zhao, J., and Meng, J. 2003. Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 106:759-764.

Zhao, J., Udall, J. A., Quijada, P. A., Grau, C. R., Meng, J., and Osborn, T.C. 2006. Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. 112:509-516.

Zheng, C., Boer, M. P., and van Eeuwijk, F. A. 2018. Accurate genotype imputation in multiparental populations from low-coverage sequence. Genetics 210:71-82.

Zheng, M. S., Takahashi, H., Miyazaki, A., Hamamoto, H., Shah, J., Yamaguchi, I., and Kusano, T. 2004. Up-regulation of *Arabidopsis thaliana* NHL10 in the hypersensitive response to

Cucumber mosaic virus infection and in senescing leaves is controlled by signaling pathways that differ in salicylate involvement. Planta 218:740-750.

Zhu, W., Wei, W., Fu, Y., Cheng, J., Xie, J., Li, G., Yi, X., Kang, Z., Dickman, M. B., and Jiang, D. 2013. A secretory protein of necrotrophic fungus *Sclerotinia sclerotiorum* that suppresses host resistance. PLoS One 8:e53901.

Zitnick-Anderson, K., Oladzadabbasabadi, A., Jain, S., Modderman, C., Osorno, J. M., McClean, P. E., and Pasche, J. S. 2020. Sources of resistance to *Fusarium solani* and associated genomic regions in common bean diversity panels. Front. Genet. 11:475-488.

Zuo, J. F., Niu, Y., Cheng, P., Feng, J. Y., Han, S. F., Zhang, Y. H., Shu, G., Wang, Y., and Zhang, Y. M. 2019. Effect of marker segregation distortion on high density linkage map construction and QTL mapping in Soybean (*Glycine max* L.). Heredity 123:579-592

CHAPTER 4. EFFECT OF WETNESS DURATION AND INCUBATION TEMPERATURE ON DEVELOPMENT OF ASCOSPORIC INFECTION BY *SCLEROTINIA SCLEROTIORUM* [1]

Abstract

The impact of wetness duration and incubation temperatures on *Sclerotinia sclerotiorum* ascospore germination and ascosporic infection efficiency were evaluated. Ascospore germination was optimal when incubated in continuous moisture (free water) at 21 °C. Significantly lower germination was observed at 10 or 30 °C. In infection efficiency studies, canola flowers were inoculated with dry ascospores and placed on leaves of canola plants which were incubated for 240 h at 10, 15, 20, 25, or 30 °C in alternating 6 to 18 h wet/18 to 6 h dry periods. Interrupting wet incubation delayed symptom appearance and hindered development of the epidemics. A logistic regression model estimated at 50% the probability of disease development when 48 h of wet incubation at 20 °C accumulated in a period of 6 days. The selected model was validated using data from field trials. Results of these studies will contribute to develop more accurate warning models for diseases caused by *S. sclerotiorum*.

Introduction

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is endemic to the state of North Dakota where it causes economically significant losses in canola, dry bean, and sunflower (del Río et al. 2007). In addition to these crops, however, *S. sclerotiorum* can attack more than 400

---

[1]This chapter published as: Shahoveisi, F., and del Río Mendoza, L. E. 2020. Effect of wetness duration and incubation temperature on the development of ascosporic infections by Sclerotinia sclerotiorum. Plant Dis. 104:1817-1823. Shahoveisi had primary responsibility for conducting experiments, collecting and analyzing data, and writing the manuscript. del Rio Mendoza served as a proofreader and checked the statistical analysis and edited the manuscript.

other plant species including some currently under evaluation as alternative crops for North Dakota (Chapara et al. 2018; del Río et al. 2005).

Most diseases caused by *S. sclerotiorum* are initiated by ascospores (Abawi and Grogan 1975), although on sunflowers, myceliogenic germination of sclerotia could also result in Sclerotinia wilt (Foley et al. 2016). Earlier studies indicated that "successful inoculation with ascospores required free moisture for approximately 48 to 72 h, at optimum temperature of 20 to 25 °C, before signs of infection were evident" (Abawi and Grogan 1975); later, observations by other researchers widened that range to 40 to 112 h, at temperatures of 12 to 24 °C (Boland and Hall 1988; Young et al. 2004). Under field conditions, however, continuously wet periods of such lengths are the exception rather than the norm; instead, periods of high humidity, usually at night and dawn, are alternated with periods of low humidity during the day. The impact of alternating wet and dry periods on disease development varies depending not only on the length and timing of dry period occurrence but also on the pathogen involved (Magarey et al. 2005), with *S. sclerotiorum* being very tolerant to desiccation once established in floral tissues (Harikrishnan and del Río 2006); however, very little is known about the moisture requirements and the impact of interrupted wetness on ascospore germination and the initial stages of disease development. Incubation temperature is another important factor affecting the establishment and development of *S. sclerotiorum* in the host. The lower and upper temperature limits for growth and development of this fungus are 7 to 30 °C, with an optimum of 20 to 25 °C (Abawi and Grogan 1975; Harikrishnan and del Río 2006; Uloth et al. 2015). However, its effect in combination with wetness periods has not been thoroughly investigated. We conducted studies to address the effect of wetness periods and incubation temperatures on ascospore germination and incidence of *S. sclerotiorum* on canola (*Brassica napus*).

Material and methods

All experiments were conducted using *S. sclerotiorum* ascospores produced under laboratory conditions. Briefly, sclerotia produced in cornmeal-sand medium were conditioned for carpogenic germination by three successive cycles of 24 h freeze at –20 °C then 24 h thaw at 21 °C (Harikrishnan and del Río 2006). Conditioned sclerotia were stored at 0 °C until used. To produce ascospores, conditioned sclerotia were buried in sterile white sand contained in 9 cm diameter polystyrene Petri dishes. Sand was saturated with sterile distilled water and the sclerotia incubated at 21 °C and 12 h light/dark for 5 weeks. Apothecia were usually formed within 30 days.

Ascospore germination study

Apothecia used for the germination study discharged the ascospores on the dish lids. Lids were replaced daily, and ascospores-laden lids were stored in dry conditions at 4 °C until used. At the time of use, the ascospores were washed off the lids with sterile distilled water and collected in vials. Ascospore concentrations were estimated using a hemacytometer and adjusted to 5×10^4 ascospores ml^{-1}. This study was conducted to evaluate the effect of different incubation temperatures on ascospore germination of *S. sclerotiorum* isolates WM031 from Nebraska and WM061 and WM192 from North Dakota. These isolates had been collected from soybean, lupins, and canola, respectively. The study was conducted twice in a CRD with four replications for each temperature. Forty microliter aliquots of spore suspensions were placed on glass cover slides and the covers were placed on wet paper towel in Petri dishes. The dishes were closed and incubated at 10, 21, and 30 °C for 8 h. After this period, the number of germinated spores and length of the germ tubes were measured using 50 spores per sample. Spore germination for isolates incubated at 30 °C were also assessed after 48 h. The latter were made to

verify recent observations suggesting ascospores were not able to germinate at 30 °C (Clarkson et al. 2014). Isolates within each temperature were compared based on mean of spore germination.

Infection efficiency study

Flowers used in these studies were inoculated with dry ascospores. Briefly, flowers were placed in the top two levels of a six-stage Andersen spore sampler (Andersen 1958). A funnel, attached to the intake orifice of the sampler, was placed on top of a dish containing mature apothecia and the sampler activated for approximately 30 s (Figure 4.1A). A small number of inoculated flowers were incubated for 72 h at 21 °C in Petri dishes containing wet paper towels. After incubation, flowers with visible mycelial growth were counted and mycelia were transferred to dishes containing potato dextrose agar (PDA) or blue medium (Steadman et al. 1994). Presence of sclerotia on PDA and development of yellow halo on blue medium were used as confirmation that they belonged to *S. sclerotiorum*.

Canola flowers from cv. Westar, collected on the day of inoculation, were inoculated with dry ascospores of isolate WM031 as described. Groups of 10 inoculated flowers were deposited on leaves of single canola plants, cv. Westar, at the six-leaf growth stage (Figure 4.1B). These plants were subjected to four combinations of alternating wet and dry conditions (Table 4.1), while being incubated at 10, 15, 20, 25 or 30 °C for 10 days. For each temperature, treatments were evaluated using a randomized complete block design with three replications. A plastic bag containing single plants from each treatment was considered a replication (Figure 4.1C). The bags were kept closed to keep wet incubation conditions constant and were opened momentarily only when a treatment was either ending or starting wet incubation conditions. For treatments starting dry incubation, plants were removed from the bags but kept in the growth

chamber. Visible moisture on leaves of plants outside the bags disappeared within 20 to 90 min. Plants starting a new wet incubation cycle were sprayed with distilled water until runoff and put back in the corresponding plastic bag. The number of foliar lesions caused by *S. sclerotiorum* on each plant was recorded daily and expressed as a percentage (Figure 4.1D).

Table 4.1. Impact of interrupted wetness period and incubation temperature regimes on length of latent period, and disease incidence caused by *Sclerotinia sclerotiorum* ascosporic infection on canola 144 h post inoculation.

Temperature	Wetness duration (hours)[a]		Latent period	Incidence
(C°)	Daily	Accumulated	(h)	(%)
10	6	42	168	3
10	10	70	168	3
10	14	98	158	2
10	18	126	158	9
15	6	42	156	48
15	10	70	124	50
15	14	98	114	64
15	18	126	114	73
20	6	42	112	55
20	10	70	116	55
20	14	98	110	65
20	18	126	90	68
25	6	42	119	80
25	10	70	100	73
25	14	98	98	78
25	18	126	102	79
30	6	42	88	39
30	10	70	76	35
30	14	98	58	33
30	18	126	46	75
l.s.d. (P = 0.05)[b]				
10			6.2	ns
15			24	28
20			18	19
25			21	ns
30			ns	23

[a] Each treatment mean represents the average of 6 replications.
[b] l.s.d.= least significant difference calculated at P = 0.05 for each temperature were rounded to whole numbers; ns= not significant.

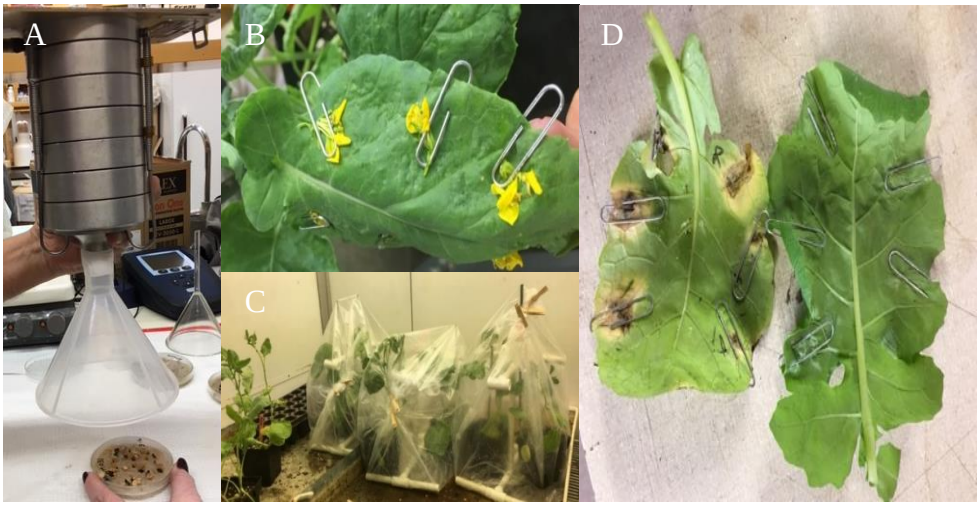


Figure 4.1. Dry-ascospore inoculation of canola flowers and incubation of inoculated plants under different wetness duration and temperature regimes in the infection efficacy study. A) flower inoculation using an Andersen sampler, B) inoculation of leaves using ascospore bearing flowers, C) incubation of plants under different temperature and wetness durations in the growth chamber, and D) recording the disease incidence.

Data analyses and model development

All data analyses were conducted using SAS software (version 9.4; SAS Institute, Cary, NC). For both the ascospore germination and the infection efficiency studies, Levene's test for homogeneity of variances ($P \leq 0.05$) was conducted to determine whether trials within each study could be combined for analysis. When permitted, combined analyses of variance were conducted using the general linear model (GLM) procedure considering trials, and the interaction between trials with treatments as random effects. Treatments (e.g., isolates, incubation temperatures, and wetness durations) were considered as fixed effects. F-tests for treatments were conducted using their interactions with trials as denominators and Fischer's protected least significant difference (LSD) tests at $P = 0.05$ were conducted to discriminate between treatment means using proper sources of error. For the infection efficiency experiment, these tests were conducted to compare the length of the latent periods, defined as the time between inoculation and the expression of first symptoms, and the highest amount of disease incidence that was calculated 144 h after inoculation. The association between duration of the wet period and

disease incidence was modeled using logistic regression analysis (SAS version 9.4). Logistic regression provides estimates of the probability that a foliar lesion will be produced from ascospore-inoculated flowers. For the analyses, data were classified as either 0 or 1, depending on whether disease incidence was ≤ 20% or > 20%, respectively (Harikrishnan and del Río 2008; Rothmann and McLaren 2018). Disease data collection started after 24 h of incubation and continued until 240 h. Models using wetness duration, and the linear and quadratic effects of incubation temperatures as the predictor were developed. In disease favorable temperatures, data collection was started 24 h after inoculation.

Models were evaluated following standard procedures that included comparing fitness statistics like Akaike Information Criterion, Schwarz Criterion, and the –2 Log L; their percentage of concordant and discordant pairs, Sommer's D, Gamma, Tau-a, and *c* statistics; and the Hosmer and Lemeshow test that evaluates the model's predictive capability (Hosmer and Lemeshow 2000). The selected model was validated on a data set not used for model development. The sensitivity and specificity of the model were calculated as described by Coughlin et al. (1992) and the overall accuracy estimated using the equation:

$$\text{Accuracy} = \left(\text{Sensitivity} * \frac{\text{observed controls}}{\text{total observations}}\right) + \left(\text{Specificity} * \frac{\text{observed cases}}{\text{total observations}}\right)$$

Field validation of canola model

The model developed for canola was validated in field trials conducted at Prosper, Carrington, Langdon, and Hansboro, North Dakota, between the second and third weeks of July 2018. Trials at each location had five replications and six flowers per replication and were conducted three times, except at Carrington where they were conducted twice. Flowers inoculated with dry ascospores of *S. sclerotiorum* as described, were placed on leaves in the lower third of canola plants growing in commercial fields or research plots and fastened in

position using paper clips. Leaves with attached flowers were observed 7 days post inoculation (dpi) for lesion development. Weather data from stations of the North Dakota Agricultural Weather Network (https://ndawn.ndsu.nodak.edu/) closest to each trial were used to calculate the number of daily hours when leaf moisture was likely to occur. A 2 °C difference between air temperature and dew point temperature was considered as indicator of dew formation on leaves (Huber and Gillespie 1992; Wang et al. 2019) and was used in lieu of leaf wetness sensors. χ^2 test of independence was conducted to evaluate the null hypothesis that observed and predicted values were independent from each other.

Results

Ascospore germination study

Significant differences ($P = 0.05$) in spore germination were observed among isolates when incubated at 21 °C; after 8 h at this temperature, isolates WM31 and WM61 had mean germinations of 85% each while WM192 had a mean of 69% (Figure 4.2). After 24 h of incubation at 21 °C, profuse hyphal development and branching were observed on all isolates. No observations were made at longer incubation times. Spore germination after 8 h of incubation at 10 and 30 °C was <10% for all isolates. After 24 h of incubation at 30 °C, the average germination for isolates WM31, WM61, and WM192 was 13%. No branching was observed at this time and germ tubes were typically no longer than the diameter of the spore. After 48 h of incubation at 30 °C, significant differences ($P = 0.05$) were observed in germination among isolates; isolates WM61 and WM192 had average germinations of 10% while isolate WM31 had 35% germination and an average germ tube length of 7 µm.

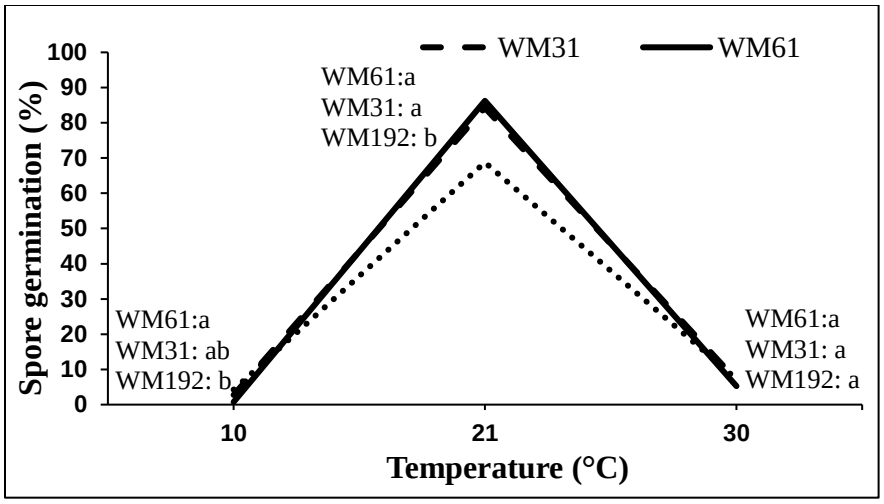


Figure 4.2. Spore germination of *Sclerotinia sclerotiorum* ascospores, incubated at 10, 21, and 30 °C in constant moisture conditions. Means with the same letter within each incubation temperature are not significantly different (P < 0.05).

Infection efficiency study

All inoculated canola flowers were colonized by *S. sclerotiorum* when incubated in wet chambers for 72 h.

The disease developed on leaves at all incubation temperatures and wetness duration periods. The latent period ranged from 2 days at 30 °C and 18 h of wetness to 7 days at 10 °C and 6 h of wetness. At 15, 20, and 25 °C, the latent period ranged between approximately 4 and 6 days and the various evaluated wetness durations did not cause significant differences among them (Table 4.1). Results of infection efficiency studies showed that 25 °C is the optimum temperature for disease development with the lowest disease incidences being observed on plants incubated at 10 °C regardless of wetness duration (Table 4.1). The model ($P < 0.001$) that best described the association between temperature, wetness periods, and disease development was

$$P = \frac{1}{1 + e^{-(-20.62 + 0.046 * moist + 1.57 * temp - 0.033 * temp^2)}}$$

Where: P = probability of disease development, moist = cumulative number of hours of wet conditions in a 144-h period, temp = incubation temperature (°C), and temp^2 = quadratic effect of temperature.

This model, based on 144 h of incubation, used a probability threshold of $P = 0.38$ instead of the default value of $P = 0.50$ (Hosmer and Lemeshow 2000) to classify predictions as cases and had an estimated sensitivity and specificity of 77% and 74%, respectively (Table 4.2). Incubation temperature heavily influenced the probability of disease development. As the incubation temperature departed from the optimum, the moisture requirement to have 50% probability of disease development increased from 36 h at 25 °C to 48 h at 20 °C, 60 h at 30 °C, and 96 h at 15 °C (Figure 4.3). When validated on the independent data set, the accuracy of the model was estimated at 73% with a sensitivity of 79%, and a specificity of 70% (Table 4.2).

Field validation of canola model

The independence test showed that observed and predicted values were not independent from each other and null hypothesis was rejected at $P = 0.05$. During field validation, the model predicted with a 77% accuracy the instances when disease would occur (i.e., sensitivity) and with a 72% accuracy the instances when diseases would not occur (i.e., specificity). The overall accuracy of the model during validation was 75% (Table 4.2).

Table 4.2. Fitness parameters of logistic regression models that estimate the probability of Sclerotinia disease occurrence from ascosporic infections by *Sclerotinia sclerotiorum* on canola plants [a]

	Canola model			
Fitness parameters	Model	Model	Validation	Field validation
Sensitivity (%)	88	77	79	77
Specificity (%)	86	74	70	72
Overall accuracy	87	75	73	75

[a] The model used to calculate the probability of disease development was $P = 1/(1+e^{-[-20.62+(0.046*moist)+(1.57*temp)+(-0.033*temp2)]})$. Here, P is probability of disease development, moist is the cumulative number of wet incubation hours in a period of 144 h, temp is incubation temperature, and temp^2 is the quadratic effect of temperature.

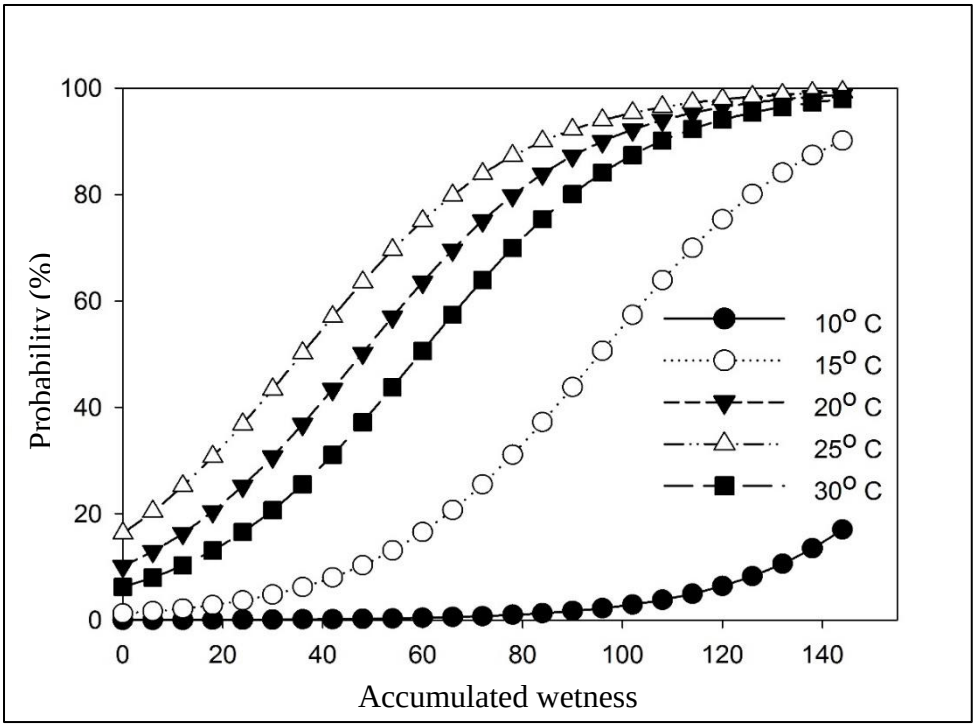


Figure 4.3. Influence of leaf wetness duration and incubation temperature during a six-day period on the probability of *Sclerotinia sclerotiorum* leaf lesion development on canola (Westar cultivar).

Discussion

This work characterized the effect of incubation temperatures on ascospore germination and modeled the relationship of wetness duration and temperature regimes on development of foliar lesions by *S. sclerotiorum* on canola plants. Ascospore germination was highest when incubated at 21 °C and marginal at 10 or 30 °C. Similarly, lesion development was hampered when wet incubation conditions were interrupted for six or more hours daily. Logistic regression models estimated at 50% the chance of lesion development on canola leaves when the number of cumulative wet incubation hours were at 48 h in a period of 6 days.

Incubation of ascospores at 10 or 30 °C slowed germination; however, it did not stop it. Young et al. (2004) indicated that at the latter temperature, little to no germination was observed; however, their observations were made after 8 h of incubation. In our study, up to 35%

of spores from isolate WM31 had germinated after 48 h of incubation and the other two had 10% germination. This differential behavior may be a sign that WM31, which was originally collected from Nebraska, has better adaptation to higher temperatures than the North Dakota isolates (Uloth et al. 2015). The germ tubes observed after 48 h of incubation at 30 °C were shorter than those produced at 21 °C after 8 h of incubation; however, that may not necessarily be an impediment for penetration of plant tissues as observed by Jamaux et al. (1995).

The inoculation method used to evaluate the role of interrupted wet incubation conditions on ascospore infection efficiency was appropriate, effective, and simulated the natural inoculation process. The use of the Andersen sampler facilitated a uniform distribution of spores on the dishes carrying the flowers and consequently the pathogen was detected on all flowers tested for its presence. Further, the method allowed incubation conditions to be the sole source of moisture for priming ascospores for germination and did not wound tissues that facilitate penetration into plant tissues as other methods (Botha et al. 2009).

Alternating wet and dry incubation conditions increased the duration of the latent period and reduced the final amount of disease compared with continuous-moisture incubation but did not suppress the disease. According to Harikrishnan and del Río (2006), *S. sclerotiorum* hyphae is very tolerant to desiccation and could resume growth even when colonized flowers have been dried for a few days. This resiliency was observed in this study too.

The magnitude of the delay in symptom appearance depended on the duration of the wet and dry periods as observed by Phillips (1994) but also on incubation temperature and type of host. The accumulation of 36 h of leaf wetness at 15 °C resulted in 6% chance of disease development; the same number of wet hours would result in a 50% chance at 25 °C. Increasing the accumulated wetness duration from 36 to 60 h would raise the chance of disease

development to 17% at 15 °C and to 75% at 25 °C. Interestingly, disease developed at 30 °C, a temperature that many researchers consider the upper limit for this pathogen (Abawi and Grogan 1975; Koch et al. 2007, Clarkson et al. 2014; Uloth et al. 2015). Incubating plants for 18 wet hours at 30 °C resulted in similar disease incidence to incubating plants for 6 h in 25 °C; however, the lesions were much smaller and did not represent a threat to the plant. However, the fungus could continue developing at faster rates once temperatures cooled down.

The importance of leaf wetness in the development of ascosporic infections by *S. sclerotiorum* was pointed out earlier by researchers who studied its role in experiments where wetness conditions were continuous (Abawi and Grogan 1975; Blad et al. 1978; Boland and Hall 1988) and by observations that compared disease intensities in irrigated versus non-irrigated conditions (Weiss et al. 1980). Phillips (1994) studied the impact of discontinuous moisture on the development of *S. sclerotiorum*; however, his experiments only included a single interruption of the wet incubation conditions although under variable temperature regimes. More recently, Clarkson et al. (2014) evaluated the effect of temperature and relative humidity on disease development but did not evaluate the effect of discontinuous wetness duration. To our knowledge, this is the first study that reports the effect of multiple cycles of wet and dry incubation conditions and uses inoculation of dry ascospores to model disease development.

Repeated cycles of wet and dry conditions not only reduced the ability of ascospores to infect and colonize floral tissues but also allowed for saprophytes to compete for the dead tissues. During a similar infection efficiency study conducted on dry bean (data not shown), it was noted that flowers that were not associated with a leaf lesion did not exhibit typical *S. sclerotiorum* mycelial growth; instead, they were colonized by saprophytes that produced dark brown to black mycelium and/or had a mushy texture, a symptom of tissue degradation. It is very

likely that under field conditions, where multiple ascospore showers are possible (Qandah and del Río 2011; Schwartz and Steadman 1978), similar competition would take place limiting the potentially additive effect that said showers would have on the probability of a flower yielding a leaf lesion.

Numerous models have been produced to estimate the risk of Sclerotinia development. Some of them were produced by associating cultural practices and/or weather variables with high intensity epidemics (Clarkson et al. 2014; Harikrishnan and del Río 2008; Workneh and Yang 2000) while others were produced associating the detection of infected plant parts with final disease intensity (Bom and Boland 2000; Turkington and Morrall 1993). While the information generated by these models is useful, their ability to accurately predict disease development is hampered by the fact that none address "disease establishment" in the calculation of the risk of disease development. This issue was addressed in this manuscript.

The developed logistic regression model in this study showed high sensitivity and specificity. Sensitivity is defined as the true proportion of cases (e.g., disease present) correctly predicted by the model; specificity refers to the proportion of controls (e.g., disease absent) correctly predicted by the model (Coughlin et al. 1992; Hosmer and Lemeshow 2000).

The model developed in this study was validated under field conditions and it will contribute to our understanding of the epidemiology of diseases initiated by *S. sclerotiorum* ascospores. Dew formation was estimated as an indicator of leaf wetness duration. This approach has been used by other researchers in lieu of leaf wetness sensors (Huber and Gillespie 1992; Sentelhas et al. 2008) and is easier to implement by growers. The model proved to be accurate in the field and thus could be used as part of a disease-warning system for growers.

References

Abawi, G. S., and Grogan, R. G. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. Phytopathology 65:300-309.

Andersen, A. A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol 76:471-484.

Blad, B. J., Steadman, J. R., and Weiss, A. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible bean. Phytopathology 68:1431-1437.

Boland, G. J., and Hall, R. 1988. Epidemiology of Sclerotinia stem rot of soybean in Ontario. Phytopathology 78:1241-1245.

Bom, M., and Boland, J. G. 2000. Evaluation of disease forecasting variables for Sclerotinia stem rot (*Sclerotinia sclerotiorum*) of canola. Can. J. Plant Sci. 80:889-898.

Botha, C., McLaren, N. W., and Swart, W. J. 2009. Evaluation of greenhouse inoculation techniques used to screen for Sclerotinia stem rot resistance in soybeans. S. Afr. J. Plant Soil 26: 48-50.

Chapara, V., Chittem, K., and del Río, L. E. 2018. First report of white mold caused by *Sclerotinia sclerotiorum* on faba beans in North Dakota. Plant Dis. 102:1669-1669.

Clarkson, J. P., Fawcett, L., Anthony, S. G., and Young, C. 2014. A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. PLoS One 9:e94049.

Coughlin, S. S., Trock, B., Criqui, M. H., Pickle, L. W., Browner, D., and Tefft, M. C. 1992. The logistic modeling of sensitivity, specificity, and predictive value of a diagnostic test. J. Clin. Epidemiol. 45:1-7.

del Río, L. E., Bradley, C. A., Henson, R. A., Endres, G. J., Hanson, B. K., McKay, K., Halvorson, M., Porter, P. M., LeGare, D. G., and Lamey, H. A. 2007. Impact of Sclerotinia stem rot on yield of canola. Plant Dis. 91:191-194.

del Río, L. E., Bradley, C. A., and Johnson, B. L. 2005. First report of white mold caused by *Sclerotinia sclerotiorum* on Echium (*Echium vulgare)*. Plant Dis. 89:684-684.

Foley, M. E., Doğramacı, M., West, M., and Underwood, W. R. 2016. Environmental factors for germination of *Sclerotinia sclerotiorum* sclerotia. J. Plant Pathol. Microbiol. 7:379-383.

Harikrishnan, R., and del Río, L. E. 2008. A logistic regression model for predicting risk of white mold incidence on dry bean in North Dakota. Plant Dis. 92:42-46.

Harikrishnan, R., and del Río, L. E. 2006. Influence of temperature, relative humidity, ascospore concentration, and length of drying of colonized dry bean flowers on white mold development. Plant Dis. 90:946-950.

Hosmer, D. W., and Lemeshow, S. 2000. Applied Logistic Regression, second ed. John Wiley and Sons, Hoboken, NJ.

Huber, L., and Gillespie, T. J. 1992. Modeling leaf wetness in relation to plant disease epidemiology. Annu. Rev. Phytopathol. 30:553-577.

Jamaux, I., Gelie, B., and Lamarque, C. 1995. Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by scanning electron microscopy. Plant Pathol. 44:22-30.

Koch, S., Dunker, S., Kleinhenz, B., Rohrig, M., and Tiedemann, A. 2007. A crop loss-related forecasting model for Sclerotinia stem rot in winter oilseed rape. Phytopathology 97:1186-1194.

Magarey, R. D., Sutton, T. B., and Thayer, C. L. 2005. A simple generic infection model for foliar fungal plant pathogens. Phytopathology 95:92-100.

Phillips, A. J. L. 1994. Influence of fluctuating temperatures and interrupted periods of plant surface wetness on infection of bean leaves by ascospores of *Sclerotinia sclerotiorum*. Ann. Appl. Biol. 124:413-427.

Qandah, I., and del Río, L. E. 2011. Temporal dispersal patterns of *Sclerotinia sclerotiorum* ascospores during canola flowering. Can. J. Plant Pathol. 33:159-167.

Rothmann, L. A., and McLaren, N. W. 2018. *Sclerotinia sclerotiorum* disease prediction: A review and potential applications in South Africa. S. Afr. J. Sci. 114:1-9.

Schwartz, H. F., and Steadman, J. R. 1978. Factors affecting sclerotium populations of, and apothecium production by, *Sclerotinia sclerotiorum*. Phytopathology 68:383-388.

Sentelhas, P. C., Marta, A. D., Orlandini, S., Santos, E. A., Gillespie, T. J., and Gleason, M. L. 2008. Suitability of relative humidity as an estimator of leaf wetness duration. Agric. Forest Meteor. 148:392-400.

Steadman, J. R., Marcinkowska, J., and Rutledge, S. 1994. A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 16:68-70.

Turkington, T. K., and Morrall, R. A. A. 1993. Use of petal infestation to forecast Sclerotinia stem rot of canola: the influence of inoculum variation over the flowering period and canopy density. Phytopathology 83:682-689.

Uloth, M. B., You, M. P., Cawthray, G., and Barbetti, M. J. 2015. Temperature adaptation in isolates of *Sclerotinia sclerotiorum* affects their ability to infect *Brassica carinata*. Plant Pathol. 64:1140-1148.

Wang, H., Sanchez-Molina, J. A., Li, M., and Rodríguez Díaz, F. 2019. Improving the performance of vegetable leaf wetness duration models in greenhouses using decision tree learning. Water 11:158-177.

Weiss, A., Kerr, E. D., and Steadman, J. R. 1980. Temperature and moisture influences on development of white mold disease (*Sclerotinia sclerotiorum*) on Great Northern beans. Plant Dis. 64:757-759.

Workneh, F., and Yang, X. B. 2000. Prevalence of Sclerotinia stem rot of soybeans in the north-central United States in relation to tillage, climate, and latitudinal positions. Phytopathology 90:1375-1382.

Young, C. S., Clarkson, J. P., Smith, J. A., Watling, M., Phelps, K., and Whipps, J. M. 2004. Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. Plant Pathol. 53:387-397.

CHAPTER 5. EFFECT OF TEMPERATURE ON RESPONSE OF *BRASSICA NAPUS* TO INFECTION BY *SCLEROTINIA SCLEROTIORUM*

Abstract

Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is endemic to North Dakota canola production areas. Environmental factors affect the disease development significantly where temperature is one of the most determinant factors. The objectives of this study were to evaluate the role of temperature on myceliogenic germination and growth of *S. sclerotiorum* isolates collected from different geographical regions and also on SSR development on *Brassica napus* germplasms with different level of resistance to the disease. Both studies were conducted twice in growth chambers using randomized complete block designs with three and five replications for the first and second studies, respectively. Isolates showed the maximum colony growth under 25 °C followed by 20 °C. Among the 19 isolates tested for myceliogenic germination under 10, 15, 20, 25, and 30 °C, isolates WM031 and WM731, with small and large colony areas of 1.9-14.3 cm and 4.1-45 cm, respectively, were used for the inoculation of the plants. Three germplasms and a susceptible hybrid line were inoculated under three temperature regimes including 10, 25, 30 °C. The largest relative area under the disease progress curve (rAUDPC) values of plants were observed at 25 °C. Two germplasms, Gan You No1 and Chisaya natane, were constantly resistant under all temperature regimes with the rAUDPC range of 4.2-21.1 cm and 8.6-24.6 cm at 25 °C, respectively. While the reaction of the plants to the disease was significantly different at 10 °C, there was no differences between the resistant germplasms and the susceptible check (45H28) at 30 °C. Colt, with rAUDPC range of 30.2-64.7 cm was highly susceptible even at extreme temperatures. Results of these studies will provide insights into the role of temperature on mycelial growth of isolates collected from different

geographical regions and impact of temperature and host resistance level on *S. sclerotiorum* disease development.

Introduction

The fungus *S. sclerotiorum* (Lib.) de Bary has the potential of infecting more than 500 plant species (Sharma et al. 2016) including several economically important crops such as canola, soybean, dry bean, lettuce, and sunflower (Saharan and Mehta 2008). Canola is one of the primary risk crops in North Dakota where every unit of the disease incidence could reduce the yield by 0.5% (equal to 12.75 kg/ha) (del Río et al. 2007). Yield losses caused by *S. sclerotiorum* on canola in Germany, Sweden and the United Kingdom could be as high as 50-70% under favorable environmental conditions (Pope et al. 1989; Koch et al. 2007).

S. sclerotiorum growth and host infection are highly affected by environmental factors (Rothmann and McLaren 2018). Among weather variables, temperature has a significant impact on all developmental stages of the pathogen including carpogenic and myceliogenic germination of sclerotia, ascospore survival, disease establishment on the plant, and subsequent disease development (Abawi and Grogan 1975; Boland and Hall 1988; Young et al. 2004; Harikrishnan and del Río 2006; Saharan and Mehta 2008; Lane et al. 2019; Shahoveisi and del Río Mendoza 2020). The range of favorable temperatures may vary depending on the type of germination and developmental stage of the pathogen. Temperatures of 10-20 °C promote carpogenic germination of sclerotia (Sun and Yang 2000; Wu and Subbarao 2008); however, the range is wider, 7- 30 °C, for myceliogenic germination if sclerotia are incubated in moist sand (Huang and Kokko 1989; Lane et al. 2019). While myceliogenic germination could occur at a broad range of temperatures, mycelial growth is optimum at 20-22 °C (Domingues et al. 2016; Godoy et al. 2017). A narrower temperature range, 20-25 °C, is considered optimum for ascospore

germination, and for disease initiation and development on the plant (Abawi and Grogan 1975, 1979; Uloth et al. 2015). While the disease could initiate at high temperature regimes, e.g. 30 °C, lesion expansion on leaves does not occur at this temperature (Abawi and Grogan 1979; Shahoveisi and del Río Mendoza 2020). The role of temperature on mycelial growth of *S. sclerotiorum* has been evaluated previously; however, the relationship between isolates' origin and temperature and their role on mycelial growth has been studied to a lesser extent (Godoy et al. 2017).

The average air temperature during the canola flowering period (July) in North Dakota ranges between 19 and 23 °C. This range is closer to the favorable temperature for disease development. While the effect of temperature on disease development has been evaluated in previous studies, the role of this variable on the reaction of plants with different level of resistance to the disease has not been thoroughly investigated in *B. napus*. Uloth et al. (2015) studied the role of temperature on development of lesions on *B. carinata* cotyledons using two resistant and susceptible cultivars while fungal isolates from cool and warm geographical origins were used for inoculation. Increasing the temperature from 25/21 °C to 28/24 °C (day/night) resulted in smaller mean lesion size (for both resistant and susceptible plants) in plants inoculated with the cool region isolate while the lesion size increased in plants inoculated with the warmer region isolate. In general, disease development on the stem of the resistant and susceptible genotypes was greater at 18 and 25 °C compared to 14 and 28 °C when the cool temperature isolate was used; however, using the warm region isolate, lesion size on the stem was larger at 18, 25, and 28 °C compared to 14 °C (Uloth et al. 2015). From these studies, it is not clear whether temperature has an effect on the activity of genes associated with resistance to *S. sclerotiorum*, even though several studies have proven that temperature plays an important

role in reaction of plants to other fungal and bacterial pathogens (Fu et al. 2009; Li et al. 2020; Zhu et al. 2010). The evaluation of the reaction of susceptible and resistant plant genotypes at different incubation temperatures will provide insights on whether these interactions exist.

The present study was designed to I) characterize the role of temperature on mycelial growth of *S. Sclerotinia* isolates collected from different geographical regions and II) evaluate the reaction of *B. napus* germplasms with different level of host resistance to SSR disease under different temperature regimes.

Material and methods

Effect of temperature on fungal isolates

The effect of different temperature regimes on sclerotia germination and mycelial growth of 19 *S. sclerotiorum* isolates was studied with the objective of identifying isolates whose growth would not be significantly inhibited by temperatures ranging between 10 and 30 °C. The isolates used in this study were collected from different hosts in different states of the country (Table 5.1). Sclerotia were surface disinfested by immersing them in a 10% aqueous solution of NaOCl for 1 minute. Subsequently, they were rinsed with distilled sterile water twice. Sclerotia from each isolate were placed on potato dextrose agar (PDA) medium and incubated at five temperature regimes, e.g. 10, 15, 20, 25, and 30 °C. The study was conducted twice with three replications in each and using a randomized complete block design (RCBD). After 36 hours, colony diameters were measured in perpendicular directions to each other. The average radius was obtained from these measurements and used to calculate the area of each colony using the following equation:

$$A = \pi r^2$$

where A represents area of the colony, and r is average radius of the colony.

Table 5.1. List of *Sclerotinia sclerotiorum* isolates tested for sclerotia germination and mycelium growth under different temperature regimes.

Isolate	Host	Year of collection	Location were collected	Range of summer temperature (°C)
WM031	Soybean	2011	Nebraska	18-32
WM217	Canola	2005	Georgia	22-32
WM347	Canola	2009	Cavalier County North Dakota (ND)	15-28
WM412	Soybean	2008	Iowa	25-30
WM585	Soybean	2008	Illinois	16-27
WM603	Tobacco	2003	Wisconsin	23-27
WM623	Canola	2012	Cavalier County (ND)	15-28
WM636	Canola	2012	Cavalier County (ND)	15-28
WM644	Sugar beet	2013	Imperial Valley California	22-40
WM721	Canola	2014	Wyoming	23-32
WM731	Canola	2014	Bottineau County (ND)	15-28
WM746	Canola	2014	Towner county (ND)	15-28
WM766	Canola	2014	Cavalier County (ND)	15-28
WM812	Canola	2014	Bottineau County (ND)	15-28
WM841	Canola	2014	Ward County (ND)	15-28
WM842	Canola	2014	Burke County (ND)	15-28
WM848	Canola	2012	Cavalier County (ND)	15-28
WM876	Chickpea	2016	Montana	21-26
WM877	Lentil	2016	Montana	21-26

All data were analyzed using SAS software, version 9.4 (SAS Institute, Cary, NC). Levene's test for homogeneity of variances ($P \leq 0.05$) was run and trials were combined when it was permitted. Combined analyses of variance were conducted using the general linear model (GLM) procedure. Trials and their interactions with treatments (isolates and temperature) were considered random effects with treatments having fixed effects. F-tests were run for treatments and their interaction using proper sources of error as the denominators. Colony areas were compared using least squares means (lsmeans) in GLM procedure. Two isolates, one with slow colony growth and one with fast growth, were selected for the next study to evaluate the reaction of *B. napus* germplasms at different temperature regimes. Further, to better characterize the effect of incubation temperature on mycelial growth, linear regression analysis was used on sets

of data containing colonies areas obtained at incubation temperatures between 10-20, 20-25, and 25-30 °C.

Effect of temperature on disease development

Growth chamber studies were conducted to evaluate the response of three plant genotypes, Colt (USA), Gan You No 1 (China), and Chisaya natane (Japan), and a susceptible commercial check (hybrid 45H28) to two *S. sclerotiorum* isolates, WM031 and WM731 at 10, 25, and 30 °C. Using agar stem method, plants were inoculated 45-50 days after planting. Six-millimeter agar plugs containing hyphal tips of 36-hours old *S. sclerotiorum* colonies. The agar plug of each isolate was deposited on the stem between the third and fourth leaves. Using a piece of Parafilm (Bemis Company) the inoculum was secured on the stem. The experiment was conducted twice using a randomized complete block design (RCBD) with five replications. Plants incubated under high temperature regimes (25 and 30 °C) were watered two to three times a day to prevent water stress and wilting while plants incubated at 10 °C were watered once a day. Lesion size and plant mortality were assessed starting two days post inoculation (dpi) and repeated five more times at two-day intervals (i.e. 2, 4, 6, 8, 10, and 12 dpi). Plants that showed wilting or lodging at the point of inoculation were considered dead and the lesion length of those plants were not measured anymore. Lesion size data was used to calculate a modified relative area under the disease progress curve (rAUDPC) where missing values caused by death of the plants were replaced by the maximum lesion size in each trial, temperature, and isolate. Using this approach, the effect of mortality on the rAUDPC value was minimized. All data were analyzed using SAS software, version 9.4 (SAS Institute, Cary, NC). Levene's test for homogeneity of variances ($P \leq 0.05$) and combined analyses of variance were conducted as indicated for the last experiment. After calculating average rAUDPC and mortality percentage,

interaction between isolates and genotypes and the main effect of each treatment were evaluated within each temperature regime. Least square mean (lsmeans) in GLM procedure was used for mean separation. The temporal progress of plant mortality of each line was visualized by plotting them over time.

Results

Effect of temperature on fungal isolates

Variances of two trials were homogenous ($P = 0.64$) and therefore, they were combined. The interaction between isolates and temperatures was not significant ($P = 0.08$); therefore, the main effect of treatments will be discussed. While the isolates were not statistically different ($P = 0.27$), with the mean colony area of 15.4±5.5 cm^2, the main effect of temperature regimes was significant ($P = 0.0002$) (Table 5.2). The maximum average colony area of 32.3 cm^2 was observed at 25 °C, followed by 25.8 cm^2 at 20 °C, and 13.6 cm^2 at 15 °C. Extreme temperature regimes (10 and 30 °C) resulted in the smallest colony areas of 2.4 and 3.2 cm^2, respectively (Figure 5.1). All isolates except WM644 showed maximum colony areas at 25 °C (Figure 5.2). Isolate WM644 produced its largest colonies at 20 °C. Seventeen isolates had a rate of growth that ranged between 8.6- 18.1 cm^2 at 10 to 20 °C while isolate WM721 had the lowest rate of growth of 2.4 cm^2 (Appendix D). All isolates except WM031 demonstrated notably larger colony areas at 20 °C compared to 15 °C; this is while the growth rate of WM031 was only 0.6 cm^2 between these two temperature regimes. Isolates could be grouped into three growth categories when incubated at 20 to 25 °C: significant growth, moderate growth and poor growth. Eleven isolates were placed in the first group. These isolates had a better performance at 25 °C and a growth rate ranging between 6.3 and 12.8 cm^2 between these temperatures; 7 isolates showed slightly larger colony area at 25 °C with the growth rate ranged between 0.6 and 3.4 cm^2

; and one isolate had a negative growth rate of - 3.4 cm^2 between 20 and 25 °C. All isolates formed smaller colonies at 30 °C with a negative growth rate of - 6.6 to - 48.6 cm^2. Two isolates (WM031 and WM731) with small and large colony areas, respectively, were used for the next study. These isolates were not significantly different at 10 and 30 °C; however, their growth rate were statistically different (*P* <.0001) at 15, 20, and 25 °C (Figure 5.3). Isolate WM731 was among the top three fast growing isolates and isolate WM031 had the second smallest colony area after isolate WM721 at all temperature regimes.

Table 5.2. Analysis of variance of effect of temperature and isolates on *Sclerotinia sclerotiorum* colony areas after 36 hours under in vitro condition.

Sources of variation	df[a]	Mean Square	F value	Pr > F
Isolate	18	904.46	1.33	0.27
Trial ×Isolate	18	677.71		
Temperature	4	20445.41	110.26	0.0002
Trial ×Temperature	4	185.43		
Isolate ×Temperature	72	12831.04	1.40	0.08
Trial ×Isolate ×Temperature	72	9180.71		

[a] Degrees of freedom

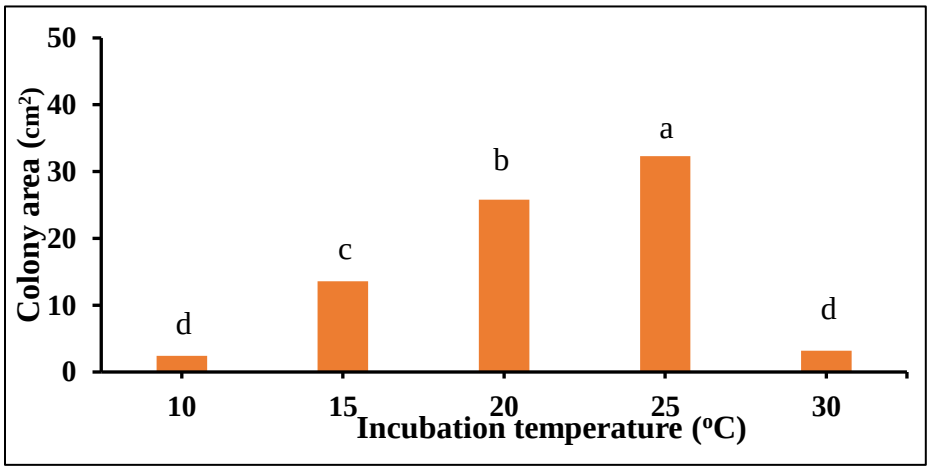


Figure 5.1. Main effect of temperature regimes on colony area of *Sclerotinia sclerotiorum* isolates. Means of colony area with the same letter are not significantly different at *P* =0.05.

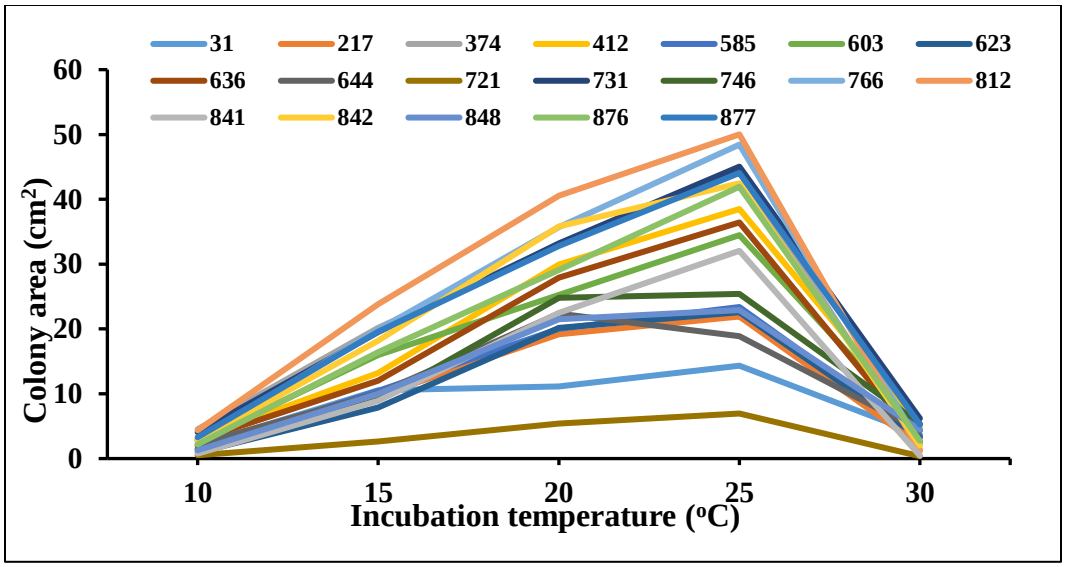


Figure 5.2. Mycelial growth of 19 *Sclerotinia sclerotiorum* isolates (shown with colored lines) on potato dextrose agar (PDA) medium incubated in different temperature regimes after 36 hours.

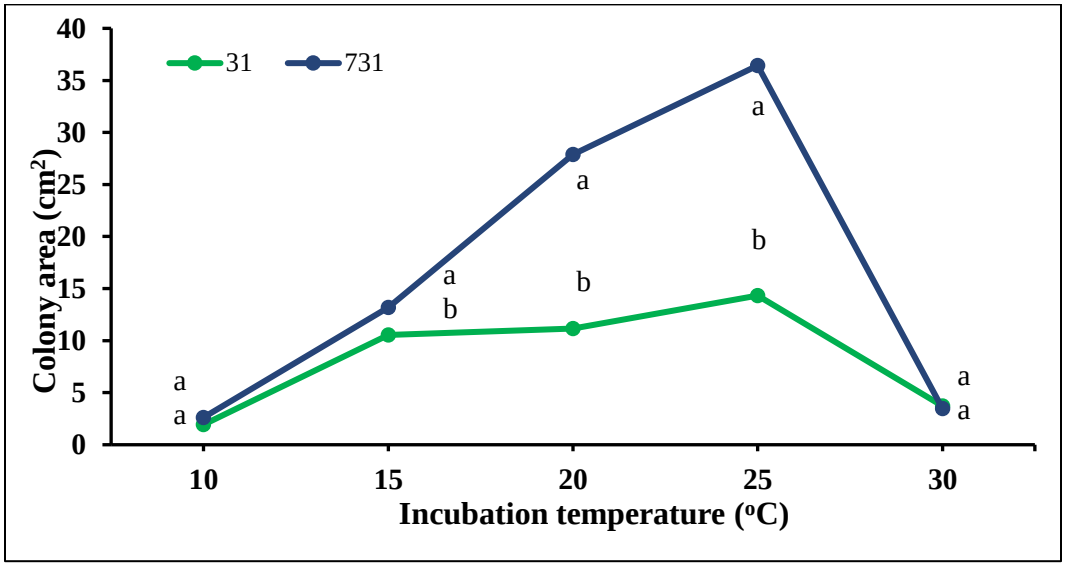


Figure 5.3. Mycelial growth of two *Sclerotinia sclerotiorum* isolates on potato dextrose agar (PDA) medium incubated in different temperature regimes after 36 hours. Means of colony area with the same letter within each temperature are not significantly different as per t-test of least square means comparison at $P \geq 0.19$.

Effect of temperature on disease development

Levene's test showed that the variances of trials were not significantly different from each other ($P = 0.48$) and therefore, trials were combined. Also, the interaction between isolates

and genotypes within incubation temperature regimes was not significant ($P > 0.05$); hence main effect of genotypes and isolates will be discussed (Table 5.3).

Isolate WM731 caused a 44% larger rAUDPCs compared to WM031 at 10 °C but not at the other temperatures (Figure 5.4). Both isolates could infect the plants at the two extreme temperature regimes evaluated; isolate WM031 resulted in a mortality range of 0-50% and 0-70% at 10 and 30 °C while these ranges increased to 0-80% and 0-70% for WM731. These results suggest that isolate WM031 performs better at warmer environments. At 10 and 30 °C, WM031 could cause mortality only in the susceptible check and Colt while WM731 could cause up to 30% mortality in resistant genotypes, Gan You No 1 and Chisaya natane, as well.

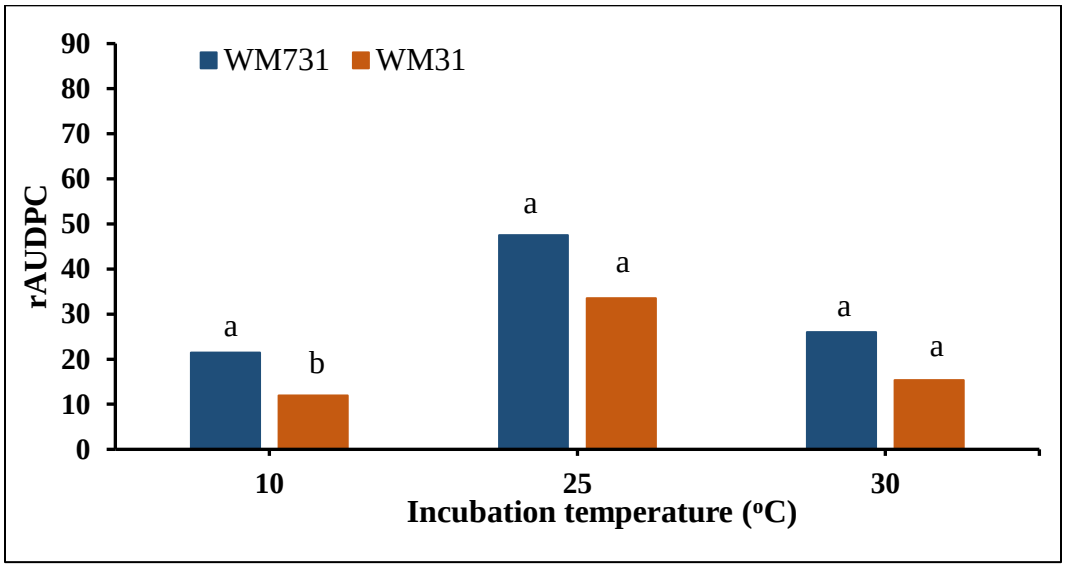


Figure 5.4. Effect of two *Sclerotinia sclerotiorum* isolates (WM731 and WM031) on relative area under disease progress curve (rAUDPC) means of *Brassica napus* genotypes under three temperature regimes. rAUDPC means with the same letter within each temperature are not significantly different.

Table 5.3. Analysis of variance of effect of isolates and plant genotypes on average relative area under the disease progress curve in plants inoculated with *Sclerotinia sclerotiorum* isolates under three temperature regimes.

Temperature	Source	df[a]	Mean Square	F value	Pr>F
10 °C	Isolate	1	1813.56	345.23	0.034
	Trial ×Isolate	1	5.25		
	Genotypes	3	3043.76	163.59	0.0008
	Trial ×Genotype	3	18.61		
	Isolate ×Genotypes	3	400.00	2.02	0.29
	Trial ×Isolate ×Genotypes	3	198.28		
25 °C	Isolate	1	3920.00	7.38	0.22
	Trial ×Isolate	1	531.48		
	Genotypes	3	8944.73	78.69	0.002
	Trial ×Genotype	3	113.68		
	Isolate ×Genotypes	3	330.85	0.97	0.51
	Trial ×Isolate ×Genotypes	3	339.39		
30 °C	Isolate	1	2288.73	74.73	0.07
	Trial ×Isolate	1	30.63		
	Genotypes	3	3408.04	9.25	0.05
	Trial ×Genotype	3	368.33		
	Isolate ×Genotypes	3	495.72	1.29	0.42
	Trial ×Isolate ×Genotypes	3	384.05		

[a] Degrees of freedom

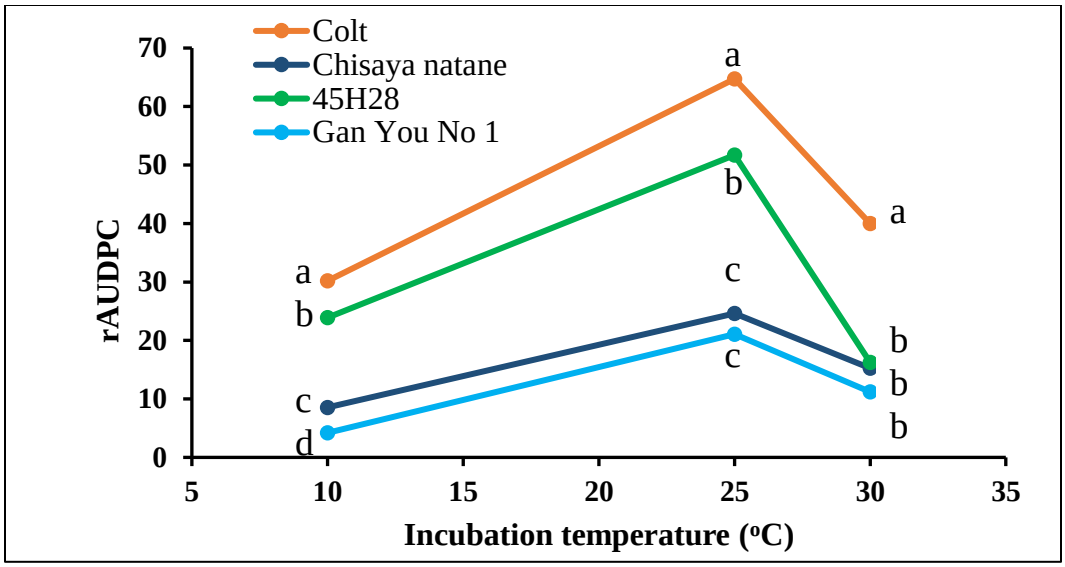


Figure 5.5. Relative area under the disease progress curve (rAUDPC) of the reaction of *Brassica napus* genotypes, Colt, Chisaya natane, Gan You No 1, and 45H28 (susceptible hybrid check) to *Sclerotinia sclerotiorum* under five temperature regimes. rAUDPC means with the same letter within each temperature are not significantly different.

Reaction of plant genotypes to the disease varied depending on the incubation temperature. At 10 °C, Colt and the susceptible check (45H28) with rAUDPCs of 30.2 and 23.9 cm, respectively, were significantly more susceptible than Chisaya natane and Gan You No 1 with rAUPDC range of 4.2-8.6 cm. At 25 °C, Colt and the susceptible check developed larger lesions while the other two genotypes were statistically more resistant ($P \leq 0.02$). The range of rAUDPC at the optimum temperature regime was 21.1-64.7 cm with Colt and Gan You No 1 as the most susceptible and resistant genotypes, respectively. Interestingly, the reaction of the resistant genotypes and susceptible checks was not significantly different at 30 °C; this is while Colt was still highly susceptible, with rAUDPC of 40 cm (Figure 5.5). These results suggest that 30 °C is not favorable for disease development as long as the host has some level of resistance but disease incidence could be considerably high if the plant is very susceptible to the pathogen.

Plotting plant mortality percentage over time revealed that Chisaya natane and Gan You No 1 had 0-15% mortality rate at 12 dpi at 10 and 30 °C; however, the range increased to 65-

70% for Colt and 0-45% for hybrid 45H28 at the same reading date. The highest mortality rates were observed at 25 °C where Colt and hybrid 45H28 showed 90% and 60% mortality at 12 dpi, respectively, and Gan You No 1 and Chisaya natane had 20% and 30% dead plants, respectively. While the percentage of plant mortality was not significantly different until 4 dpi at 25 °C, resistant plants, Colt and the susceptible check started to show differences after 6 dpi. Two resistant genotypes were more resistant than the susceptible check at 10 and 25 °C while their mortality percentage was not significantly different than the hybrid 45H28 at 30 °C (Figure 5.6). This finding verifies the results of the rAUDPC analysis where reactions of the resistant and semi-susceptible plants to the disease at high temperatures were not significantly different (Figure 5.6).

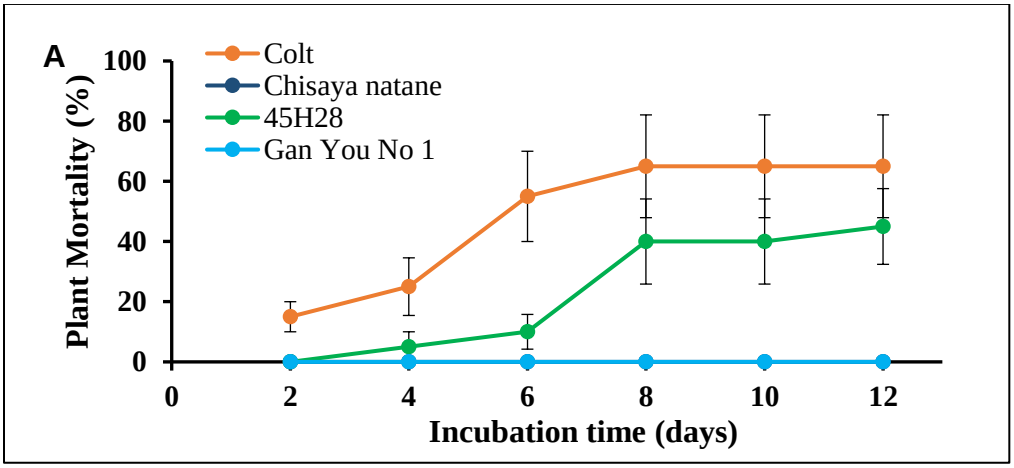


Figure 5.6. Percentage of plant mortality of *Brassica napus* genotypes, Colt, Chisaya natane, Gan You No 1, and 45H28 (susceptible hybrid check) over time. Plants were inoculated with *Sclerotinia sclerotiorum* isolates under three temperature regimes A) 10 °C B) 25 °C and C) 30 °C. Error bars represent standard error of means.

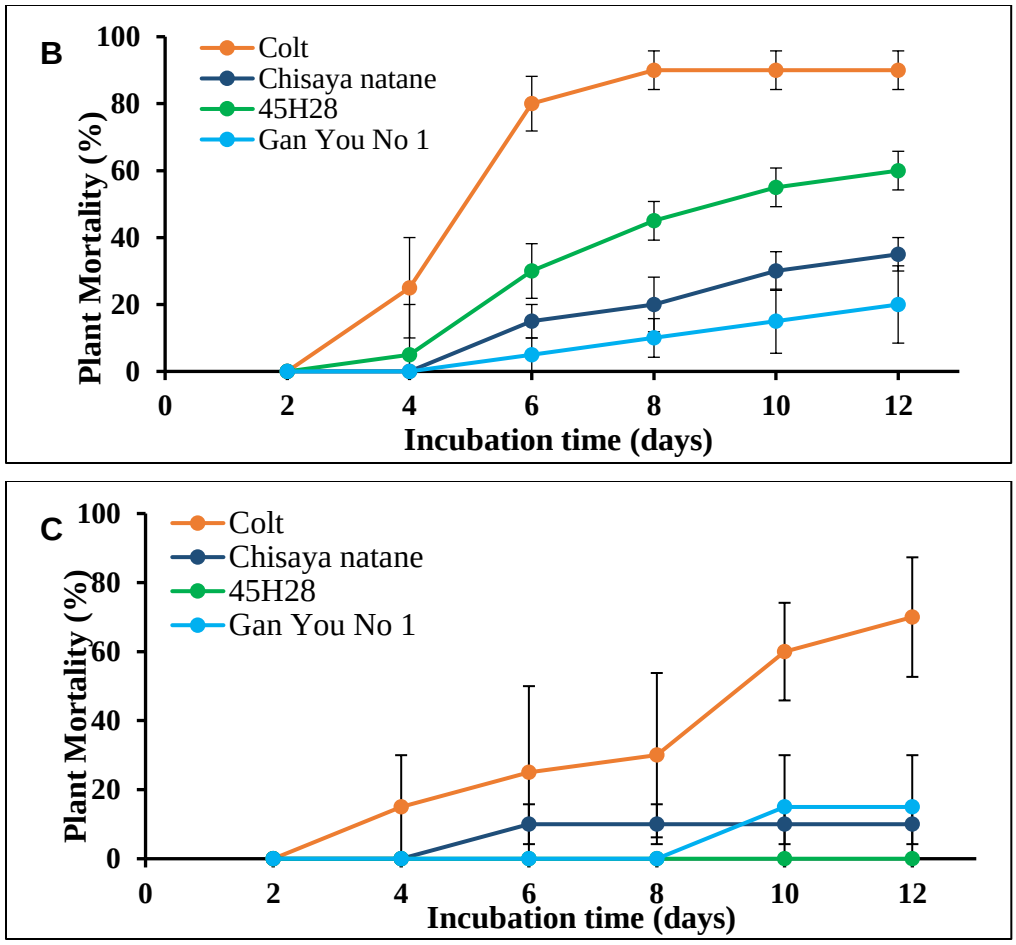


Figure 5.6. Percentage of plant mortality of *Brassica napus* genotypes, Colt, Chisaya natane, Gan You No 1, and 45H28 (susceptible hybrid check) over time (continued). Plants were inoculated with *Sclerotinia sclerotiorum* isolates under three temperature regimes A) 10 °C B) 25 °C and C) 30 °C. Error bars represent standard error of means.

Discussion

This study characterized the role of temperature on mycelial growth of 19 *S. sclerotiorum* isolates and disease development in four *B. napus* genotypes with different levels of resistance. Growth of *S. sclerotiorum* isolates collected from various geographical regions in the US was highly affected by temperature regimes; the colony area of isolates ranged between 7-50 cm^2 at 25 °C; the range of colony area decreased to 0.5-4.5 cm^2 at 10 °C and 0.4-6 cm^2 at 30 °C, respectively. Using rAUDPC and mortality, Colt was significantly more susceptible than the other resistant genotypes and hybrid 45H28 under all incubation temperatures. While Chisaya

natane and Gan You No 1 were statistically more resistant than the hybrid 45H28 at lower temperatures, the reaction of these resistant lines and the susceptible check was not significantly different at 30 °C.

The results of the first experiment indicated that 25 °C was the optimum temperature for 18 isolates which were collected from geographical regions with the low summer temperature range of 15-25 °C and the high range between 26 and 32 °C. Isolate WM644 was the only exception that showed maximum colony growth at 20 °C which is in contrast with the high summer temperature range, 22-40 °C, of its place of collection. However, different results were observed in a study conducted by Krishnamoorthy et al. (2017). In their study, 20 °C was the optimum temperature for the mycelial growth of a *S. sclerotiorum* isolate collected from a cool and wet climate (higher elevation region of Kothagiri, India) with an average summer temperature of 20 °C. In another study, the effect of two temperature regimes, i.e. 20 and 27 °C, on mycelial growth of 16 isolates was evaluated under controlled conditions. The isolates used in that study had been collected from different geographical regions in Brazil including Paraná, Goiás Distrrito Federal, Minas Gerais, Mato Grosso do Sul, Bahia, Mato Grosso and the United States including Iowa, Wisconsin, Ohio, Florida, and Minnesota. Results of this work indicated that two isolates collected from Ohio, with the summer temperature range of 26-29 °C, and Goiás, with the growing season average temperature of 25 °C, had the smallest mycelial growth at 20 °C while the largest colony size belonged to an isolate collected from Okeechobee County in Florida, with the summer temperature range of 22-32 °C. However, at 27 °C, the isolate from Florida showed the smallest lesion size and an isolate collected from Iowa had the largest colony (Godoy et al. 2017). In a slightly different study, Uloth et al. (2015) evaluated the role of temperature on lesion expansion in *B. carinata* inoculated with isolates collected from cool and

warm geographical regions. The results demonstrated that, in general, isolates originated from warm climates have a better adaptation to high temperatures. While similar results were observed for most of the isolates tested in this study, isolate WM644 was an exception. One of the possible reasons for that could be an error in recording the name of the origin for the isolate. Further, the host's canopy structure has a determinant role in the microclimate; dense canopies provide shades and the temperature under the canopy would be significantly lower than the average temperature reported for the region.

From the first study, isolates WM031 and WM731 were selected for inoculating the PIs and the susceptible check. While WM812 showed the largest mycelial growth at 15, 20, and 25 °C, isolate WM731 had the highest growth rate at 30 °C; since the objective of the work was to identify isolates with minimum influence at 10 and 30 °C, WM 731 was used for inoculation of the plants. Isolates WM721 and WM031 showed the smallest colony sizes across temperatures; WM031 was used in the following experiment since its growth rate was not significantly different from WM721 at extreme temperatures and more sclerotia were available for the next study.

When the effect of temperature on disease development was evaluated, isolates and resistant genotypes did not show a significant interaction and main effects of the treatments were discussed. Isolate WM031 had a better performance at higher temperatures than isolate WM731, and that could be associated with the origin of the isolate; WM031 was collected from Nebraska which has a generally warmer climate compared to North Dakota, the place of origin of isolate WM731. At the same time, WM731 caused larger lesions at 10 °C than WM031. However, this association did not appear to be consistent among isolates throughout this study.

Other researchers have shown that growth on artificial media could be positively correlated to stem lesion expansion on plants (Ge et al. 2012). While correlation analyses were not conducted in this study, comparing the growth of isolates WM031 and WM731 on PDA medium with their growth on plants did not indicate a constant relationship between growing on the medium and plants at all temperatures. In other words, two isolates were significantly different at 25 °C but not at 10 and 30 °C on PDA; however, they showed differences at 10 °C but not at 25 and 30 °C on plants.

Colt was identified as a semi-resistant genotype in the previous study conducted by Khot et al. (2011) while it showed a high level of susceptibility in our study. The difference in the level of resistance observed in different studies could be due to a number of reasons including segregation of the plants produced by a single seed source (Zhang et al. 2011). Using double haploid plants or recombinant inbred lines that are completely homozygous (Xu et al. 2017) could secure obtaining similar results in future studies. It is noteworthy that Khot et al. (2011) evaluated and verified the resistance of Chisaya natane and Gan You No 1 under filed conditions while Colt resistance was concluded only under growth chamber and field experiments; suggesting that environmental conditions have an important role on resistance level of genotypes.

Temperatures equal or greater than 30 °C have been reported as the upper limit of the lesion formation and development (Abawi and Grogan 1975; Koch et al. 2007, Clarkson et al. 2014; Uloth et al. 2015; Shahoveisi and del Río Mendoza 2020). However, our results indicated that this threshold is highly dependent on the host genotype. While two resistant genotypes, Chisaya natane and Gan You No 1, and the susceptible check had a rAUDPC range of 11.2-16.3 cm at 30 °C, Colt showed a rAUDPC of 40 cm which is approximately twice as those on the

resistant genotypes. These results suggest that the pathogen could grow noticeably at high temperatures when the host is highly susceptible.

In general, the optimum temperature for mycelial growth of isolates and disease development on plants was 25 °C. Further, results of this study showed that resistant and susceptible genotypes were consistently resistant and susceptible, respectively, at all temperature regimes. Therefore, no evidence verified that genes conferring resistance in canola-*S. sclerotiorum* pathosystems could be affected by temperature regimes. While literatures have verified the presence of temperature-dependent defense and resistance genes in other pathosystems such as wheat-stripe rust (Fu et al. 2009), canola-blackleg (Huang et al. 2006), and Arabidopsis- *Pseudomonas syringae* (Li et al. 2020), information about the role of temperature on defense genes activity is not available for canola-SSR pathosystems. Complementary experiments to evaluate the reaction of more genotypes with different levels of resistance are required.

References

Abawi, G. S., and Grogan, R. G. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. Phytopathology 65:300-309.

Abawi, G. S., and Grogan, R. G. 1979. Epidemiology of diseases caused by Sclerotinia species. Phytopathology 69:899-904.

Boland, G. J., and Hall, R. 1988. Epidemiology of Sclerotinia stem rot of soybean in Ontario. Phytopathology 78:1241-1245.

Clarkson, J. P., Fawcett, L., Anthony, S. G., and Young, C. 2014. A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. PLoS One 9:e94049.

del Río, L. E., Bradley, C. A., Henson, R. A., Endres, G. J., Hanson, B. K., McKay, K., Halvorson, M., Porter, P. M., Le Gare, D. G., and Lamey, H. A. 2007. Impact of Sclerotinia stem rot on yield of canola. Plant Dis. 91:191-194.

Domingues, M. V. P. F., Moura, K. E. D., Salomão, D., Elias, L. M., and Patricio, F. R. A. 2016. Effect of temperature on mycelial growth of *Trichoderma*, *Sclerotinia minor* and *S. sclerotiorum*, as well as on mycoparasitism. Summa Phytopathol. 42:222-227.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., and Dubcovsky, J. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357-1360.

Ge, X. T., Li, Y. P., Wan, Z. J., You, M. P., Finnegan, P. M., Banga, S. S., Sandhu, P. S., Garg, H., Salisbury, P. A., and Barbetti, M. J. 2012. Delineation of *Sclerotinia sclerotiorum* pathotypes using differential resistance responses on *Brassica napus* and *B. juncea* genotypes enables identification of resistance to prevailing pathotypes. Field Crops Res. 127:248-258.

Godoy, C. V., Koga, L. J., Oliveira, M. C. N. D., Hill, C. B., and Hartman, G. L. 2017. Mycelial growth, pathogenicity, aggressiveness and apothecial development of *Sclerotinia sclerotiorum* isolates from Brazil and the United States in contrasting temperature regimes. Summa Phytopathol. 43:263-268.

Harikrishnan, R., and del Río, L. E. 2006. Influence of temperature, relative humidity, ascospore concentration, and length of drying of colonized dry bean flowers on white mold development. Plant Dis. 90:946-950.

Huang, H. C., and Kokko, E. G. 1989. Effect of temperature on melanization and myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 67:1387-1394.

Huang, Y. J., Evans, N., Li, Z. Q., Eckert, M., Chèvre, A. M., Renard, M., and Fitt, B. D. 2006. Temperature and leaf wetness duration affect phenotypic expression of Rlm6-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytol. 170:129-141.

Khot, S. D., Bilgi, V. N., del Río, L. E., and Bradley, C. A. 2011. Identification of *Brassica napus* lines with partial resistance to *Sclerotinia sclerotiorum*. Plant Health Prog. 12:15-24.

Koch, S., Dunker, S., Kleinhenz, B., Röhrig, M., and Tiedemann, A.V. 2007. A crop loss-related forecasting model for Sclerotinia stem rot in winter oilseed rape. Phytopathology 97:1186-1194.

Krishnamoorthy, K. K., Sankaralingam, A., and Nakkeeran, S. 2017. Effect of temperature and salinity on the growth of *Sclerotinia sclerotiorum* causing head rot of cabbage. Int. J. Curr. Microbiol. Appl. Sci. 6: 950-954.

Lane, D., Denton-Giles, M., Derbyshire, M., and Kamphuis, L. G. 2019. Abiotic conditions governing the myceliogenic germination of *Sclerotinia sclerotiorum* allowing the basal infection of *Brassica napus*. Australas. Plant Path. 48:85-91.

Li, Z., Liu, H., Ding, Z., Yan, J., Yu, H., Pan, R., Hu, J., Guan, Y., and Hua, J. 2020. Low temperature enhances plant immunity via salicylic acid pathway genes that are repressed by ethylene. Plant Physiol. 182:626-639.

Pope, S. J., Varney, P. L., and Sweet, J. B. 1989. Susceptibility of cultivars of oilseed rape to *Sclerotinia sclerotiorum* and the effect of infection on yield. Asp. Appl. Biol. 23:451-456.

Rothmann, L. A., and McLaren, N. W. 2018. *Sclerotinia sclerotiorum* disease prediction: A review and potential applications in South Africa. S. Afr. J. Sci. 114:1-9.

Saharan, G. S., and Mehta, N. 2008. Sclerotinia diseases of crop plants: Biology, ecology and disease management. Springer Science+Busines Media B.V. The Netherlands.

Shahoveisi, F., and del Río Mendoza, L. E. 2020. Effect of Wetness Duration and Incubation Temperature on Development of Ascosporic Infections by Sclerotinia sclerotiorum. Plant Dis. 104:1817-1823.

Sharma, P., Verma, P. R., Meena, P. D., Kumar, V., and Singh, D. 2016. Research progress analysis of Sclerotinia rot (*Sclerotinia sclerotiorum*) of oilseed brassicas through bibliography. J. oilseed Brassica 1:45-125.

Sun, P., and Yang, X. B. 2000. Light, temperature, and moisture effects on apothecium production of *Sclerotinia sclerotiorum*. Plant Dis. 84:1287-1293.

Uloth, M. B., You, M. P., Cawthray, G., and Barbetti, M. J. 2015. Temperature adaptation in isolates of *Sclerotinia sclerotiorum* affects their ability to infect *Brassica carinata*. Plant Pathol. 64:1140-1148.

Wu, B. M., and Subbarao, K. V. 2008. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathology 98:1144-1152.

Xu, Y., Li, P., Yang, Z., and Xu, C. 2017. Genetic mapping of quantitative trait loci in crops. Crop J. 5:175-184.

Young, C. S., Clarkson, J. P., Smith, J. A., Watling, M., Phelps, K., and Whipps, J. M. 2004. Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. Plant Pathol. 53:387-397.

Zhang, J., Qi, C., Pu, H., Chen, S., and Chen, F. 2011. Genetic map construction and Sclerotinia resistance QTLs identification in rapeseed (*Brassica napus* L.). Proc. Int. Rapeseed Cong. 59: 673676-673680.

Zhu, Y., Qian, W., and Hua, J. 2010. Temperature modulates plant defense responses through NB-LRR proteins. PLoS Pathog. 6:e1000844.

CHAPTER 6. CONCLUSIONS

Canola is the second major oilseed crop in the world and the United States is one of the top producers worldwide. North Dakota with 85% of the US production is the leading state nationwide. While several diseases can affect canola, SSR is one of the most important and endemic diseases in North Dakota. SSR could cause sever yield losses under favorable environmental conditions and presence of the susceptible host. Objectives of this dissertation were to identify genetic regions associated with response to SSR through various phenotyping and genotyping methods and to investigate the role of environmental factors such as temperature and wetness duration on epidemiology of the disease.

In the first study, two double haploid mapping populations were produced and used for QTL bi parental analyses. Stem lesion and mortality were the original disease rating methods which were used to generate other eight phenotyping scoring systems including binomial, polynomial, and rAUDPC data sets. In general, lesion size, rAUDPC, and mortality binomial were the most efficient systems in identification of QTL. Among three genotyping pipelines, data imputation could increase the number of QTL (with high R^2) in one population but decreased the efficiency in the other population. Unique mapping reads did not affect the result of the QTL analysis in either populations suggesting double haploid populations may not require this variant filtering step. Using no segregation distortion Chi-Square test or less stringent levels (α= 0.0001) had a positive impact on the number of the detected QTL while R^2 values remained unchanged. Overall, 19 QTL on chromosomes A02, A07, A09, C01, and C03 were identified in two populations where different methods were applied. While QTL associated with SSR on the same physical range of A02, A09, C01, and C03 have been reported in the previous studies, the QTL on A07 is reported for the first time and it could be considered a novel QTL. Findings of

this study are useful in future breeding programs; however, the next step is to evaluate the efficiency of these markers in other populations under field conditions.

In the second study, the role of two significant weather factors including temperature and wetness duration in *S. sclerotiorum* ascospore germination and lesion establishment on leaves were investigated. Ascospore germination was significantly higher at 21 °C. Less than 10 % of ascospores germinated at 10 and 30 °C after 8 h; however, increasing the incubation period to 24 h resulted in average germination of 13% at this temperature. While 25 °C was the optimum temperature for the disease development, ascospores could germinate and form lesions at 10 and 30 °C. Using wetness duration, temperature, and quadratic effects of temperature, a logistic regression model with 77% sensitivity, 72% specificity, and 75% accuracy under field conditions was developed. This model will be incorporated into the future SSR warning systems to assist growers for fungicide applications.

In the third study, the role of temperature in mycelial growth of 19 *S. sclerotiorum* isolates collected from different climates in the US was evaluated. All isolates formed colonies at 10, 15, 20, 25, and 30 °C; however, the optimum temperature for mycelial growth was 25 °C. Two fast and slow growing isolates, which their growth were not highly affected at 30 °C, were selected for the next study. The last study was designed to evaluate the role of host resistance and temperature on the disease development where two different isolates were used for inoculation. Results indicated that 25 °C was the optimum temperature for the pathogen regardless of the host resistance level and isolates. However, disease development at 30 °C was highly affected by the host susceptibility. In other worlds, disease progress in resistant and semi susceptible plants was hampered at this temperature while high level of infections were observed in a highly susceptible

plant material. Results of this study indicated that host resistance affects the role of temperature in disease development.

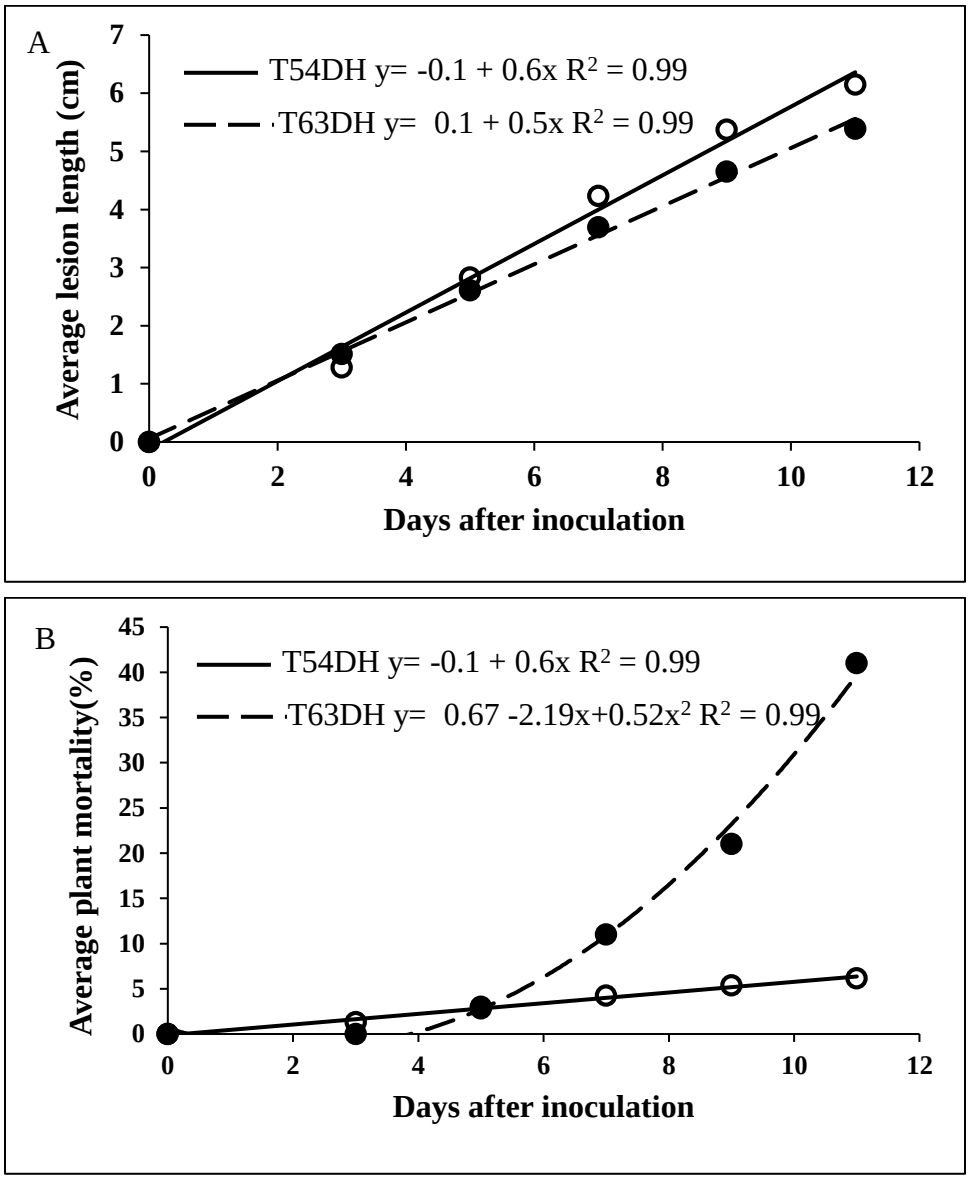
In summary, understanding the epidemiology of SSR disease, genetic of host resistance, and combined impact of these two factors on SSR development facilitates the management of the disease and sustainable production of canola.

APPENDIX A. NINE DIFFERENT SCORING SYSTEMS USED AS THE PHENOTYPING INPUT FOR THE QUANTITAIVE TRAIT LOCI (QTL) ANALYSES

Scoring system	Description
Lesion size	Length of the lesion at day 9 (original lesion size)
Lesion size, binomial	Binomial dataset of the original lesion size with values of 0 ($\leq$ 3cm) and 1 (> 3cm)
Lesion size, polynomial	Polynomial dataset of the original lesion size with values of 1 ($\leq$ 3cm), 2 (> 3 and $\leq$ 6cm), and 3 (> 6cm)
rAUDPC1[a]	Area under mortality curve using data from day 3, 5, 7, 9, and 11
rAUDPC2	Area under mortality curve using data from day 5, 7, 9, and 11
rAUDPC3	Area under mortality curve using data from day 3, 7, and 11
Mortality, binomial	Binomial dataset of the original mortality percentage with values of 0 ($\leq$ 20% cm) and 1 (> 20%)
Mortality, polynomial	Polynomial dataset of the mortality percentage with values of 1 ($\leq$ 20%), 2 (> 20 and $\leq$ 60%), and 3 (> 60%)

[a] relative area under mortality curve

APPENDIX B. DAILY STEM LESION EXPANSION RATES (A) AND MORTALITY RATES (B) OF SCLEROTINIA STEM ROT ON DOUBLED HAPLOID MAPPING POPULATIONS T63DH and T54DH



APPENDIX C. *BRASSICA NAPUS* CANDIDATE GENES LOCATED WITHIN FLANKING MARKERS OF THE QTL WITH HIGH SELECTION EFFICIENCY IN SEPARATION OF SUSCEPTIBLE AND RESISTANT REACTIONS TO *SCLEROTINIA SCLEROTIORUM*

Gene Model	QTL	Gene position	Protein name	Function	Reference (function/ expression)
BnaA02g12230D	SR63.A2.1	6476887 to 6477963 (- stand)	NDR1/HIN1 like protein	Response to bacteria, viruses, salicilic acid, wounding, hypoxia	**Zhu et al. 2013 * Zheng et al. 2004 * Varet et al. 2002 * Century et al. 1997
BnaA02g12320D	SR63.A2.1	6521004 to 6524302 (+ strand)	Cytosolic isocitrate dehydrogenase	Plant defense, Stress response	* Mhamdi et al. 2010
BnaA02g12540D	SR63.A2.1	6726474 to 6729239 (+ strand)	Farnesoic acid carboxyl-O-methyltransferase	Response to molecule of fungal origin/ response to SA/ to jasmonic acid/ to wounding	*Yang et al. 2006
BnaA02g13340D	SR63.A2.1 SR63.A2.2	7333458 to 7335568 (+ strand)	Probable pectate lyase 13	Defense response, incompatible interaction/pectin catabolic process/cell wall modification	*Vogel et al. 2002
BnaA02g13590D	SR63.A2.1 SR63.A2.2	7458166 to 7459260 (+ strand)	Clathrin assembly protein At1g68110	Defense response to fungus/endocytosis	*Chatukuta et al. 2018
BnaA02g14050D	SR54.A2.1	7884003 to 7885500 (- strand)	Peroxidase	Biosynthesis and degradation of lignin, response to environmental stresses such as wounding, pathogen attack and oxidative stress	Vasconcellos et al. 2017 **Leite et al. 2014 *O'Brien et al. 2012 * Bindschedler et al. 2006
BnaA02g14120D	SR54.A2.1	7937710 to 7938221 (- strand)	Putative defensin-like protein 263	Defense response to fungus/killing of cells of other organism/Antimicrobial, Fungicide/Plant defense	Lacerda et al. 2014 *Seo et al. 2014 Sels et al. 2008

Gene Model	QTL	Gene position	Protein name	Function	Reference (function/ expression)
BnaA02g14130D	SR54.A2.1	7952175 to 7952680 (- strand)	Putative defensin-like protein 264	Defense response to fungus/killing of cells of other organism/Antimicrobial, Fungicide/Plant defense	Lacerda et al. 2014 *Seo et al. 2014 Sels et al. 2008
BnaA02g14390D	SR54.A2.1	8103071 to 8103603 (- strand)	Putative defensin-like protein 184	Defense response to fungus/killing of cells of other organism/Antimicrobial, Fungicide/Plant defense	Lacerda et al. 2014 *Seo et al. 2014 Sels et al. 2008
BnaA02g14780D	SR54.A2.1	8452393 to 8454564 (- strand)	WAT1-related protein At1g70260	Negative regulation of defense response to bacterium/negative regulation of defense response to oomycetes/regulation of defense response to fungus	*Pan et al. 2016 *Denancé et al. 2013
BnaA02g15190D	SR54.A2.1	8731495 to 8732680 (- strand)	BnaC02g20340D protein	Defense response	-
BnaA02g15240D	SR54.A2.1	8772454 to 8773392 (- strand)	BnaC02g20370D protein	Defense response	-
BnaA02g15250D	SR54.A2.1	8775119 to 8775838 (- strand)	MLP-like protein 31	Defense response	**Garg et al. 2013 *Yang et al. 2015
BnaA02g15660D	SR54.A2.1	9138032 to 9139782 (+ strand)	NAC domain-containing protein	Defense response to fungus/ abscisic acid/ auxin/and cytokinin	** Qasim et al. 2020 * Nuruzzaman et al. 2013 *Wang et al. 2009 *Lin et al. 2007 *Nakashima et al. 2007

Gene Model	QTL	Gene position	Protein name	Function	Reference (function/ expression)
BnaA02g16350D	SR54.A2.1	9722016 to 9722255 (- strand)	Protein CLAVATA 3 Auxin efflux carrier component	Innate immune response/receptor serine/threonine kinase binding	Tena et al. 2011 *Lee et al. 2011
BnaA02g17500D	SR54.A2.1	10566250 to 10566946 (+ strand)	Defensin like protein	Response to biotic stimulus/defense response	Lacerda et al. 2014 *Seo et al. 2014 Sels et al. 2008
BnaA02g17510D	SR54.A2.1	10569933 to 10570266 (+ strand)	Defensin-like protein	Defense response/defense response to fungus/killing of cells of other organism	Lacerda et al. 2014 *Seo et al. 2014 Sels et al. 2008

References with (*) represent studies conducted on expression analysis of the genes coding the same proteins identified in this study. References with (**) represent studies conducted on expression analysis of the genes related to resistance response to *Sclerotinia sclerotiorum*.

APPENDIX D. GROWTH RATE (SLOPE OF THE LINEAR REGRESSION EQUATIN) OF 19 *SCLEROTINIA SCLEROTIORUM* ISOLATES ON POTATO DEXTROSE AGAR(PDA) AT 10-20 °C, 20-25 °C, AND 25-30 °C AFTER 36 HOURS OF INCUBATION

Isolate	Growth rate (cm^2)		
	10-20 °C	20-25 °C	25-30 °C
WM31	4.6	3.2	- 10.6
WM 217	8.2	2.7	- 19.9
WM 374	14.3	11.3	- 42.9
WM 412	13.4	8.6	- 33.5
WM 585	9.3	3.4	- 19.9
WM 603	11.3	9.2	- 30.1
WM 623	9.6	2.4	- 19.4
WM 636	12.5	8.5	- 33.9
WM 644	10.9	- 3.5	- 14.6
WM 721	2.4	1.5	- 6.6
WM 731	14.6	11.8	- 38.9
WM 746	11.7	0.6	- 20.1
WM 766	16.4	12.7	- 46.2
WM 812	18.1	9.5	- 48.9
WM 841	10.9	9.6	- 31.6
WM 842	16.6	6.6	- 40.5
WM 848	10.1	1.4	- 18.5
WM 876	13.5	12.8	- 39.2
WM 877	14.8	11.3	- 38.8