Screening and quantification of disease responses in *Lens ervoides* against multiple fungal pathogens

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon

By

Karan Kapoor

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work, or in their absence, by the Head of the Department or the Dean of the College in which my thesis was done. It is understood that any copying or publication or use of the thesis, in whole or in part, for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Head of the Department of Plant Sciences

University of Saskatchewan

51 Campus Drive

Saskatoon, SK S7N 5A8

OR

Dean

College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 – 110 Science Place

Saskatoon SK S7N 5C9

ABSTRACT

Lens ervoides is a potential source of novel disease resistance genes against the three major lentil pathogens *Ascochyta lentis, Colletotrichum lentis* and *Stemphylium botryosum*. Experiments were conducted to evaluate 157 *L. ervoides* accessions for resistance to *A. lentis* under field and greenhouse conditions to determine whether *L. ervoides* has non-host resistance and revealed that *A. lentis* isolate AL-61 was able to complete its life cycle on all accessions. This confirmed that *L. ervoides* does not possess non-host resistance against the pathogen. Six out of 157 accessions were identified as highly susceptible, 34 as moderately susceptible, 38 as intermediate, 67 as moderately resistant and 12 as highly resistant. The wide range of resistance levels among *L. ervoides* accessions warranted further histopathological investigations into the infection process of *A. lentis*, as well as *C. lentis* and *S. botryosum*. Leaflet samples of selected resistant and susceptible recombinant inbred lines (RILs) of intraspecific *Lens ervoides* population LR-66, its parents and susceptible *L. culinaris* check Eston and resistant check CDC Robin were collected from 6 to 240 hours post inoculation (hpi) to determine whether resistance in *L. ervoides* is quantitative or qualitative. Conidial germination of *A. lentis* was significantly higher on susceptible RIL LR-66-570 compared to resistant RIL LR-66-629 from 6 to 24 hpi but not at 48 hpi. Pycnidia formed on all *A. lentis*-infected leaflets of included genotypes, further confirming that there is no non-host resistance in *L. ervoides*. The development of infection vesicles and primary hyphae by *C. lentis* were significantly higher on anthracnose-susceptible RIL LR-66-524 compared to resistant LR-66-528 at 24 and 48 hpi. Conidial germination, germ tube length and germ tube penetration by *S. botryosum* were not significantly different on the Stemphylium blight-resistant and susceptible RILs, but the area of dead tissue per leaflet was significantly higher in Stemphylium blight-susceptible RIL LR-66-577 compared to resistant LR-66-637 from 96 to 144 hpi. Histopathology data revealed quantitative and not qualitative differences among LR-66 RILs against the three pathogens. Ascochyta blight screening and histopathology on all three pathogens provide a foundation for further research into the molecular control of resistance in *L. ervoides*.

ACKNOWLEDGEMENTS

To begin with, I would like to thank my supervisor Dr. Sabine Banniza for her help, support and time in completing my research project and thesis. I would also like to thank my committee members, Drs. Aaron Bettie, Kirstin Bett and Pierre Hucl for their suggestions regarding the experimental design and their useful review and comments regarding my thesis. I am also grateful to my external examiner Dr. Sally Vail for the critical review and inputs for improving this thesis.

I would like to thank Pulse Pathology Lab members, especially Cheryl Armstrong-Cho, Stephanie Boechler, Nimllash Sivachandra Kumar, Kiela Caudillo Ruiz and Kamal Pathirannehelage for their help in conducting the experiments.

I would also like to express my gratitude towards the members of Crop Science Field Lab, especially Allison Sackville, Devini De Silva, Thiago Do Prado, Stacey Wagenhoffer, Scott Ife, Kevin Mikituk for their help with the field experiments.

I would like to thank Eldon Siemens and Jacqueline Bantle for their help in facilitating my experiments in the greenhouse.

I am grateful to the funding agencies Natural Sciences and Engineering Research Council (NSERC), Saskatchewan Pulse Growers (SPG), Western Grains Research Foundation (WGRF) and the Government of Saskatchewan.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Chapter 1

1.0 Introduction

Lentil is one of the most important legume crops in Canada, accounting for a major share of total agricultural exports. Lentil is the sixth most important legume crop regarding production with 4.8 million metric tons worldwide in 2014 (FAOSTAT, 2014). It gains its importance from the fact that it is a rich source of protein and nutrients for the large vegetarian population in Indian sub-continent where it is consumed with rice or wheat. Breeding efforts in the past three decades have immensely increased the production of lentil all over the world, especially in North America. This improvement through breeding, however, also resulted in a narrowing of the genetic base of lentil germplasm through continuous selection for desired agronomic traits. As the number of diverse alleles was reduced in cultivated lentil germplasm, crops have become more prone to different biotic stresses. This prompted scientists to focus on wild relatives of lentil as a potential source for novel resistance genes against different diseases. Ascochyta blight (caused by *Ascochyta lentis*), anthracnose (caused by *Colletotrichum lentis*) and Stemphylium blight (caused by *Stemphylium botryosum*) are threats to current and future lentil production in Canada and the northern United States of America. Although interspecific populations have been successfully developed, segregation distortion in these populations has made it difficult to determine the genetic control of disease resistance and to develop markers. To avoid these problems encountered in interspecific populations, the intraspecific *Lens ervoides* (Brign.) Grande. recombinant inbred line (RIL) population LR-66 was developed from a cross of accessions IG 72815 and L01-827A. LR-66 is of interest because IG 72815 has high levels of resistance against race 0 of *C lentis*, which has not been found in *L. culinaris* to date (citation). Novel resistance genes against Ascochyta blight and Stemphylium blight are also expected to be present in these accessions. Ninety-four RILs of LR-66 along with their parents were previously screened for all three diseases, and based on disease scores, resistant and susceptible RILs were identified (Bhadauria et al. 2017). The parents have contrasting disease resistance for anthracnose and Stemphylium blight (IG 72815 is highly resistant and L01-827A is moderately susceptible to both diseases), but not for Ascochyta blight for which both have resistance.

Genotyping-by-sequencing of LR-66 has already been completed, and a linkage map consisting of 7 linkage groups (LGs) has been generated based on recombination frequencies between single nucleotide polymorphisms (SNPs). Quantitative Trait Loci (QTLs) were mapped for *C. lentis* with five for race 0 and 6 QTLs for race 1 resistance. Of the five QTLs mapped for race 0, one was mapped on LG3 explaining 14% of the variation, two on LG5 together explaining 33%, one on LG2 explaining 11%, and another one on LG7 explaining 9% of the phenotypic variation. For race 1 resistance, two QTLs were mapped on LG3 explaining 39% of the variation, two on LG5 explaining 31% of the variation, and 2 on LG2 explaining 19% of the variation. Two QTLs for Stemphylium blight explaining 28% of the phenotypic variation were mapped on LG2 and one explaining 12% of the variation to LG3. In another study, while studying the genetics for Ascochyta blight resistance in an interspecific population (*Lens culinaris* cv. Eston x *L. ervoides* L01-827A), Sari (2014) observed absence of macroscopic disease symptoms on L01-827A plants even though the same plants were inoculated twice, thus ruling out disease escape and age dependant resistance. He therefore hypothesized that L01-827A might have genes mediating non-host resistance against *A. lentis*.

The first objective of this project was to determine whether the nature of resistance to *A. lentis* in *L. ervoides* is based on non-host resistance by screening *L. ervoides* accessions for Ascochyta blight resistance under greenhouse and field conditions. Based on the work done so far on LR-66, this project was also aimed at studying the development of *C. lentis*, *A. lentis* and *S. botryosum* at the cellular level to identify qualitative or quantitative differences among selected RILs of LR-66 and *L. culinaris* controls.

1.1. Research Hypothesis

1) The nature of resistance in *L. ervoides* against Ascochyta blight is based on non-host resistance.

2) Resistance to Stemphylium blight and anthracnose is quantitative whereas for Ascochyta blight it is qualitative.

1.2 Research objectives

1 To screen the *L. ervoides* germplasm collection for Ascochyta blight resistance with the objective to identify susceptible accessions;

2 To assess disease progression through histopathology studies of Ascochyta blight, anthracnose and Stemphylium blight on selected *L. ervoides* LR-66 RILs;

Chapter 2

Literature Review

2.1 Lentil

2.1.1 Lentil biology

Lentil is an annual self-pollinating diploid (2n = 14) legume crop with a genome size of ~4 Gbp (Arumuganathan and Earle 1991). Cultivated lentil (*L. culinaris* ssp. *culinaris*) are bushy annual herbaceous plants with erect, semi-erect or spreading growth habit ranging from 15 - 75 cm. Stems and branches are slender in shape, and the root system consists of a taproot with fibrous lateral roots with nodules. Leaves are pinnate or imparipinnate with sessile leaflets varying from 1 - 3 cm. Each leaf has two small stipules at its base and usually terminates in a tendril depending upon genotype. The flower is complete, papilionaceous on peduncles and develops at the upper nodes of the plant. The colour of flower varies from white to purple-blue. Pods are oblong, compressed, ranging in length from 6 - 22 mm and in width from 3.5 - 11 mm, and contain one or two seeds. Seeds are classified based on size into macrosperma type (6 to 9 mm) or small and medium-sized microsperma (2 to 6 mm) (Barulina 1930, Muehlbauer et al. 1995).

2.1.2 The genus *Lens*

The genus *Lens* (Miller) belongs to the tribe Viciae, subfamily Papilionaceae and family Fabaceae (Leguminoseae). Although the genus *Lens* was originally classified in 1740 by Miller the taxonomy of, and phylogenetic relationships among, *Lens* species is still the subject of discussion (Cubero et al. 2009). Ladizinsky (1979) classified the genus *Lens* into the four species *L. culinaris*, *L. orientalis*, *L. nigricans*, and *L. ervoides*. However, later some accessions of *L. nigricans* were reclassified as *L. odemensis* thus a new classification of *L. culinaris* into the cultigen subspecies *culinaris* and the wild ssp. *orientalis* and *odemensis* were established, whereas *L. nigricans* obtained the two subspecies *nigricans* and *ervoides* (Ladizinsky et al. 1984). Species were reclassified again as *L. culinaris*, with subspecies *culinaris* and *orientalis*, *L. odemensis*, *L. ervoides* and *L. nigricans* (Ladizinsky 1993a).

Later, two new species were added to the genus, *L. tomentosus* (Ladizinsky 1997) and *L. lamottei* (Van Oss et al. 1997). The most recent species classification was developed by Ferguson et al. (2000) on the basis of molecular and morphological markers identifying *L. culinaris*, with the four subspecies *culinaris*, *orientalis*, *tomentosus* and *odemensis*, *L. ervoides*, *L. nigricans* and *L. lamottei*. This classification was also confirmed by Zimniak-Przybylska et al. (2001). Therefore, there are currently six species in the genus *Lens*: *L. culinaris* ssp. *culinaris* and *orientalis* (Boiss.) Ponert; *L. odemensis* (Godr.) Ladiz; *L. tomentosus* Ladiz; *L. nigricans* (Bieb.) Godr; *L. ervoides* and *L. lamottei* Czfr. (Cubero et al. 2009, Richard et al. 2014).

2.1.3 The concept of genepool in the genus *Lens*

The genus Lens is classified into three genepools based upon the ability of species to form fertile hybrids (Harlan and de Wet 1971). Based on the latest classification, the primary genepool contains *L. culinaris* ssp. *culinaris* and *orientalis* both of which form fertile hybrids, and of which ssp. *orientalis* is designated as the progenitor of lentil. The secondary genepool contains species that can form partially fertile hybrids with the cultivated lentil species through embryo rescue techniques and includes *L. odemensis*, *L. ervoides*, and *L. tomentosus*. The tertiary genepool comprises *L. nigricans* and *L. lamottei* as these are not able to form hybrids with cultivated lentil (Ladizinsky and Abbo 2015). In another study (Wong et al. 2015) gene pools were classified based on genotype-by-sequencing (GBS) analysis concluding that *L. culinaris*, *L. orientalis* and *L. tomentosus* are in primary, *L. lamottei* and *L. odemensis* are in the secondary, *L. ervoides* is in the tertiary and *L. nigricans* is in the quaternary gene pools which is different from earlier classifications. From an evolutionary point of view, the genus *Lens* is active and therefore it is always possible to have some interchanges among the genepools (Cubero et al. 2009).

2.1.4 Wild relatives as repository for crop improvement

Cultivated lentil germplasm has become more prone to diseases possibly due to a narrow genetic base. Therefore, it has been considered necessary to exploit wild relatives for genetic improvement. The use of wild relatives will result in the widening of the genetic base of cultivated germplasm as

these are potential novel sources of resistance to various biotic stresses. Though efforts for the development of hybrids started in the 1970's, trait-based screening of wild germplasm was not initiated until the 1990's. ICARDA took the lead and reported the first results from testing of wild lentil species. Bayaa and Erskine (1991) screened accessions of *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis*, *L. nigricans*, and *L. ervoides* for vascular wilt resistance, among which three accessions of *L. culinaris* ssp. *orientalis* and *L. nigricans* and two accessions of *L. ervoides* were found to be resistant at both the seedling and adult stages. Similarly, for Ascochyta blight, 86 *L. culinaris* ssp. *orientalis*, 35 *L. odemensis*, 35 *L. nigricans* and 89 *L. ervoides* accessions were screened and 24, 12, 3 and 36 accessions, respectively, were found to be resistant (Bayaa et al.1994) (Table 4.1). For Fusarium wilt, Bayaa et al. (1995) screened 109 accessions of *L. culinaris* ssp. *orientalis*, 17 of *L. culinaris* ssp. *odemensis*, 30 of *L. nigricans* ssp. *nigricans*, and 63 of *L. nigricans* ssp. *ervoides* at the seedling stage. All accessions of *L. culinaris* ssp. *odemensis* were susceptible, and only three accessions (ILWL 70, 79, 113) of *L. culinaris* ssp. *orientalis* and two (ILWL 59, ILWL 138) of *L. ervoides* had good resistance to Fusarium wilt. ILWL 138 showed resistance to both, wilt and Ascochyta blight (Bayaa et al. 1994, 1995). Similarly, Nasir (1998) screened 99 accessions of *Lens*, among which 36 *L. culinaris* ssp. *orientalis*, 4 *L. culinaris* spp. *odemensis*, 14 *L. ervoides* and 11 *L. nigricans* accessions were found resistant to Fusarium wilt. At ICARDA scientists tested 247 wild relatives for Fusarium wilt, among which accessions of *L. culinaris* ssp. *orientalis* and *L. ervoides* were found to be resistant to this disease (Gupta et al. 2011). Twelve wild lentil accessions and F_2 progenies of 10 interspecific crosses were screened by Ahmad et al. (1997) for Ascochyta blight resistance and identified two wild accessions each from *L. culinaris* ssp. *orientalis* (W6 3241, W6 3261) and *L. ervoides* (W6 3173, W6 3176), three accessions of *L. nigricans* (W6 3208, W6 3210, W6 3218) and one accession of *L. odemensis* (W6 3244) with high resistance. A genetic study of interspecific F_2 progenies revealed that host resistance to Ascochyta blight was controlled by two complementary dominant gene pairs in wild species of *L. ervoides* and *L. odemensis* (Ahmad et al. 1997). Further, it was determined by Ye et al. (2000), that two genes are responsible for resistance to Ascochyta blight from interspecific F_3 families from a cross between W 63261 (*L. orientalis*) and Titore (*L. culinaris*). While screening 375 accessions of six wild species of lentil for resistance to *Ascochyta lentis*, Tullu et al. (2010) observed that some accessions of *L. ervoides* (Table 4.1), *L. nigricans*, *L. culinaris* subsp. *orientalis* and *L. culinaris* subsp. *odemensis* were resistant while those of *L. culinaris* subsp. *tomentosus* were

susceptible. For anthracnose resistance Tullu et al. (2006) screened 574 accessions of all six wild *Lens* species against race 1 and race 0 of *C. lentis*. Accessions of *L. ervoides* (Table 4.1) and *L. lamottei* showed resistance against both races whereas those of *L. orientalis*, *L. odemensis*, *L. nigricans* and *L. tomentosus* were highly susceptible to race 0. In another study, Fiala et al. (2009) reported introgression of anthracnose resistance into RILs developed from *L. ervoides* accession L01-827A and cultivar Eston through embryo rescue. Genetic analysis indicated that resistance to races 0 and 1 was conferred by two recessive genes. This study was further confirmed by Vail et al. (2012) who identified specific RILs that conferred resistance to race 0. The authors also reported that *L. ervoides* accession IG 72815 has better resistance for anthracnose than the *L. ervoides* accession L01-827A. The involvement of two genes in resistance against race 0 of *C. lentis* was confirmed by Tullu et al. (2013) from an interspecific cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815. Recently Singh et al. (2014) screened 401 diverse wild lentil accessions comprising 171 of *L. culinaris* ssp. *orientalis*, 42 of *L. culinaris* ssp. *odemensis*, 35 of *L. nigricans*, 20 of *L. culinaris* ssp. *tomentosus*, 124 of *L. ervoides* and nine of *L. lamottei* for agronomic traits, rust and powdery mildew resistance. Accessions belonging to *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis*, *L. nigricans* and *L. ervoides* were highly resistant to both diseases (Table 4.1). Similarly, Podder et al. (2013) screened 56 wild relatives (from all six *Lens* species) for Stemphylium blight (caused by *Stemphylium botryosum* Wallr.) resistance, among which *L. lamottei* and *L. ervoides* accessions were found most resistant (Table 2.1). In another study, Singh et al. (2017) screened an interspecific F_5 generation of RIL population ILL 10829 (*L. culinaris*) × ILWL 30 (*L. ervoides*) against *Fusarium oxysporum* f. sp. *lentis* (Vasd. Srin.) Gord and identified two highly resistant RILs (RIL18, RIL86) in addition to thirteen resistant RILs. Recently 30 wild lentil accessions, which included 7 accessions each from *L. orientalis, L. odomensis, L. ervoides,* 8 of *L. nigricans* and one of *L. lamottei* were screened against two *A. lentis* isolates (FT13037, FT13038) among which two highly resistant *L. orientalis* accessions (ILWL 180, ILWL 7) to both the isolates were identified. Whereas six *L. nigricans* accessions (PI 572351, PI 572348, PI 615677, ILWL 37, PI 572359) were found to be resistant to *A. lentis* isolate FT13038 and moderately resistant to FT13037, one accession each of *L. odomensis* (PI 572360), *L. ervoides* (PI 572333), *L. lamottei* (ILWL 437), *L. orientalis* (ILWL 146) and *L. nigricans* (PI 572347) were moderately resistant to both *A. lentis* isolates (Dadu et al. 2017).

Table 2.1. *Lens ervoides* accessions as potential source of resistance against different fungal diseases

Source	Disease Resistance	Accessions	Disease Score (scale)
Singh et al. (2014)	Rust	ILWL 50, 54, 56, 58	≤ 5 (1 – 9)
Singh et al. (2014)	Rust & Powdery mildew (together)	ILWL 40, 41, 42, 159, 269, 292, 294, 321, 398, 418, EC series 718428, 718439	≤ 5 (1 – 9)
Singh et al. (2014)	Powdery mildew	ILWL 91	≤ 5 (1 – 9)
Bayaa et al. (1994)	Ascochyta blight	ILWL 41, 45, 50, 58, 63, 123, 128, 129, 130, 133, 134, 136, 138, 139, 141, 142, 158, 184, 185, 186, 193, 206, 204, 208, 259, 261, 262, 263, 269, 273, 274, 285, 294, 303, 318, 323	< 3 (1 – 9)
Ahmad et al. (1997)	Ascochyta blight	W6 3173 and W6 3176	< 5 (1 – 9)
Tullu et al. (2010)	Ascochyta blight	IG 72846, 107445, 72564, 72826, 72914, 72707, 72646, 72567, 72792, 72784, 72665, 72565, 72576, 72921, 72577, 72910, 72579, 72566, 72590, 72924, 72815, 72571, 72859, 75575, 72570, 72730, 72731, 72576, 72578, 72841	≤ 5 (0 – 11)
Dadu et al. (2017)	Ascochyta blight	ILWL 180, 7, 37, 437,146, PI 572351, 572348, 615677, 572359, 572360, 572333, PI 572347	≤ 5 (0 – 9)[a]
Podder et al. (2013)	Stemphylium blight	IG 72646, 72654, 72803, 72799, 72651, 72815, 116033	≤ 3 or 30% (0 – 10 or 0 – 100%)
Tullu et al. (2006)	Anthracnose (race 0)	IG 72579, 72653, 72654, 72655, 72665, 72815, 116014, 72659, 107441, 72651, 72792, 72588, 72793, 116023, 72646, 72664, 72660, 72799	< 5 (1 – 9)

[a] Numbers in brackets indicates rating scale which was used by authors for disease scoring.

2.1.5 Genome analysis of lentil germplasm

In the 1980's the first reports on the use of morphological and isozyme markers were published for lentil germplasm (Muehlbauer and Slinkard 1981; Zamir and Ladizinsky 1984; Tadmor et al. 1987; Muehlbauer et al. 1989). The first genetic map of lentil was developed by using restriction fragment length polymorphisms (RFLPs), isozyme and morphological markers (Havey and Muehlbauer 1988). Later, an interspecific genetic map of *L. ervoides* x *L. culinaris* was developed by Weeden et al. (1992) using 64 morphological, isozyme, and RFLP markers. A genetic map of lentil using three morphological, 89 random amplified polymorphic DNA (RAPD), 79 amplified fragment length polymorphism (AFLP) and six restriction fragment length polymorphism (RFLP) markers was developed by Eujayl et al. (1998a), whereas Rubeena et al. (2003) used 100 RAPDs, 11 inter-simple sequence repeats (ISSRs) and three resistance gene analog (RGA) markers. A further contribution was made by Duran et al. (2004) who developed a genetic map of lentil using five morphological, two SSRs, 71 RAPDs, 39 ISSRs and 83 AFLPs markers. Using 56 RAPDs, 106 ISSRs, 94 AFLPs and three morphological markers, Kahraman (2004b) developed a linkage map of lentil for winter hardiness. Another genetic map was reported by Hamwieh et al. (2005) which was developed using 41 microsatellites and 45 AFLP markers. Based on 207 AFLP, simple sequence repeat (SSRs) and RAPD markers, a linkage map of lentil for earliness and plant height was constructed by Tullu et al. (2008). A diversity analysis of a core set of lentil germplasm was carried out by Hamwieh et al. (2009), using 14 newly developed SSR markers. In another study, a linkage map was developed using 79 intron-targeted amplified polymorphic (ITAP) and 18 genomic simple sequence repeat (SSR) markers (Phan et al. 2007). Based on transcriptomics data, 192 EST-SSRs were designed by Kaur et al. (2011), out of which 166 primers were amplified from 12 cultivated lentil genotypes. Using Conserved Primers (CPs) from *Medicago truncatula* EST sequences Alo et al. (2011) carried out diversity analysis among 175 wild and 133 domesticated lentil accessions. In a similar study, Gupta et al. (2012) utilised 196 EST-SSR markers from *M. truncatula* and used them for generating a linkage map of lentil. Single Nucleotide Polymorphism (SNP) markers were developed by Sharpe et al. (2013) from transcriptomic sequences obtained from wild and cultivated lentil. A genetic map of *L. ervoides* comprising 377 SNP markers from a GoldenGate genotyping array and single SNP marker assays was developed by Gujaria-Verma et al. (2014). In a significant development, the lentil genome was sequenced at 125x coverage for lentil cultivar CDC Redberry and SNPs are being identified (Bett et al. 2014). Recently, Bett et al.

(2016) completed the sequencing of the lentil genome and sequence data was made available on the knowpulse portal (http://knowpulse.usask.ca/portal/lentil genome). One hundred and twenty–two functional SSR markers were developed by Verma et al. (2014), using a genomic library enriched for GA/CT. Recently, 50,960 SNPs were identified by Temel et al. (2014) through transcriptome sequencing, using the lentil genotypes Precoz and WA8649041, and were used to construct a SNP–based linkage map of lentil. In another study, Verma et al. (2015) developed 501 genomic SSR markers from two microsatellite genomic libraries enriched for GA/CT and GAA/CTT motifs generated a linkage map and mapped QTLs for seed weight and seed size on LG4 of lentil.

2.2 Interspecific Hybridization

Lens germplasm is divided into three genepools based on their ability to generate hybrids which may be completely or partially sterile (Ladizinsky and Abbo 2015). Attempts to develop crosses between *Lens* species of different genepools mostly resulted in embryo abortion (Abbo and Ladizinsky 1991). Embryo rescue techniques have been used to recover hybrids (Cohen et al. 1984), but in many cases, progeny derived from such hybrids eventually die due to meiotic irregularities. Cohen et al. (1984) developed an embryo rescue protocol in lentil and was able to develop interspecific hybrids between *L. culinaris* x *L. ervoides.* Ladizinsky et al. (1985) studied the cytogenetics of F_2 plants from a hybrid between *L. culinaris* (No.7) and *L. ervoides* (No. 32) using embryo rescue technique and found that hybrids were heterozygous for a reciprocal translocation which resulted in 50% gamete viability and a 1:1 ratio of aborted to viable embryos.

Based on cytogenetic studies it was determined that *L. ervoides, L. nigricans* and *L. odemensis* were isolated from each other as a result of which hybrid embryos can collapse before reaching maturity. Nonviable seeds, chlorophyll–deficient seedlings or meiotic aberrations leading to sterility have been observed (Ladizinsky 1993a). In contrast, successful intraspecific hybridization between *L. culinaris* ssp. *culinaris* and its subspecies have been achieved, although with some degree of variability in fertility of the hybrid progeny (Muehlbauer et al. 1989; Vandenberg and Slinkard 1989; Fratini et al. 2004, Ladizinsky 1979; Ladizinsky et al. 1984). When studying the two interspecific populations *L. ervoides* (No.32) x *L. culinaris* (No.7) and *L. orientalis* (No. 133) x *L. culinaris* (No 2), Abbo and Ladizinsky (1994) reported that F_1 embryo abortion was affected by dominant gene action and was not caused by chromosomal aberrations. Crosses between

cultivated lentil and *L. culinaris* ssp. *orientalis* were developed for introgression of genes from the wild parent (Hamdi and Erskine 1994). Among 30 interspecific crosses between cultivated lentil and four wild species (*L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis*, *L. ervoides*, and *L. nigricans*), 100% successful hybridization was achieved with *L. culinaris* ssp. *culinaris* and *L. nigricans* whereas with *L. orientalis* the success rate of hybridization was 66%, and it was 50% with the other species, where all hybrids died after three weeks (Ahmad et al. 1995).

Cultivated lentil was shown to be more cross-compatible with *L. culinaris* ssp. *orientalis* than with *L. culinaris* ssp. *odemensis* (Gupta 2003). Intra–specific and inter–sub–specific hybrids among *L. culinaris* ssp. *culinaris* and *L. culinaris* ssp. *orientalis* were developed by Fratini et al. (2004) under both field and greenhouse conditions. An embryo rescue protocol was developed by Fratini and Ruiz (2006), with which interspecific hybrids between the cultivated lentil and *L. ervoides*, *L. nigricans* and *L. odemensis* were recovered. They successfully developed intraspecific *(L. culinaris* ssp. *culinaris*) Tetir x ILL 323 and interspecific *L. culinaris* ssp. *culinaris* Alpo x *L. odemensis* lentil hybrids and multiplied them *in vitro* to produce F_2 seeds (Fratini and Ruiz 2008). An interspecific population between Eston (*L. culinaris* subsp. *culinaris*) x L01–827A (*L. ervoides*) was developed by Fiala et al. (2009) using embryo rescue technique and advanced the RIL population to an $F_{7:8}$ generation but many RILs could not be advanced due to variability in fertility with the consequence that the size of the RIL population was reduced from 150 RILs at F_2 to 85 RILs at the $F_{7:8}$ generation. Using an embryo rescue technique Tullu et al. (2013) developed an interspecific RIL population derived from a cross between *L. culinaris* ssp. *culinaris* Eston and *L. ervoides* IG 72815. In order to broaden the genetic base of *Lens* taxa, Singh et al. (2013) made 9 interspecific crosses, using 4 *L. culinaris* cultivars (Precoz, ILL 10829, L 830 and ILL 8006) and 7 wild lentil accessions which included 2 accessions each of *L. orientalis* (ILWL 7, ILWL 62), *L. odemensis* (ILWL 20, ILWL 81), *L. ervoides* (ILWL 30, ILWL 55) and one of *L. lamottei* (ILWL 14).

Further in this research, effects of the environment on the success of interspecific crosses, heterosis in the F_1 and transgressive segregants in the F_2 generations for morpho-physiological traits were studied (Singh et al. 2013, 2014). Using ovule culture, Suvorova (2014) developed an interspecific population from a cross between *L. culinaris* ssp. *culinaris* and *L. tomentosus* ILWL 120 and advanced the generation from F_2 to F_7. Interspecific hybrids were recovered by Saha et al. (2015)

between *L. culinaris* and *L. tomentosus*, *L. lamottei* and *L. odemensis* using embryo rescue technique using 4–chloroindole–3 acetic acid in combination with *in vivo* grafting and developed five interspecific F_2 populations.

2.3 Biotic stresses of lentils

2.3.1 Ascochyta Blight

Lentil plants are affected by fungal and viral diseases in the major regions around the world but Ascochyta blight is the most important one (Taylor et al. 2007). It is caused by *Ascochyta lentis* Vassilievsky (teleomorph: *Didymella lentis* W.J. Kaiser, B.C. Wang, and J.D. Rogers). Ascochyta blight was first reported in 1938 (Bondartzeva–Monteverde and Vassilievsky 1940, as reviewed in Ye et al. 2000), and since then its occurrence has been reported from all major lentil growing regions such as Australia, North America, the Middle East and South East Asia. Yield losses due to Ascochyta blight can be up to 70% (Gossen and Morrall, 1983).

2.3.1.1 Infection process of Ascochyta lentis

Roundhill et al. (1995) studied the infection process of *A. lentis* on lentil by inoculating detached leaflets with conidia. Conidia had germinated within 6 h after inoculation. Appressoria were formed after 10 h of inoculation. The penetration peg penetrated the cuticle near the junction of two epidermal cells, and the plasmalemma was disrupted within 40 h of inoculation. All cells were invaded by fungal structures by 52 h after inoculation and no nucleus or cytoplasm was intact anymore. After colonizing the epidermis, the fungus invaded mesophyll cells and by nine days symptoms became clearly visible. Sari et al. (2017) conducted descriptive microscopy on the three *Lens culinaris* ssp. *culinaris* accessions Eston, CDC Robin and 964a–46 at 6, 12, 18, 24, 36, 48, 60 and 90 h post–inoculation (hpi) with *A. lentis*. Host reactions were the same up to 48 hpi but differences in penetration of epidermal cells, papillae formation, massive colonization and disruptions of cytoplasm by the fungus could be seen among genotypes at 60 and 90 hpi. The authors reported that beneath the site of penetration, there was a reinforcement of the cell wall and formation of papillae in Eston and 964a–46 but not in CDC Robin. In contrast to Eston, 964a–46 developed thinner papillae. In addition, at 90 hpi colonizing fungal mycelium was denser in 964a–46 than Eston whereas it was not observed in CDC Robin.

In a recent study, histopathology was conducted on detached leaves of *L. culinaris* lines ILL 7537, ILL 5588 (cv. Northfield) and ILL 6002 (Sambasivam et al. 2017). Detached leaves were collected and fixed in a Petri dish and inoculated with *A. lentis* isolates AL4 (high virulence) or Kewell (low virulence) and incubated for 2, 6, 12, 24, 36 and 48 hours. Spore germination at 2 and 6 hpi, germ tube length at 2, 6, 12 and 24 hpi, and appressoria formation at 6, 12 and 24 hpi were measured. At 2 hpi, significant differences were observed in spore germination between isolates AL4 and Kewell on all three genotypes but at 6 hpi significant differences were only observed in ILL 7537 for both isolates. Isolate AL4 had significantly longer germ tube length than Kewell on all three genotypes for the period of 2 to 24 hpi. Isolate AL4 and Kewell differed significantly in appressoria formation in all three genotypes at 12 hpi.

2.3.1.2 Genetics and mapping of Ascochyta Blight resistance in lentil

The first report on the genetic control of Ascochyta blight resistance was published by Tay (1989) who studied five *L. culinaris ssp. culinaris* F_2 populations derived from Eston x Laird, Laird x ILL 5588, Eston x ILL 5588, Laird x ILL 5684 and Eston x ILL 5684. Results suggested that Laird has one recessive gene named ral_1, ILL 5684 contains the two dominant genes Ral_2 and Ral_3, and ILL 5588 contains the recessive gene ral_1 and the two dominant genes Ral_2 and Ral_3. Two duplicate recessive genes responsible for resistance to Ascochyta blight infection in an Indianhead x PI 345635 derived population were identified by Andrahennadi (1994). In the same year, Sakr (1994) reported two genes to be involved in resistance to Ascochyta blight, one dominant and one recessive.

In an attempt to use wild relatives as a source of resistance, Ahmad et al. (1997) studied the genetics of Ascochyta blight resistance using populations generated from crosses between cultivated lentil (*L. culinaris* ssp *culinaris*) and wild lentil species (*L. culinaris* ssp. *orientalis, L. ervoides and L. odemensis*). In *L. culinaris* ssp. *orientalis* a single dominant gene pair and in *L. ervoides and L. odemensis* two complementary dominant gene pairs were identified. While studying intraspecific cultivated lentil population, Andrahennadi (1997) identified the dominant gene Ral_1 and the recessive gene ral_2 in IL5588 and Indianhead, which were screened using RAPD markers in bulk segregant analysis. The RAPD marker $UBC227_{1290}$ was found to be linked with ral_2, which was also confirmed by Vakulabharanam et al. (1997).

Studying the genetics of Ascochyta blight resistance in the F_3 interspecific population derived from Titore (*L. culinaris* ssp. *culinaris*) x W6 3261 (*L. culinaris* ssp. *orientalis*), Parh (1998) observed a single dominant gene responsible for resistance to Ascochyta blight but found no RAPD marker linked to it. In another study conducted by Ford et al. (1999) it was reported that a single major locus with a dominant gene (*AbR1*) controls resistance in lentil accession ILL 5588, and seven RAPD markers linked to the resistance locus in coupling phase were identified, of which two markers, RB18, and RV01, are flanking the resistance locus. Two major genes were reported by Ye et al. (2000), one partially dominant with a large effect and one dominant gene with a lower effect. In a similar study, Ye et al. (2001) conducted a genetic study using populations generated from crosses of lentil cultivars Indianhead, Laird, ILL 5588 and ILL 5684 with susceptible cultivar Titore. In addition to these, Indianhead x Laird, and ILL 5684 x ILL 5588 were also generated. The F_1 was advanced to the F_2 generation and BC_1, BC_2 and 3–way crosses were developed by crossing with both susceptible and resistant genotypes. Two dominant genes, one for resistance and one for moderate resistance were present in ILL 5588, one dominant gene was identified in ILL 5684, one recessive gene for resistance was present in cultivar Laird, and two recessive genes with additive effects were responsible for the resistant reaction in Indianhead.

These results were confirmed by Ye et al. (2003) using the additional susceptible parent W6 3192 (*L. ervoides*) and crosses were generated in all possible combinations. F_2–derived plants from a cross between two susceptible parents showed that there was one resistance gene in W6 3192 (*L. ervoides*) and one resistance gene in Titore in complementary gene interaction for Ascochyta blight resistance. Gene ral_2 was confirmed by Chowdhury et al. (2001) in Indianhead, who identified the two flanking RAPD markers $UBC227_{1290}$ and $OPD–10_{870}$ linked in repulsion phase with the gene. By using the two F_2 populations of ILL 5588 x ILL 7537 and of ILL 7537 x ILL 6002, Rubeena et al. (2006) mapped five and three QTLs, respectively, for Ascochyta blight resistance. One QTL on linkage group LG6 was mapped by Tullu et al. (2006), in close proximity to anthracnose resistance genes which explained 41% of the phenotypic variation for Ascochyta blight resistance. In another study, Gupta et al. (2012) mapped three QTLs for seedling resistance to Ascochyta blight, two on LG1 and one on LG9, and another three were detected for resistance at the pod or maturity stage, one each on LG1, LG4 and LG5.

Recently, Sari (2014) studied recombinant inbred lines (RILs) from all possible crosses among Ascochyta blight resistant *L. culinaris* genotypes CDC Robin, 964a–46, ILL 7537 and ILL 1704 for allelism. QTL analysis of RILs indicated that CDC Robin and 964a–46 had two different QTLs mapped for resistance. In addition, wild *L. ervoides* accession L01–827A had two complementary recessive resistance genes for Ascochyta blight resistance (Sari 2014). While studying RIL populations for Ascochyta blight resistance derived from Indianhead x Northfield, Indianhead x Digger and Northfield x Digger, Sudheesh et al. (2017) developed three genetic linkage maps and a consensus map. The authors were able to map three disease resistance QTLs in Indianhead x Northfield RIL populations on LG 2, 3 and 6, explaining 47%, 15% and 7% phenotypic variation, respectively while two QTLs were mapped on LG 2 and 3 in the RIL population derived from Indianhead x Digger, explaining 30% and 22% variation. No disease resistance QTLs were mapped for the RIL population derived from Northfield X Digger.

2.3.2 Stemphylium Blight

Stemphylium blight of lentil is caused by ascomycete *Stemphylium* spp. Recently, it was discovered that *Stemphylium botryosum* and another *Stemphylium* sp. infects lentil crops in Saskatchewan, Canada (Caudillo et al. 2017). In lentil the disease was first reported by Bakr and Zahid in 1987 in Bangladesh. Under epidemic conditions it can cause yield losses up to 90% (Sinha and Singh, 1993). Stemphylium blight has emerged as a new threat to lentil production in Canada and the northern USA after Ascochyta blight and anthracnose (Hashemi et al. 2005a). *Stemphylium botryosum* has a broad host range (Booth and Pirozynski 1967) and infects other plant species such as alfalfa (*M sativa*) (Rokaibah 1996) and spinach (*Spinacia oleracea* L.) (Koike et al. 2001).

2.3.2.1 Infection process of *Stemphylium botryosum*

Mwakutuya and Banniza (2010) studied the infection process of *Stemphylium botryosum* on lentil leaflets. Conidial germination started within the first two hours of inoculation. As conidia were polyspermic in nature, up to 6 germ tubes emerged from a single conidium growing in random directions on the leaf surface. The majority of germ tubes penetrated through stomata within 12 h. The authors did not observe appressoria during the study. At 48 h after inoculation stemphylium blight severity reached more than 80%.

2.3.2.2 Genetics and mapping of Stemphylium Blight resistance in lentil

Work on Stemphylium blight in lentil was initiated two decades ago. It was reported that variation for resistance in lentil for Stemphylium blight depends upon the morphological structures such as epidermal hairs, thickness of the epidermis and cortical layers (Chowdhury et al. 1997). While studying Stemphylium blight resistance in a lentil RIL population derived from a cross between Barimasur–4 x CDC Milestone, resistance was found to be quantitative in nature (Kumar 2007). Based on phenotypic data from two years, Saha et al. (2010) mapped four QTLs related to Stemphylium blight resistance using 206 RILs derived from ILL 5888 x ILL 6002. QTL QLG480–81 was common for phenotypic data from both years, explaining 25.2 and 46.0% of the variation in disease scores of those two years. Sequence-related amplified polymorphism (SRAP) marker ME4XR16c was most tightly linked to QTL QLG480–81 on LG4.

Podder (2012) screened intra (*L. culinaris* subsp. *culinaris* LR-36) and interspecific (*L. culinaris* subsp. *culinaris* x *L. ervoides* LR-26) RIL populations of lentil for Stemphylium blight resistance and found quantitative resistance in both populations. Recently Bhadauria et al. (2017) mapped two QTLs for Stemphylium blight resistance in the intraspecific *L. ervoides* population LR-66, one on LG2 explaining 28% of the phenotypic variation and one on LG3 explaining 12% of the variation. In order to find resistance in *L. culinaris* for Stemphylium blight, Kant et al. (2017) screened 300 accessions and found 6 (ILL 6408, ILL 0133, ILL 0379, ILL 0426, ILL 0427, ILL 0215) of them to be resistant. In another study 185 genotypes of *L. culinaris* were screened for Fusarium wilts and Stemphylium blight out of which 16 were resistant to Fusarium wilt and 87 genotypes to Stemphylium blight. Moderate resistance to Fusarium wilts was identified in another 23 and to Stemphylium blight in 36 genotypes, while nine genotypes (Maheswarbharti, RL 13, ILL 6468, Arun, ILL 9996, ILL 6024, ILL 6811, ILL 7164 and RL 21) were found to be resistant to both Fusarium wilts and Stemphylium blight (Yadav et al. 2017).

2.3.3 Anthracnose

Anthracnose was first reported in Manitoba in 1987 and in Saskatchewan in 1990 (Morrall 1988, Morrall and Pedersen 1991). Anthracnose can cause yield losses of up to 70% (Gibson et al. 1991). *Colletotrichum lentis*, the causal organism of lentil anthracnose belongs to the class Sordariomycetes in the phylum Ascomycota. No sexual stage of *C. lentis* has been reported under natural conditions but could be induced *in vitro* (Armstrong–Cho and Banniza 2006). Buchwald et

al. (2004) reported two pathogenic races of *C. lentis* namely Ct1 and Ct0 based on disease severity on lentil genotypes. Ct0 is more virulent than Ct1 as it causes severe symptoms on all differential genotypes. Armstrong–Cho et al. (2012) renamed both races as race 0 and race 1 to avoid confusion with gene designations.

2.3.3.1 Infection process of *Colletotrichum lentis*

The infection process of *C. lentis* on resistant and susceptible lentil cultivar leaflets at 1, 3, 6, 12, 24, 48, 72, 96, 120, and 144 hpi was described by Chongo et al. (2002). Conidia germinated from 3 to 6 hpi, appressoria were formed within 6 to 12 hpi and infection pegs were observed beneath appressoria penetrating the epidermal cells at 24 hpi. For the first 72 hpi, there were no visible symptoms, which developed at 96 to 144 hpi. In another study, primary and secondary hyphae were clearly observed at 48 hpi in lentil leaflets (Armstrong–Cho et al. 2012). The authors found significant differences between the two races in conidial germination, appressorium formation and penetration on the surface of detached lentil leaflets. There was no significant difference between the two races in the size of primary hyphae assessed as the area of epidermal cells occupied by these structures on detached lentil leaflets, but primary hypha size was significantly different on attached lentil leaflets. However, when comparing 3 isolates of each race, it was shown that not all race 1 isolates were clearly distinguishable from race 0 isolates indicating that there is overlap between both races for certain characters assessed (Armstrong–Cho et al. 2012).

2.3.3.2 Genetics and mapping of Anthracnose resistance in lentil

Extensive screening for resistance to anthracnose was carried out in cultivated lentil germplasm, but only a few lentil lines were identified with high levels of partial resistance, such as PI 320937, PI 345629, breeding line 458–57 and cultivar Indianhead (Chongo and Bernier 1999). No cultivated lentil is immune to anthracnose (Bernier et al. 1992). Buchwaldt et al. (2001) developed three populations by crossing resistant Indianhead, PI 320937 and PI 345629 with susceptible Eston and reported one recessive and two dominant genes for resistance against anthracnose based on F_3 family segregation in the populations. A major gene for resistance against anthracnose, *LCt–2*, whose effect was influenced by minor genes was reported by Tullu et al. (2003) based on a RIL population derived from lentil cv Eston (susceptible) and PI 320937 (resistant). Locus *LCt–2* was flanked by the two RAPD markers $OPEO6_{1250}$ and $UBC–704_{700}$ that were linked to the *LCt–2* locus in repulsion (6.4 cM) and in coupling (10.5 cM), respectively. In addition to RAPD markers, AFLP

markers EMCTTACA$_{350}$ and EMCTTAGG$_{375}$ were linked in coupling, whereas EMCTAAAG$_{175}$ was linked in repulsion phase to the *LCt–2* locus.

Tar'an et al. (2003) confirmed marker-assisted selection for anthracnose resistance in a RIL population derived from CDC Robin and breeding line 964a–46 using RAPD marker OPEO6$_{1250}$. Linkage between resistance genes for Ascochyta blight and anthracnose was reported based on the observation that a QTL on LG 6 which explained 41 % of the variation for Ascochyta blight resistance was localized between an AFLP marker (ctcaccB) and the anthracnose resistance gene *LCt2* (Tullu et al. 2006). Fiala et al. (2009) studied the genetics of anthracnose resistance for race 1 and race 0 in an interspecific RIL population derived from the cross of Eston (*L. culinaris*) x L01–827A (*L. ervoides*). In this study it was predicted that two recessive genes are involved in resistance to both the races, however results were skewed due to segregation distortion because of loss of some RILs during population advancement.

Plant age-dependent resistance against anthracnose was studied in lentil cultivars CDC Redberry, CDC Robin, VIR421, Eston (*L. culinaris*) and an interspecific *L. culinaris* x *L. ervoides* hybrid LR-59-81 using race 0 and race 1 isolates of *C. lentis*. VIR421 had partial resistance to race 0, CDC Robin and CDC Redberry were susceptible to race 0 but resistant to race 1, whereas Eston was susceptible to both races. It was reported that the podding stage was best to study disease reaction in CDC Redberry and CDC Robin but not for LR-59-81 and VIR421 (Vail and Vandenberg 2012). In another report, three populations were generated from Eston as the common susceptible, and Indianhead, PI 320937 and PI 345629 as the resistant parents (Buchwaldt et al. 2013). In order to determine the allelic variation of resistance genes three more populations derived from Indianhead × PI 320937, Indianhead × PI 345629 and PI 320937 × PI 345629 were also generated. Studying these populations at the F_1, F_2, BC_1R, BC_1S and F_3 by screening them against the two *C. lentis* race 1 isolates 91IH and 95B36, two recessive genes, *ctr1* and *ctr2*, and three dominant genes, *CtR3*, *CtR4* and *CtR5*, were identified. Resistance genes *ctr2* and *CtR5* were present in PI 345629, *CtR4* was present in PI 320937 for both isolates, while *ctr1* and *CtR3* present in Indianhead were detected by isolate 91IH and 95B36, respectively (Buchwaldt et al. 2013).

Vail et al. (2012) identified specific RILs derived from a cross of Eston x L01–827A (*L. ervoides*), which were resistant to race 0 under field conditions. Two genes were reported for anthracnose resistance in the interspecific population Eston x *L. ervoides* accession IG 72815 (Tullu et al. 2013).

Shaikh et al. (2012) reported resistance in landraces (*L. culinaris*) for both races of anthracnose. On the pathogen side, a total of 2,857 ESTs from *C. lentis* infected leaf tissues were assembled into 1,682 unigenes of which 101 encoded membrane and transport associated proteins, 159 coded for proteins in signal transduction and 387 for stress and defence–related proteins (Bhadauria et al. 2013). In a recent study, using the intraspecific *L. ervoides* (L01–827A x IG 72815) RIL population LR-66, quantitative trait loci (QTLs) were mapped for *C. lentis* with 5 for race 0 and 6 QTLs for race 1 resistance. Of the 5 QTLs mapped for race 0, one was mapped on LG3 explaining 14% of the variation, two on LG5 explaining 33%, one on LG2 explaining 11%, and another one on LG7 explaining 9% of the phenotypic variation. For race 1 resistance, two QTLs were mapped on LG3 explaining 39%, two on LG5 explaining 31%, and 2 on LG2 explaining 19% of the phenotypic variation (Bhadauria et al. 2017).

Chapter 3

Characterization of Ascochyta blight resistance among accessions of *Lens ervoides*

3.1 Introduction

In the past four decades, pulse crops have been recognized as a rich source of protein, especially in developing countries where meat as a source of protein is either culturally not popular (e.g. India) or human populations cannot afford it (e.g. African countries). Due to the potential role of pulse crops in global food and nutritional security, there has been a resurrection of cultivation of many pulse crops in their native areas of cultivation, such as the Middle East and the Indian sub-continent, and they were introduced to new geographic regions such as Australia, Canada and the USA. Lentil is one such pulse crop which currently plays an important role as a nutritious food source in the developing world. Lentil is a self-pollinating diploid (2n = 14) annual legume crop with a genome size of 4 Gbp (Arumuganathan and Earle 1991) and is the fifth most important legume crop in terms of grain production with 4.8 million tons worldwide in 2014 and an average yield of 1.06 tonnes ha^{-1} (FAOSTAT, 2014).

Similar to other crops, lentil is subjected to different biotic constraints, which reduce the productivity of the crop. Since the inception of lentil cultivation in western Canada in the late 1970's, Ascochyta blight caused by the necrotrophic fungus *Ascochyta lentis* has been a major biotic stress for production in Saskatchewan. Apart from affecting seed quality, Ascochyta blight can cause yield losses of up to 70% (Gossen and Morrall, 1983). In the past three decades, breeding efforts have contributed significantly to increasing lentil production all over the world, especially in North America. The process of yield improvement through plant breeding, however, also resulted in the narrowing of the genetic base of lentil germplasm through continuous selection for desired agronomic traits. This narrowing of the genetic base of lentil germplasm was due to a reduction in the number of diverse alleles in cultivated lentil germplasm, which resulted in crops being more prone to different biotic stresses and possible break-down of existing resistance to known pathogens when confronted with new pathotypes or races.

In order to counter this problem, scientists started to focus on wild relatives of lentil as a potential source for novel resistance genes against different diseases. These novel resistance genes can play an important role in reducing the number and cost of fungicide applications for farmers, especially in case of necrotrophic fungi, for which complete resistance is usually not present. According to a recent classification based on genotyping by sequencing (GBS) by Wong et al. (2015), the genus *Lens* consists of seven species with *Lens culinaris* (cultivated species), *Lens orientalis* and *Lens tomentosus* in the primary, *Lens lamottei* and *Lens odemensis* in the secondary, *Lens ervoides* in the tertiary and *Lens nigricans* in the quaternary gene pool. In contrast, *Lens* germplasm was divided into three genepools based on their ability to generate hybrids, which may be completely or partially sterile (Ladizinsky and Abbo 2015). In past studies, it was shown that five wild relatives of lentil (*L. orientalis, L. lamottei, L. odemensis, L. ervoides* and *L. nigricans*) are a depository for disease resistance genes for Ascochyta blight (Bayaa et al. 1994, Ahmad et al. 1997, Tullu et al. 2010, Dadu et al. 2017). In addition to Ascochyta blight, disease resistance in the wild lentil species has also been reported for Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lentis*, anthracnose caused by *Colletotrichum lentis*, rust caused by *Uromyces fabae* (Grev.) Fuckel., Stemphylium blight caused by *Stemphylium botryosum* and powdery mildew caused by *Erysiphe polygoni* DC. (Bayaa and Erskine 1991, Bayaa et al. 1995, Nasir 1998, Tullu et al. 2006, Podder et al. 2013, Singh et al. 2014).

While identifying disease resistance in wild lentil relatives is relatively easy, transferring these traits into cultivars poses difficulties. Limited success has been made with interspecific hybridization primarily among the species of the primary genepool, i.e. *L. culinaris* and *L. orientalis* (Ladizinsky et al. 1984, Hamdi and Erskine 1994, Ladizinsky 1999; Gupta 2003, Fratini et al. 2004; Gupta and Sharma 2007). Embryo rescue technique made it possible to transfer genes successfully from other genepools of *Lens* to cultivated lentil (Cohen et al. 1984, Ahmad et al. 1995, Fratini and Ruiz 2006, Fiala et al. 2009, Tullu et al. 2013, Saha et al. 2015). Although embryo rescue made it possible to recover interspecific hybrids, individual recombinant inbred lines were often lost in subsequent generations, which resulted in genetic distortion and prevented studies of

the genetic control of traits of interest (Fiala et al. 2009, Tullu et al. 2013). Only one study on an interspecific population developed from *L. culinaris* and *L. ervoides* did not report any genetic distortions (Singh et al. 2017).

In order to avoid this problem, the intraspecific *L. ervoides* RIL population LR-66 was developed from a cross of accessions IG 72815 and L01-827A. *Lens ervoides* population LR-66 is of interest because parents have high levels of resistance against race 0 of *C. lentis*, Ascochyta blight and Stemphylium blight. Parental accession IG 72815 is highly resistant and L01-827A is moderately susceptible to anthracnose and Stemphylium blight, so Bhadauria et al. (2017) was able to map disease resistance QTLs for both, anthracnose (race 0 and 1 of *C. lentis*) and Stemphylium blight in LR-66. Both accessions are resistant to Ascochyta blight, and indeed, Sari (2014) hypothesized that L01-827A might have genes mediating non-host resistance against *A. lentis*. The objective of this project was to determine whether the nature of resistance to *A. lentis* in *L. ervoides* is based on non-host resistance by screening *L. ervoides* accessions for Ascochyta blight resistance under greenhouse and field conditions.

3.2 Material and methods

3.2.1 Seed multiplication

All 166 accessions classified as *L. ervoides* that are stored at the Crop Development Centre (CDC) were grown under controlled conditions for seed increases, some of which had been previously evaluated for resistance to various diseases (Appendix 1). As seed supplies were limited and accessions are known to have irregular and unreliable germination, two seeds were pre-germinated in a Light Emitting Diode (LED) chamber while a third was directly seeded in the greenhouse. Two scarified seeds of each *L. ervoides* accession were seeded into a single cup of a 50-cup-tray filled with Sunshine Mix #3 (Sun Gro® Horticulture) and were exposed to LED light. Growing conditions in the LED chamber were maintained at a constant temperature of 20 ^{o}C and a 16 / 8 h day/night cycle. To maintain sufficient moisture levels, trays were watered twice a day and covered with plastic domes in order to reduce evaporation. Similarly, one scarified seed was seeded in the Agriculture Greenhouse, University of Saskatchewan, in a 150 x 180 mm pot (Listo Products Ltd, Canada) containing a mix of Sunshine Mix #4 (Sun Gro® Horticulture) and perlite (Sun Gro® Horticulture) in a ratio of 3:1 with an 18 / 6 h day/night cycle and day/night temperatures of 25^{o}C / 22^{o}C. Once the *L. ervoides* seedlings in the LED chamber were two weeks old, they were

transplanted into the 150 x 180 mm plastic pots (Listo Products Ltd, Canada) already containing one seedling of the same accession. The timing of *L. ervoides* seed germination was highly variable among individual seeds and among accessions under LED lights. Out of 166 accessions seeded, three *L. ervoides* accessions (IG 72569, IG 72580 and IG 72807) did not germinate under LED or greenhouse conditions. Two attempts of germinating IG 72569, IG 72580 and IG 72807 failed. The remaining 163 germinated accessions were morphologically compared to accessions IG 72815 and L01-827A, previously confirmed as *L. ervoides*. Of those, 157 accessions were identified as *L. ervoides* based on the narrow leaflet character whereas the remaining six accessions were not. Among these 157 *L. ervoides* accessions, nine (IG 72568, IG 72783, IG 72784, IG 72797, IG 72861, IG 116022, IG 140884, IG 140910 and IG 140927) have leaves similar to IG 72815 and L01-827A but different plant structures.

Each plant was individually bagged with mesh net bags in order to avoid loss of seeds due to shattering of pods. Once the majority of seeds were released from the pods, seeds of each individual plant were collected in a separate envelope. From each *L. ervoides* accessions, one of the three plants with the maximum number of seeds was selected for further seed multiplication and Ascochyta blight greenhouse screening.

A second round of seed multiplication was initiated to generate sufficient seeds for field testing. Four scarified seeds per accession were grown under LED lights in 144-cup-trays (one seed per cup), and two scarified seeds of each *L. ervoides* accession were grown under greenhouse conditions in 210 mm x 210 mm plastic pots (Listo Products Ltd, Canada) as described before. Once the seedlings under LED light were two weeks old, they were transplanted into the 210 x 210 mm plastic pots in the greenhouse already seeded with two seeds of the same accession for a total of 6 plants. At podding, plants were individually bagged with the mesh net bags to avoid seed loss due to shattering of pods. Upon reaching maturity, seeds from plants of each accession in each pot were bulk harvested and collected in the single envelope.

3.2.2 Refreshing of *Ascochyta lentis* isolate AL-61 and inoculum preparation

Ascochyta lentis isolate AL-61 is the standard isolate used for screening lentil germplasm at the CDC. To ensure virulence of AL-61, it was decided to refresh the isolate from the cryogenic vial (Nalgene^{TM}) containing cryopreservation fluid (a combination of skim milk powder, warm deionized water, glycerol and deionized water), stored at -80^{o}C. The standard susceptible check

Eston used in all Ascochyta blight germplasm screening experiments at the CDC was used for this purpose. Five 98.4 x 98.4 x 88. 9 mm plastic pots (4" Kord Traditional Square Pot) were seeded with six Eston seeds per pot in the greenhouse with a day/night cycle of 18 / 6 h and 18 / 22 ºC. Two weeks after seeding, plants were thinned to four per pot.

Petri dishes (Fisherbrand) with 50% oatmeal agar (OMA) (BD Difco) were prepared and inoculated with a conidial suspension of *A. lentis* isolate AL-61 from the cryogenic vial and incubated for ten days at room temperature under incandescent light. After ten days, Petri dishes were flooded with sterile water and conidia were scraped with sterile glass slides. The conidial suspension was collected and filtered through Mira cloth into an Erlenmeyer flask. The concentration of the conidial suspension was adjusted to 5×10^5 conidia mL^{-1} using a hemocytometer for inoculation of Eston plants at a rate of 3 mL $plant^{-1}$. Inoculated plants were kept in a humidity chamber for two days post-inoculation (dpi) with 17 / 7 h day/night at 21ºC. Plants were then transferred to the misting bench with misting for 40 sec every 40 min to promote Ascochyta blight development. After two weeks, plant leaves with Ascochyta blight lesions were selected arbitrarily from each pot and plated on water agar. After three days, Petri dishes were assessed under a dissecting microscope, and an agar plug containing germinated conidia was placed on fresh agar medium. After two days, a single germinated spore was selected and a plug containing this germinated spore was placed on 50% oatmeal agar (OMA) in a Petri dish to obtain a monoconidial culture. This OMA plate was incubated under incandescent light for ten days before several small pieces (approx. 0.5 x 0.5 cm) of OMA plugs with mycelium were stored in 30 cryovials in cryopreservation fluid stored at -80ºC as stock cultures for inoculum production for this experiment.

3.2.3 Faba bean (*Vicia faba* var. *minor*) grain inoculum preparation

Faba bean (*Vicia faba* var. *minor*) grain inoculum was used as inoculum under field conditions with slow sporulation and spore release for an extended period of time when conditions (moisture) are favourable. For the preparation of faba bean grain inoculum, a plastic tub (55 L) was filled with approximately 27 L of faba bean seeds and distilled water to reach a volume of approximately 40 L. Seeds were soaked for 16 h and then transferred into one litre plastic bottles that were closed with lids with a foam-plugged hole and autoclaved twice before being inoculated with a conidial suspension of AL-61 at 5×10^5 conidia mL^{-1}. Containers were incubated for three weeks under

incandescent light and were manually shaken thrice a week to get a uniform fungal infection on faba beans. Once infection on the seeds became visible, seeds were transferred onto plastic trays for drying in a biosafety cabinet for two weeks. Seeds were broken into smaller pieces using a mechanical roller to prevent birds eating the grain in the field, and to obtain sufficient inoculum. The inoculum was packed in paper bags, which were sealed in polyethene garbage bags for storage in the fridge at 5^{o}C until use.

3.2.4 Greenhouse Screening of *L. ervoides* accessions against *Ascochyta lentis*

A total of 157 accessions identified as *L. ervoides* along with *Lens culinaris* checks Eston (susceptible) and CDC Robin (resistant) were screened with *A. lentis* isolate AL-61 in a randomized complete block design (RCBD) with five replications blocked over time due to space constraints.

Seeds were planted into 144-cup-trays with five cups per accession per replication in an LED growth chamber as described before. Cup trays were watered twice a day and covered with plastic domes to maintain humidity. As seedling emergence varied widely among accessions, accessions were subdivided into four groups of unequal numbers, solely based on the time of emergence to synchronize growth stage and were inoculated independently.

Seedlings were transplanted at two to four plants per pot into 98.4 x 98.4 x 88. 9 mm plastic pots containing a mix of Sunshine #4 (Sun Gro® Horticulture) and perlite (Sun Gro® Horticulture) in a ratio of 3:1 as they reached the 4-leaf stage and were transferred to the greenhouse with 22 / 18 ^{o}C and 18 / 6 h day /night. Four sets of Eston and CDC Robin were included in each replication and for each group. When plants reached the 14 to 16-leaf stage, they were inoculated with conidial suspensions of 5×10^{5} conidia mL^{-1} of AL-61. For the checks CDC Robin and Eston, 3 mL of conidial suspension was used per plant, but this amount was adjusted for *L. ervoides* accessions to ensure coverage until run-off. After inoculation, plants were placed in a humidity chamber containing three humidifiers for two days before being transferred to a misting bench with misting for 40 sec every 40 min for 16 days.

3.2.5 Field Screening of *L. ervoides* accessions against *Ascochyta lentis*

In order to assess ascochyta blight resistance of the 157 *L. ervoides* accessions under field conditions, an experiment was conducted at two locations in Saskatoon, an outdoor pot experiment at the Seed Farm (N 52.135402, E 106.620667) and a field experiment at Preston Avenue site (N

52.127447, E 106.615434) of the University of Saskatchewan. The experiment was conducted in a randomised complete block design (RCBD) with five replications at each location.

3.2.5.1 Pot experiment under field conditions at the Seed Farm

For the pot experiment at the Seed Farm, 157 *L. ervoides* accessions were pre-germinated under the LED lights in a growth chamber as described before. All five replications were seeded over time due to space constraints in the LED growth chamber. Once the seedlings reached the 4-leaf stage, they were transplanted into 98.4 x 98.4 x 88. 9 mm pots containing Sunshine Mix #4 (Sun Gro® Horticulture) and perlite (Sun Gro® Horticulture) at a 3:1 ratio (one pot per accessions per replication) at 2 to 4 seedlings per pot and maintained in the greenhouse for two weeks. They were then transplanted into 150 x 180 mm plastic pots filled with the same mix at the Seed Farm. Plastic pots were placed on polypropylene landscaping cover following an RCBD and anchored to the ground with U-shaped anchor pins. The experiment was divided into two strips of pots, each with six pots per row and 70 rows. Misters were placed approximately every 9 feet between the two strips of pots and were programmed for 1 min of misting every 15 min. Moisture sensing and irrigation probes were placed in each pot, but the irrigation system was used only twice for fertilizing plants during the whole experiment as misting kept pots moist.

Attempts to construct a temporary tunnel over the pots using a plastic pipe frame covered with perforated plastic sheet failed and high winds destroyed this tunnel, despite repeated reconstruction, so these efforts were abandoned after one week.

Three days after transplanting the plants into 150 x 180 mm plastic pots, *A. lentis* infested faba bean grain inoculum was spread at 10 g per pot at the base of plants. Pots were covered with a perforated plastic sheet for three days, and water was splashed on top of the perforated plastic sheet, five times a day to increase humidity and promote sporulation. A second faba bean grain inoculation occurred 3 weeks later as no disease symptoms were observed after the first inoculation. Plants were inoculated a third time with 40 L of conidial suspension (5×10^5 conidia mL^{-1}) of AL-61 for 830 pots. Disease symptoms occurred sporadically and at low levels, so absence or presence of symptoms were recorded for each pot 16 dpi with the conidial suspension, at which point the majority of the plants were at the podding stage.

3.2.5.2 Field experiment (Preston Avenue)

At Preston Avenue, scarified seeds (75 seeds per accession) of the 157 *L. ervoides* accessions and *L. culinaris* checks Eston (susceptible) and CDC Robin (resistant) were directly seeded in an RCBD design with five replications in land treated with pre-emergence herbicide (Edge™ and Pursuit®) using an SRES Step 4 planter. In this experiment, misters were placed approximately every 9 feet along the pathways and were programmed to run at 6 am and 9 pm daily for 15 min. *L. ervoides* accessions germinated and developed slowly, and many had not germinated one month after seeding. Two months after seeding, plants were inoculated with faba bean grain inoculum at 20 g m^{-2}. A substantial infestation of aphids was noticed, probably because this experiment was seeded late, hence had the greenest plants in the field at this point, which probably attracted the aphids, and an insecticide was sprayed twice. Two additional inoculations followed with conidial suspension (5×10^5 conidia mL^{-1}) after each insecticide spray, and 10 and 18 days following previous inoculations. The field was covered with a crop cover (American Nettings and fabric Inc., USA) to increase humidity for enhancing disease development after each inoculation. Unfortunately, aphid damage was so severe that the majority of the plants were beyond recovery and no disease symptoms were observed on the plants which had few green leaves left.

3.3 Disease assessment

In the greenhouse, plants were assessed individually for disease severity 18 dpi using a 0-10 scale based on 10% incremental increases in the percentage of disease severity. Individual plant scores were converted to percentage of infected plant tissue using the class midpoint values. The average disease scores of plants per replicate pot were used for data analysis. Based on reactions compared to the checks, *L. ervoides* accessions were grouped into five groups: highly susceptible (HS: significantly more susceptible than Eston), moderately susceptible (MS: similar to Eston), intermediate resistance (IM: resistance level between Eston and CDC Robin), moderately resistant (MR: similar to CDC Robin) and highly resistant (HR: significantly more resistant than CDC Robin).

For the pot experiment at the Seed Farm, disease absence and presence per pot were recorded. No disease could be assessed on plants at Preston Avenue.

3.4 Statistical analysis

Data collected in the greenhouse were tested for homogeneity of variance. Analysis of variance was conducted with the mixed model procedure of SAS (Version 9.4, SAS Institute, Cary, NC, USA) where *L. ervoides* accessions were considered a fixed effect factor and replications, blocks and interactions were regarded random factors. Heterogeneous variances were modelled with the repeated statements as required. Genotypes were compared by multiple comparisons of means using Fisher's least significant difference. Genotypes that were equally or more susceptible compared to the susceptible check Eston were considered susceptible *L. ervoides* accessions.

3.5 Results

Disease severity among *L. ervoides* accessions varied from 2.1% (IG 72918) to 55.2 % (IG 136611) (Table 3.1). Mean Disease severity in Eston ranged from 33.1 to 36.4%, and that of CDC Robin from 14.6 to 19.1%. Several *L. ervoides* accessions had higher or similar disease severity compared to the susceptible check Eston, and similarly, some accessions had disease severity equal to or less than the resistant check CDC Robin, so six *L. ervoides* accessions were grouped as HS, 34 as MS, 38 as IM, 67 as MR and 12 as HR (Fig 3.1, Table 3.1).

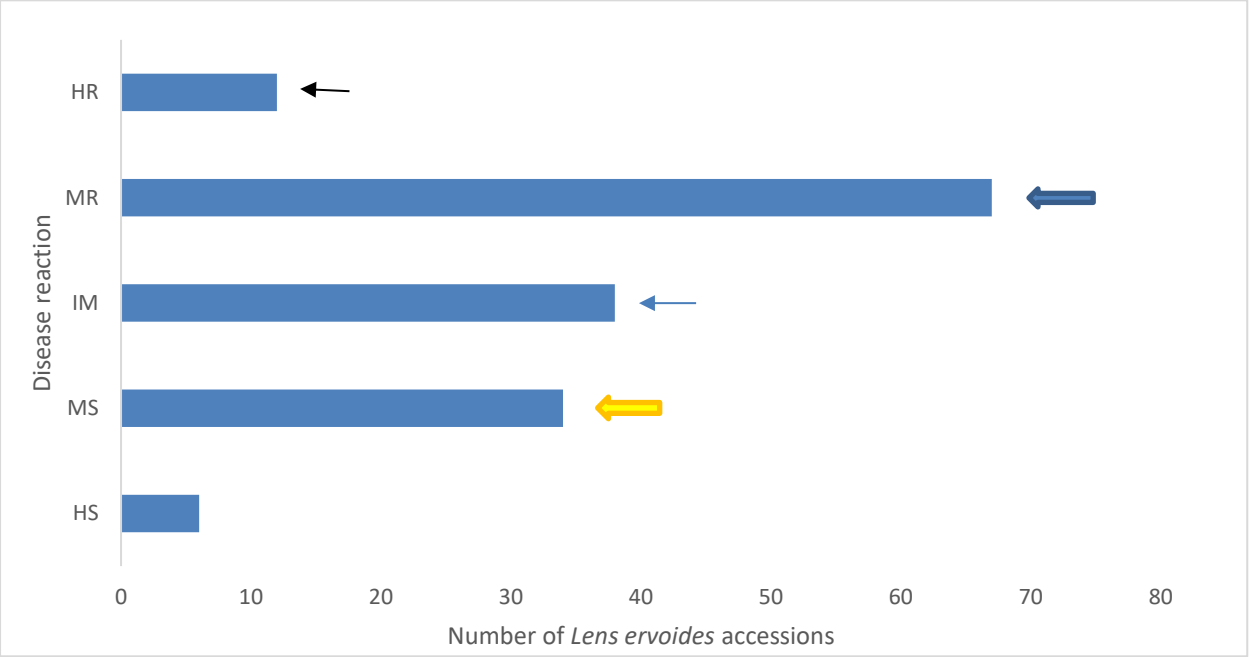


Figure 3.1. Number of *Lens ervoides* accessions classified into highly resistant (HS), moderately resistant (MR), intermediate resistance (IM), moderately susceptible (MS) and highly resistant

(HR) to Ascochyta blight based on greenhouse data. ← indicates L01-827A, ← indicates IG 72815 (LR-66 parents), ⟸ indicates CDC Robin, ⟸ indicates Eston.

In the pot experiment at the Seed Farm, all accessions showed disease symptoms in at least one of the five replicate pots (Table 3.1) indicating that they can all be infected by *A. lentis*. The number of replicated pots with symptoms of each accession did not correlate with their disease severity in the greenhouse experiment, so rather than being an indication of the level of resistance of the accessions, this is more likely a reflection of the location of individual pots relative to misting irrigation in the pot experiment considering the hot and very windy growing conditions.

Table 3.1. Ascochyta blight severity (mean and standard error of the mean [STDERR]), rated on a scale from 0 to 100 % with 10% increments, on *Lens ervoides* accessions in the greenhouse, and presence or absence of Ascochyta blight in outdoor pot experiments at the Seed Farm (Numbers in brackets indicate number of replicate pots [r = 5] with infected plants)

Accessions	Greenhouse			Seed Farm	Accessions	Greenhouse			Seed Farm
	Mean	STDERR	Group	Presence		Mean	STDERR	Group	Presence
IG 136611	55.274	1.9015	HS	Yes (5)	IG 72731	32.472	1.4255	MS	Yes (3)
IG 72863	48.007	1.6401	HS	Yes (1)	IG 72570	32.133	0.6385	MS	Yes (3)
IG 136613	44.022	2.7868	HS	Yes (4)	IG 72652	32.091	1.5343	MS	Yes (3)
IG 72583	42.79	1.4094	HS	Yes (4)	IG 107435	31.77	1.157	MS	Yes (3)
IG 136612	40.615	1.5777	HS	Yes (4)	IG 136614	31.67	3.3451	MS	Yes (3)
IG 136615	40.117	1.6256	HS	Yes (3)	IG 136619	31.111	1.693	MS	Yes (5)
IG 72589	38.366	1.5846	MS	Yes (5)	IG 72788	30.885	1.6994	MS	Yes (3)
IG 136609	38.138	1.6342	MS	Yes (4)	IG 72579	30.754	2.0634	MS	Yes (5)
IG 72587	37.82	1.5214	MS	Yes (4)	IG 136621	30.591	1.624	MS	Yes (4)
IG 72563	37.756	2.5018	MS	Yes (3)	IG 136633	30.548	1.8775	MS	Yes (4)
IG 72781	37.002	1.9685	MS	Yes (4)	IG 72710	30.133	1.6391	MS	Yes (5)
Eston1	36.493	1.7032	MS	Yes (5)	IG 72578	29.949	1.3546	MS	Yes (3)
Eston3	36.477	0.9882	MS	Yes (3)	IG 72922	29.735	1.6957	MS	Yes (5)
IG 136616	35.804	2.4677	MS	Yes (2)	IG 72564	29.59	1.3379	MS	Yes (4)
IG 116013	35.144	1.7066	MS	Yes (3)	IG 136618	29.516	1.4496	MS	Yes (3)
Eston4	35.126	0.8282	MS	Yes (4)	IG 72808	29.504	1.4217	MS	Yes (4)
IG 72565	35.122	1.4602	MS	Yes (4)	IG 72803	29.276	1.4629	MS	Yes (3)
IG 107442	34.61	2.2736	MS	Yes (5)	IG 72794	28.948	2.5992	MS	Yes (4)
IG 72755	34.342	3.3964	MS	Yes (5)	IG 72814	28.913	1.5457	IM	Yes (3)
IG 72576	34.323	1.6138	MS	Yes (4)	IG 72709	28.238	1.622	IM	Yes (4)

Accessions	Greenhouse			Seed Farm	Accessions	Greenhouse			Seed Farm
	Mean	STDERR	Group	Presence		Mean	STDERR	Group	Presence
IG 72784	34.04	1.0435	MS	Yes (4)	IG 72917	28.208	1.3139	IM	Yes (5)
IG 72727	33.362	1.1317	MS	Yes (4)	IG 136622	28.187	1.4441	IM	Yes (4)
IG 72566	33.274	2.1262	MS	Yes (4)	IG 72914	28.088	1.4735	IM	Yes (3)
Eston2	33.136	1.5461	MS	Yes (5)	IG 72797	27.947	1.5899	IM	Yes (4)
IG 136627	33.066	1.7978	MS	Yes (4)	IG 72756	27.871	1.7964	IM	Yes (3)
IG 72650	32.692	1.4536	MS	Yes (4)	IG 116012	27.409	1.6315	IM	Yes (4)
IG 136620	27.401	1.6341	IM	Yes (4)	IG 72729	24.278	2.2243	IM	Yes (4)
IG 107441	27.291	2.4638	IM	Yes (2)	IG 72665	24.274	0.8923	IM	Yes (2)
IG 72851	26.84	1.6052	IM	Yes (5)	IG 72822	24.018	0.9803	IM	Yes (5)
IG 72582	26.697	1.4503	IM	Yes (4)	IG 72923	23.875	0.8566	IM	Yes (5)
IG 72567	26.454	1.9949	IM	Yes (5)	IG 72862	22.839	2.1829	IM	Yes (4)
IG 136632	26.278	1.3781	IM	Yes (4)	IG 72920	22.664	2.0132	MR	Yes (4)
IG 72859	26.239	1.3918	IM	Yes (3)	IG 72661	22.528	1.2037	MR	Yes (4)
IG 72654	26.132	2.5151	IM	Yes (4)	IG 72679	22.228	2.2611	MR	Yes (5)
IG 72573	26.092	2.0167	IM	Yes (3)	IG 140935	22.106	2.0253	MR	Yes (3)
IG 72815	26.007	1.2904	IM	Yes (5)	IG 72656	22.073	1.7907	MR	Yes (5)
IG 72574	25.937	1.5908	IM	Yes (3)	IG 116023	22.043	1.522	MR	Yes (5)
IG 72658	25.906	1.8554	IM	Yes (5)	IG 136628	21.823	1.4222	MR	Yes (2)
IG 72590	25.797	1.9748	IM	Yes (5)	IG 72913	21.719	1.4085	MR	Yes (4)
IG 136630	25.769	0.8689	IM	Yes (3)	IG 72774	21.488	2.1253	MR	Yes (4)
IG 72857	25.748	0.6445	IM	Yes (2)	IG 72682	21.274	1.3871	MR	Yes (3)
IG 72841	25.678	0.9866	IM	Yes (5)	IG 72911	21.256	1.4238	MR	Yes (3)
IG 72783	25.661	2.2328	IM	Yes (2)	IG 72655	21.102	1.4222	MR	Yes (5)
IG 107437	25.519	0.9045	IM	Yes (3)	IG 136635	21.089	1.4317	MR	Yes (2)
IG 116022	25.468	1.5724	IM	Yes (3)	IG 72757	21.08	1.3551	MR	Yes (3)
IG 136625	25.345	0.9444	IM	Yes (4)	IG 72657	20.982	1.4059	MR	Yes (5)
IG 107443	25.324	1.8184	IM	Yes (3)	IG 72787	20.958	1.5022	MR	Yes (4)
IG 107446	25.316	0.7352	IM	Yes (4)	IG 72861	20.676	1.5242	MR	Yes (3)
IG 136624	25.219	1.7711	IM	Yes (5)	IG 136629	20.655	1.4608	MR	Yes (3)
IG 72571	24.867	1.4083	IM	Yes (1)	IG 72678	20.559	1.3014	MR	Yes (5)
IG 136608	24.737	1.8258	IM	Yes (4)	IG 72716	20.382	1.2759	MR	Yes (5)
IG 72585	24.328	1.8158	IM	Yes (4)	IG 72586	19.979	1.3097	MR	Yes (5)
IG 72525	19.873	1.5905	MR	Yes (3)	CDCRobin3	16.056	1.467	MR	Yes (5)
IG 141656	19.574	1.7616	MR	Yes (2)	IG 107445	15.86	1.627	MR	Yes (3)
IG 72664	19.411	1.4637	MR	Yes (4)	IG 72847	15.6	1.9771	MR	Yes (4)
IG 107440	19.185	1.5219	MR	Yes (3)	CDCRobin2	15.556	0.8471	MR	Yes (3)
CDCRobin 4	19.168	1.2393	MR	Yes (5)	IG 72575	15.494	1.0403	MR	Yes (3)
IG 140884	19.118	1.8194	MR	Yes (4)	IG 72681	15.461	0.9076	MR	Yes (2)

Accessions	Greenhouse			Seed Farm	Accessions	Greenhouse			Seed Farm
	Mean	STDERR	Group	Presence		Mean	STDERR	Group	Presence
IG 140927	19.109	1.5065	MR	Yes (3)	IG 72588	15.343	0.7769	MR	Yes (5)
IG 72730	19.059	1.4055	MR	Yes (2)	IG 72782	15.254	1.7562	MR	Yes (3)
IG 72786	18.926	1.7831	MR	Yes (3)	IG 72660	15.168	0.7931	MR	Yes (4)
IG 136626	18.683	1.4336	MR	Yes (4)	IG 140929	15.129	0.7535	MR	Yes (4)
IG 72821	18.615	1.7025	MR	Yes (3)	IG 136631	15.088	2.375	MR	Yes (4)
IG 72792	18.325	1.6147	MR	Yes (3)	IG 140910	14.951	2.0693	MR	Yes (5)
IG 72912	17.556	2.468	MR	Yes (4)	IG 72581	14.829	1.7066	MR	Yes (3)
IG 72577	17.504	1.314	MR	Yes (4)	IG 72844	14.744	0.6512	MR	Yes (3)
IG 72793	17.321	1.2043	MR	Yes (4)	CDCRobin1	14.602	1.7935	MR	Yes (3)
IG 72653	17.217	1.1477	MR	Yes (4)	IG 72860	14.551	1.663	MR	Yes (3)
IG 72659	17.206	1.1476	MR	Yes (4)	IG 72924	14.073	1.5822	MR	Yes (3)
IG 72916	17.02	1.6711	MR	Yes (3)	IG 72846	13.813	0.9746	MR	Yes (3)
IG 72915	16.925	1.2993	MR	Yes (4)	IG 107444	13.546	1.5759	MR	Yes (2)
IG 107438	16.632	1.1062	MR	Yes (5)	IG 72708	13.466	1.5372	MR	Yes (4)
IG 72919	16.513	1.5191	MR	Yes (4)	IG 72651	13.116	1.6856	MR	Yes (1)
IG 72799	16.349	1.7673	MR	Yes (5)	IG 136610	12.037	1.7813	MR	Yes (2)
IG 72842	16.338	1.7583	MR	Yes (3)	IG 72921	11.973	1.5481	MR	Yes (4)
IG 72817	16.214	1.6558	MR	Yes (4)	IG 72826	11.828	1.3439	MR	Yes (5)
IG 107436	16.113	0.9691	MR	Yes (3)	IG 72663	8.1496	1.2339	HR	Yes (4)
IG 72796	8.0298	1.3717	HR	Yes (3)	IG 72646	5	2.0111	HR	Yes (4)
IG 136617	7.5017	1.8706	HR	Yes (5)	IG 72568	4.6153	1.107	HR	Yes (3)
IG 72662	7.2204	1.1984	HR	Yes (5)	L-01-827	4.2194	0.9099	HR	Yes (1)
IG 72707	6.9889	1.5003	HR	Yes (3)	IG 72785	3.1888	0.8728	HR	Yes (1)
IG 107439	6.8312	1.1232	HR	Yes (2)	IG 72918	2.1854	1.1876	HR	Yes (2)
IG 72910	6.6027	1.1256	HR	Yes (4)					

3.6 Discussion

Among the 157 *L. ervoides* accessions of the current study, 95 were previously reported in different disease screening studies (Bayaa et al. 1994, 1995; Ahmad et al. 1997, Tullu et al. 2006, 2010; Podder et al. 2013; Singh et al. 2014; Dadu et al. 2017), whereas 62 accessions were screened in the current study for Ascochyta blight for the first time (Table 3.1, Appendix 1). In a recent study, no macroscopic symptoms of Ascochyta blight infection on *L. ervoides* accession L01-827A were observed in spite of two consecutive inoculations of the same plants with *A. lentis* isolate AL-57 (Sari 2014). Based on these observations, Sari (2014) hypothesized that resistance genes in L01-827A were mediating non-host resistance. In the same study, the interspecific RIL population LR-

59 (Eston × L01-827A) was screened with *A. lentis* isolate AL-57 for studying genetic control of Ascochyta blight resistance, which revealed that immunity was transferred to LR-59 RILs, and that the segregation of susceptible and resistant RILs fitted a Mendelian model of two complementary genes.

Non-host resistance is a type of resistance in which an entire plant species is immune to a non-adapted pathogen species. Non-host resistance can be of two types: In Type I non-host resistance, no visible disease symptoms are observed whereas in Type II non-host resistance, a rapid hypersensitive response with cell death in the non-host is observed (Mysore and Ryu 2004). Non-host resistance is a durable and broad spectrum in nature and its potential applications in plant breeding for disease resistant varieties has become of interest to scientists recently (Lee et al. 2016).

Results from both greenhouse and field experiments showed that all 157 *L. ervoides* accessions could be infected by *A. lentis* isolate AL-61 indicating that *L. ervoides* does not have non-host resistance, hence the null hypothesis is rejected. Results from Ascochyta blight screening of accessions also showed a wider range in disease severity among *L. ervoides* accessions compared to the *L. culinaris* checks CDC Robin and Eston. One possible reason for this variation in disease severity could be higher genetic variability among the *L. ervoides* accessions compared to *L. culinaris* checks as Eston is in the pedigree of CDC Robin (Vandenberg et al. 2002). CDC Robin is a typical example for a variety developed from a narrow germplasm base in cultivated lentil due to past breeding efforts towards adaptation in a breeding program such as that at the CDC. Many of the current Saskatchewan lentil cultivars can be traced back to a few parental lines collected from a few geographic regions. However, whether and to what degree such a narrow genetic base affects a particular trait depends on whether it is present in the species at all. For example, no high levels of resistance have been identified in *L. culinaris* for resistance to *C. lentis* race 0, so a narrow or wide genetic base in *L. culinaris* will not affect race 0 resistance.

Unlike lentil cultivars developed in Saskatchewan, *L. ervoides* accessions in the current experiment originate from a wide geographic area spanning from a latitude of 30.76°N to 46.2667°N and a longitude of 15.01°E to 46.77°E with a variation in elevation from 7 to 1810 m above sea level covering 15 countries in total, with Syria and Turkey contributing 47 *L. ervoides* accessions each. However, whether The current study is one of the largest evaluations conducted for wild *L. ervoides* species for resistance to Ascochyta blight or any other prevalent lentil diseases. Challenging 157

L. ervoides accessions with *A. lentis* isolate AL-61 identified 4% of accessions as HS and 24% as MS, whereas the majority of accessions have lower Ascochyta blight severity than the susceptible check Eston. These results are in agreement with Bayaa et al. (1994) and Tullu et al. (2010) who reported that the majority *L. ervoides* have resistance to Ascochyta blight. One of the probable reasons for this could be the origin of *L. ervoides* species in damp and shady habitats conducive to disease development in the middle-east which could have resulted in the evolution of higher resistance in *L. ervoides* compared to other *Lens* species through an arms race between pathogen and host (Bayaa et al. 1994, Tullu et al. 2010). In Canada the first observation of Ascochyta blight of lentil was made in 1978, but was not reported in earlier conducted disease surveys, and the origin of inoculum is not known (Morrall and Sheppard, 1981). One possibility is that Ascochyta blight infected lentil germplasm was imported from the Middle East as was the case for lentil-growing regions in the USA (reviewed by Kaiser 1997).

Ascochyta blight symptoms were observed on all 157 *L. ervoides* accessions when rated at 18 dpi. On HS and MS genotypes, disease symptoms were visible on the shoot of the plants, but stem girdling was observed in HS accessions only. In contrast, on HR genotypes disease symptoms were observed on a few leaves only and no symptoms were observed on the stems. Unlike reported by Tullu et al. (2006) for anthracnose, disease reactions of individual plants of accessions were consistent because they were derived from a single plant selected during seed multiplication. This indicates the importance of this step as accessions are probably genetically heterogeneous.

In the current study, among the 26 *L. ervoides* accessions (corresponding IG numbers retrieved at https://www.genesys-pgr.org) reported resistant to Ascochyta blight by Bayaa et al. (1994), four were classified as MS here, five as IM, 13 as MR and four as HR (Table 3.1, Appendix 1). Similarly, IG 136613, IG 136616 which were identified as resistant to Ascochyta blight by Ahmad et al. (1997) were classified as HS in the current study, while IG 136632 identified as susceptible by Ahmad et al. (1997) was classified as IM in the current study. Comparing the current results with Tullu et al. (2010), where accessions with disease scores of less or equal to 4 were regarded resistant and those with scores higher than 5 susceptible on the Horsfall-Barratt scale of 0-11, 40 *L. ervoides* accessions were common between both studies. Out of these 40 *L. ervoides* accessions, three were classified as HR, 15 as MR, 12 as IM, 10 as MS and none as HS in the current study. Three *L. ervoides* accessions (IG 72646, IG 72910 and IG 72707) classified as HR in the current

study were also identified as resistant by Tullu et al. (2010). Out of 15 MR *L. ervoides* accessions in the current study, Tullu et al. (2010) reported two *L. ervoides* accessions (IG 72664 and IG 72659) as susceptible and 13 resistant under field conditions whereas five (IG 72708, IG 72799, IG 72730, IG 72657 and IG 72862) as susceptible and three resistant (IG 72846, IG 72659 and IG 107445) under greenhouse conditions. Similarly, 12 IM *L. ervoides* accessions in the current study were reported to be resistant under field conditions (Tullu et al. 2010) but under greenhouse conditions six (IG 72729, IG 72783, IG 72841, IG 72654, IG 72582 and IG 72914) were identified as susceptible and five (IG 72665, IG 72571, IG 72590, IG 72815 and IG 72859) as resistant. The ten MS *L. ervoides* accessions in the current study were all reported as resistant under field conditions by Tullu et al. (2010) whereas under greenhouse conditions two (IG 72579 and IG 72565) were classified as resistant and four (IG 72578, IG 72652, IG 72570 and IG 72576) as susceptible (Table 3.1, Appendix 1). In the current study, IG 72583 classified as HS and IG 72565, IG 136627, IG 136614, IG 72578 (corresponding IG numbers retrieved at https://www.genesys-pgr.org) as MS were reported to be susceptible to two *A. lentis* isolates (FT13037, FT13038) (Dadu et al. 2017). Dadu et al. (2017) reported IG 136630 to be moderately resistant to both *A. lentis* isolates, whereas this accession was classified as IM in the current study. IG 136631 (also listed as IG 72581 in Genesys, https://www.genesys-pgr.org) was found to be susceptible to *A. lentis* isolate FT13037 and moderately resistant to isolate FT13038, which was also the case in the current study. The differences among the results of the current and the previous studies are probably due to the differences in pathogenicity of *A. lentis* isolates used and conditions in which experiments were conducted. Some *L. ervoides* accessions were found to have consistent disease resistance reactions across the different studies over time; these accessions could be of interest for future lentil breeding and cultivar development programs.

Some *L. ervoides* accessions evaluated for resistance here and elsewhere for Ascochyta blight were also assessed for resistance to other diseases, such as anthracnose, stemphylium blight, rust and powdery mildew (Bayaa et al. 1995, Tullu et al. 2006, Podder et al. 2013, Singh et al. 2014). Tullu et al. (2006) had identified six *L. ervoides* accessions identified with a HR reaction to Ascochyta blight here to have resistance to anthracnose, four of which (IG 72646, IG 107439, IG 72707, IG 72662) were found to be resistant to both races (race 0 and 1) of *C. lentis* under both field and greenhouse conditions. Similarly, *L. ervoides* accession IG 72583 was identified as HS here and was reported by Tullu et al. (2006) to be susceptible to both races of the anthracnose pathogen

under greenhouse conditions. When comparing the current results with Stemphylium blight screening conducted by Podder et al. (2013), it was observed that *L. ervoides* accessions L01-827A and IG 72646 identified as HR here were previously reported to be very resistant and resistant to Stemphylium blight, respectively. IG 72646 (HR) was reported to be resistant to Ascochyta blight, anthracnose and Stemphylium blight in several studies (Bayaa et al. 1994, Tullu et al. 2006, Tullu et al., 2010 Podder et al. 2013), whereas IG 72582 (IM) was reported to be susceptible to Fusarium wilt, Ascochyta blight and anthracnose (race 0) (Bayaa et al. 1995, Ahmad et al. 1997, Tullu et al. 2006, 2010).

Among the 62 *L. ervoides* accessions newly screened for Ascochyta blight, three (IG 72785, IG 136617, and IG 72796) were classified as HR, four (IG 136615, IG 136612, IG 72863 and IG 136611) as HS, 29 as MR, 15 as IM and 11 as MS (Table 3.1). Out of nine accessions (IG 72568, IG 72783, IG 72784, IG 72797, IG 72861, IG 116022, IG 140884, IG 140910 and IG 140927), which had leaves similar to IG 72815 and L01-827A but different plant structures, one was classified as HR, four as MR, three as IM and one as MS accessions (Table 3.1).

Results from the current study showed that the *A. lentis* pathogen is able to infect all 157 *L. ervoides* accessions both under field and greenhouse conditions. According to the Genesys database (https://www.genesys-pgr.org), there are 1,156 wild lentil accessions available in different germplasm banks around the world and many of them may have never been screened for Ascochyta blight resistance. Among these, 429 accessions are *L. orientalis*, 375 *L. ervoides*, 222 *L. nigricans*, 81 *L. odemensis*, 27 *L. lamottei* and 22 *L. tomentosus* accessions. In order to have a comprehensive overview of disease resistance in the genus *Lens* in general and for *L. ervoides* accessions in particular, it is very important to screen all available germplasm in future. First, the newly identified sources of resistance are potential sources of novel resistance genes against currently known *A. lentis* isolates. Second, with the constant change in pathogen population structure, these new sources of resistance may have resistance against more virulent *A. lentis* isolates, as demonstrated recently by Dadu et al. (2017) who showed that two *L. orientalis* accessions were highly resistant to highly virulent *A. lentis* isolates in Australia.

Previously, the intraspecific *Lens ervoides* (Brign.) Grande. Recombinant inbred line (RIL) population LR-66 was developed by crossing IG 72815 and L01-827A (Gujaria-Verma et al. 2014) and used to map resistance QTLs for anthracnose and Stemphylium blight (Bhadauria et al. 2017),

whereas mapping of Ascochyta blight resistance was not possible because of lack of segregation. *L. ervoides* accessions with an HR and HS reaction, such as IG 136611 (HS) and IG 72918 (HR), could be used to develop a new population that segregates for Ascochyta blight resistance for QTL mapping. In addition, accessions such as IG 72646 and IG 72582, which are resistant and susceptible to multiple fungal pathogens respectively could be also used for developing mapping populations for identifying novel QTLs for disease resistance.

In conclusion, disease symptoms were observed on all accessions, therefore *L. ervoides* does not possess non-host resistance against *A. lentis*, hence the null hypothesis is rejected. *L. ervoides* accessions have a wide range of Ascochyta blight reactions varying from highly susceptible to highly resistant. The majority of *L. ervoides* accessions showed significantly fewer disease symptoms than the susceptible check Eston and these accessions could be novel sources of resistance genes. In addition, some of these accessions may have resistance to multiple fungal diseases and can be used to improve resistance in lentil cultivars.

3.7 Prologue to Chapter 4

Screening of *L. ervoides* accessions confirmed that the resistance mechanism against *A. lentis* in *L. ervoides* is not based on non-host resistance considering that the pathogen was able to complete its life cycle on all accessions. While screening *L. ervoides* accessions, a wide range in disease severity from highly susceptible to highly resistant accessions were observed which warranted further research into the infection process of *A. lentis* in *L. ervoides* through histopathology. For this purpose, the intra-specific *L. ervoides* LR-66 population from a cross of IG 72815 × L01-827A was selected that had previously been developed to study resistance to several pathogens in *L. ervoides*. IG 72815 has partial resistance to *C. lentis* and *S. botryosum* whereas L01-827A is susceptible to both. Both parents have high (17% severity on IG 72815) or very high (6% severity on L01-827A) resistance to Ascochyta blight.

During previous disease severity screenings, resistant and susceptible LR-66 RILs were identified for all three diseases, which were used along with parents and the *L. culinaris* checks Eston and CDC Robin to study the infection process of the three pathogens *A. lentis, C. lentis* and S. *botryosum*. Chapter 4 presents the framework of understanding host responses to these pathogens at various stages of infection at the cellular level.

4 Histopathological study of host responses to infection with fungal pathogens

4.1 Introduction

Crop domestication and cultivation have been an important step for human civilizations to flourish and survive. During this course, humans domesticated and cultivated many grain crops such as wheat, rice, oats, flax and pulses. Among pluses, lentil has been one of the most ancient and important pulse crop cultivated to this day. In modern times, since the recognition of its nutritional value, lentil has been introduced to new areas of cultivation such as North America and Australia. With 4.8 million tons worldwide and an average yield of 1.06 tonnes ha^{-1}, lentil is the fifth most important legume crop in terms of grain production (FAOSTAT, 2014). Lentil is a self-pollinating diploid (2n = 14) annual herbaceous legume crop with a genome size of 4 Gbps (Arumuganathan and Earle 1991). Since the inception of lentil cultivation in North America, lentil yields have increased several folds due to plant breeding and crop management efforts. However, plant breeding efforts and the pursuit of desired agronomic, seed and adaptability traits have resulted in a narrowing of the genetic base of the cultivated lentil germplasm, which makes lentil more prone to both biotic and abiotic stresses. Among biotic stress, fungal diseases are a constant challenge for lentil production that results in yield loss and downgrading of seed quality (Bhadauria et al. 2017). Among the fungal lentil diseases, Ascochyta blight, anthracnose and Stemphylium blight are most important and major threats to current and future lentil production in Canada and the northern United States of America. In order to counter the narrow genetic base of lentil, scientists have looked at wild lentil species as a resource for disease resistance and for broadening the genetic base of lentil cultivars. For the past three decades reports of wild lentil species possessing resistance to various lentils diseases such as Ascochyta blight, anthracnose, Stemphylium blight, Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lentis*), rust (caused by *Uromyces fabae* (Grev.) Fuckel.) and powdery mildew caused by (*Erysiphe polygoni* DC.) have been published (Bayaa and Erskine 1991, Bayaa et al. 1994;1995, Ahmad et al. 1997, Nasir 1998, Tullu et al. 2006, Tullu et al. 2010, Podder et al. 2013, Singh et al. 2014, Dadu et al. 2017). Whereas identifying the sources of disease resistance in wild species is the first step in their exploitation, studying host-pathogen interactions

through histopathology is the next step to identify and understand critical events occurring during pathogenesis, which could lead to a better understanding of resistance mechanisms and host responses (Vleeshouwers et al. 2000, Xi et al. 2000, Sillero and Rubiales, 2002, Dita et al. 2007). Histopathological studies have been conducted previously in cultivated lentil to understand the host responses to Asocohyta blight, anthracnose and Stemphylium blight (Roundhill et al.1995, Chongo et al.2002, Mwakutuya and Banniza 2010, Armstrong–Cho et al. 2012, Sambasivam et al. 2017, Sari et al. 2017). Roundhill et al. (1995) studied the infection process of *A. lentis* on detached leaflets of cultivated lentil revealing that conidia germinated within 6 h and appressoria were formed after 10 h of inoculation. A penetration peg penetrated the cuticle near the junction of two epidermal cells, and the plasmalemma was disrupted within 40 h of inoculation. Within 52 h after inoculation, all cells invaded by fungal structures had no intact nucleus or cytoplasm anymore. Sari et al. (2017) conducted descriptive microscopy on three cultivated lentil accessions with different levels of resistance to *A. lentis* 6 to 90 h post–inoculation (hpi). Up to 48 hpi, host responses were the same in all three accessions, but differences in the penetration of epidermal cells, papillae formation, colonization and disruptions of cytoplasm by the fungus could be seen at 60 and 90 hpi. Sambasivam et al. (2017) recently studied the infection process of two isolates of *A. lentis* (highly virulent AL4; low virulent Kewell) on detached leaflets of resistant lentil cultivar ILL 7537, moderately resistant ILL 5588 and susceptible ILL 6002, and found significant differences for spore germination, germ tube length and appressoria formation among isolates and genotypes during the incubation period. Mwakutuya and Banniza (2010) studied the infection process of *S. botryosum* on cultivated lentil leaflets and reported that germination of polyspermic conidia had started by 2 hpi and up to 6 germ tubes were reported from a single conidium which penetrated by 12 hpi, mostly through stomata, and disease severity had reached more than 80% by 48 hpi. Chongo et al. (2002) studied the infection process of *C. lentis* on resistant and susceptible lentil cultivar leaflets and observed that conidia germinated by 3 to 6hpi, appressoria formed from 6 to 12 hpi and infection pegs were formed by 24 hpi. In another study conducted by Armstrong–Cho et al. (2012), primary and secondary hyphae of *C. lentis* (races 0 and 1) were observed in lentil leaflets by 48 hpi. Significant differences between the two races were observed for conidial germination, appressorium formation and penetration of the surface of leaflets.

Although histopathological studies have been conducted on *L. culinaris* in the past, there are no such reports on pathogen progression and host responses in wild lentil species or interspecific

hybrids, partially because integrating novel disease resistance genes from wild species through conventional methods has been challenging (Ladizinsky et al. 1984, Hamdi and Erskine 1994, Ladizinsky, 1999; Gupta 2003, Fratini et al., 2004; Gupta and Sharma, 2007). Through embryo rescue techniques it has been possible to obtain fertile hybrid embryos from interspecific crosses through which genes from wild lentil species were transferred to the cultivated species (Cohen et al. 1984, Ahmad et al. 1995, Fratini and Ruiz 2006, Fiala et al. 2009, Tullu et al. 2013, Saha et al. 2015). In many cases, these interspecific populations were subjected to genetic distortions, which makes it difficult to study the genetic control of the trait of interest (Fiala et al. 2009). To avoid the problem of genetic distortion in interspecific populations, an intraspecific *Lens ervoides* recombinant inbred line (RIL) population, LR-66, was developed using IG 72815 and L01-827A as parents (Gujaria-Verma et al. 2014). Both parents of LR-66 have high levels of resistance to Ascochyta blight, whereas against race 0 of *C. lentis* and stemphylium blight, IG 72815 has high levels of resistance and L01-827A is moderately susceptible (Bhadauria et al. 2017). Disease resistance QTLs were mapped for anthracnose (both race 0 and 1 of *C. lentis*) and Stemphylium blight on the genetic map of LR-66, but not for ascochyta blight, (Bhadauria et al. 2017). Building on the work already published on LR-66, this project was aimed at studying the progression of infection in selected LR-66 RILs through histopathology for the three pathogens *C. lentis, A. lentis* and *S. botryosum*, to determine whether there are quantitative differences for anthracnose and Stemphylium blight, and qualitative differences in host responses for Ascochyta blight.

4.2 Material and Methods

4.2.1 Plant material

Plant material included the parents, IG 72815 and L01-827A, six RILs of LR-66 and the two *L. culinaris* checks Eston and CDC Robin (Table 4.1). The six RILs were previously selected on the basis of their resistance levels in response to independent inoculations with *A. lentis, C. lentis* and *S. botryosum* and represent the most resistant and most susceptible genotypes to each pathogen (Table 4.1, Bhadauria et al. 2017).

Table 4.1. Severity (%) of Ascochyta blight, anthracnose (*Collteotrichum lentis* race 0) and Stemphylium blight on LR-66 parents and RILs used for histopathological studies (Bhadauria et al., 2017)

	Disease severity		
Genotype	Ascochyta blight	Anthracnose	Stemphylium blight
IG 72815	16.87 (1.0)[b]	31.87 (1.19)	32.62 (1.28)
L01-827A	6.25 (0.85)	51.87 (1.19)	53.12 (1.0)
Eston	49.37 (1.28)	95 (0.0)	61.87 (0.52)
Robin	16.25 (0.85)	95 (0.0)	67.37 (0.62)
LR-66-528	6.25 (0.85)	5 (0.0)	58.87 (1.0)
LR-66-524	6.87 (1.0)	71.87 (1.19)	52.2 (1.11)
LR-66-629	2.5 (0.64)	56.25 (0.85)	40.62 (1.28)
LR-66-570	40.62 (1.28)	53.75 (0.85)	67.5 (1.11)
LR-66-637	13.12 (1.0)	15 (0.85)	20 (1.29)
LR-66-577	8.75 (1.25)	38.12 (1.19)	76.87 (1.0)

[b] Numbers in brackets indicate standard errors of the means.

4.2.2 Experimental design

Experiments were conducted in a factorial randomized complete block design with three biological replications. Factors were genotype and incubation periods (Table 4.2). For each experiment, three biological replications were separately spray-inoculated with *C. lentis* isolate CT-30 (race 0) at a concentration of 5×10^4 conidia ml^{-1}, *A. lentis* isolate AL-61 at 5×10^5 conidia ml^{-1} or *S. botryosum* isolate SB-19 at 1×10^5 conidia ml^{-1} using approximately 3 ml of conidial suspension per plant. Due to space and sampling time constraints, replicates were blocked over time. For each time point, 20 leaflets were arbitrarily selected from 4 plants (five leaves from each plant) per pot representing a biological replication.

Table 4.2. Pathogens, accessions and RILs of *Lens ervoides* population LR-66 and incubation periods (hours post inoculation, hpi) for sampling of leaf tissue

Pathogens	Accessions and RILs	Incubation period (hpi)
Colletotrichum lentis	IG 72815, L01-827A, LR-66-524, LR-66-528, Eston, CDC Robin	Mock, 6, 12, 24, 48, 72, 96, 120, 144
Ascochyta lentis	IG 72815, L01-827A, LR-66-629, LR-66-570, Eston, CDC Robin	Mock, 6, 12, 24, 48, 72, 96, 144, 192, 240
Stemphylium botryosum	IG 72815, L01-827A, LR-66-637, LR-66-577, Eston, CDC Robin	Mock, 6, 12, 24, 48, 72, 96, 120, 144

Upon detachment from the plants, leaves were fixed and stored immediately in mail tubes containing CMAA fixative (30% chloroform, 60% methanol, 10% acetic acid) at room temperature. Inoculated leaflets were treated with CMAA fixative twice or thrice until the leaflets were cleared. Leaflets were then removed from the CMAA fixative and stored in 95% ethanol at room temperature.

4.2.3 Sample preparation

Six leaflets were arbitrarily removed from each 95% ethanol vial and were passed through decreasing ethanol concentrations of 70% (1 h), 50% (1.5 h) and 30% (1.5 h). Leaflet samples were stained with 0.05% Trypan blue and stored in 50% glycerol. Leaflets were mounted with the adaxial surface facing up on Fisherfinest premium microscope glass slides (25 x 75 x 1 mm) in 50% glycerol and covered with Fisherbrand microscope cover glass (18x18, # 2). Two leaflets were mounted on each glass slide for a total of three slides per sample. Colourless nail varnish was applied on the periphery of cover glass slides to make the glass slides permanent.

4.2.4 Microscopy

Mounted leaflets were examined visually under a Zeiss Axioskop 40 microscope. Pictures were taken using a Pixelink A686C camera and Zeiss Axiovision software (version 4.8.2 plus measurement module). Observations were made on the adaxial surface of leaflets from leaflet tips towards the petiolates to take into account the fact that inoculation droplets might have accumulated towards the tip of leaflets because of the position of attached leaflets during inoculation. All observations were made at 400X magnification. Quantitative data was collected for each pathogen.

For *A. lentis*, the number of germinated conidia per 50 conidia per leaflet, the average length of germ tube per 5 germinated conidia per leaflet, the number of appressoria formed per 25 germinated conidia per leaflet, the number of pycnidia per leaflet were recorded, and the percentage area of dead tissue per leaflet was visually estimated.

For *S. botryosum*, the number of germinated conidia per 50 conidia per leaflet, the number conidia with at least one germ tube penetrating through the leaf epidermis or stomata per 25 germinated conidia per leaflet, the average length of the longest germ tube per conidium of 5 germinated conidia per leaflet and the percentage area of dead tissue per leaflet was visually estimated.

For *C. lentis*, the number of infection vesicle (IV)/primary hyphae (PH) formed per 25 appressoria, the percentage of leaflet area covered by acervuli per leaflet and the percentage area of dead tissue per leaflet were visually estimated.

4.3 Statistical analysis

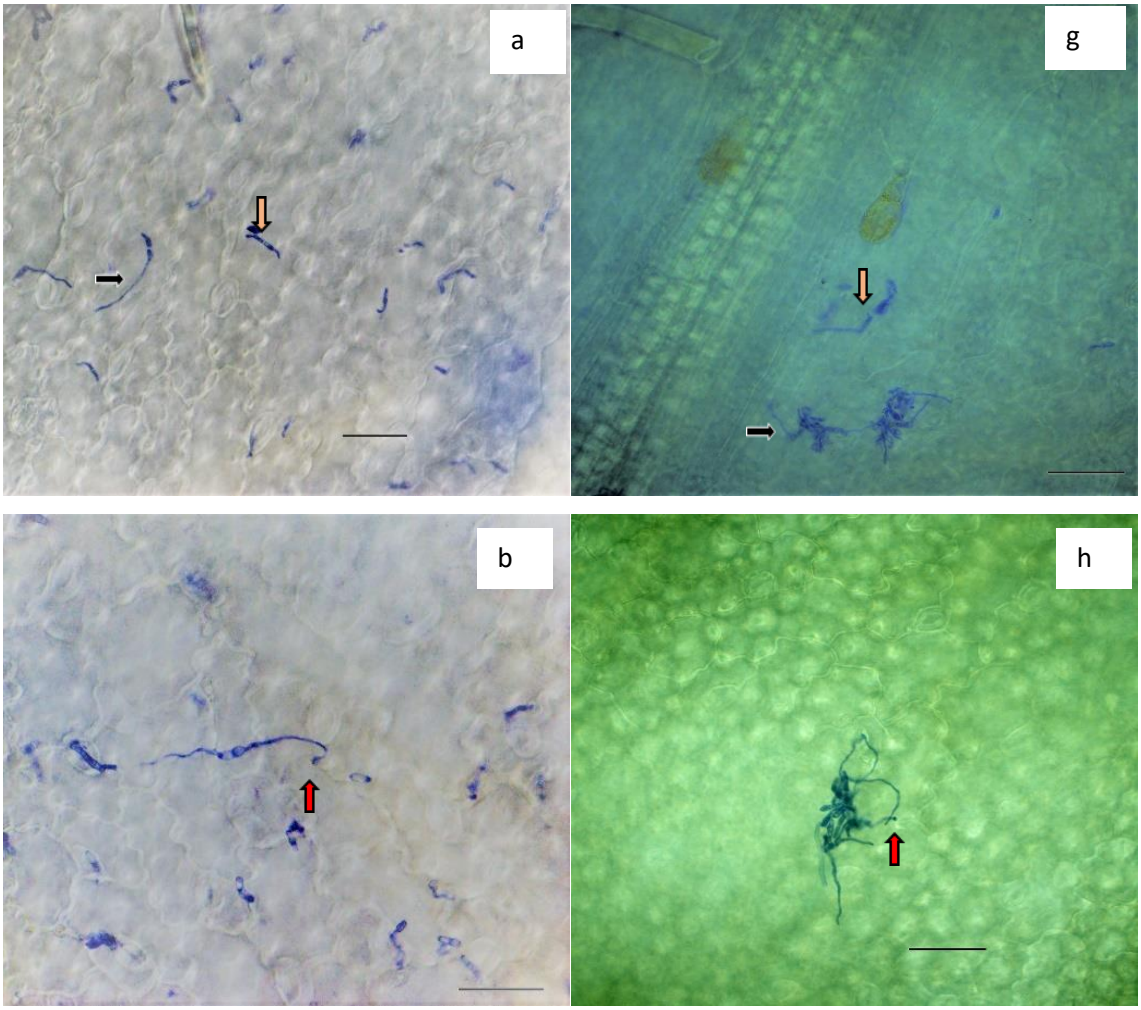
Quantitative data for different traits of pathogens for RILs, parents and checks were analysed using SAS statistical package (Version 9.4, SAS Institute, Cary, NC, USA). For testing homogeneity of variance, the Levene's test was used, followed by analysis of variances with the mixed model procedure. Each host-pathogen system was analysed separately. Accessions and time points were considered fixed effects, the time points were identified as a repeated measure, and replications were considered random effects. Regression analyses were performed where traits of interest were recorded for three or more time points. At different time points, genotypes were compared by multiple comparisons of means using Fisher's least significant difference.

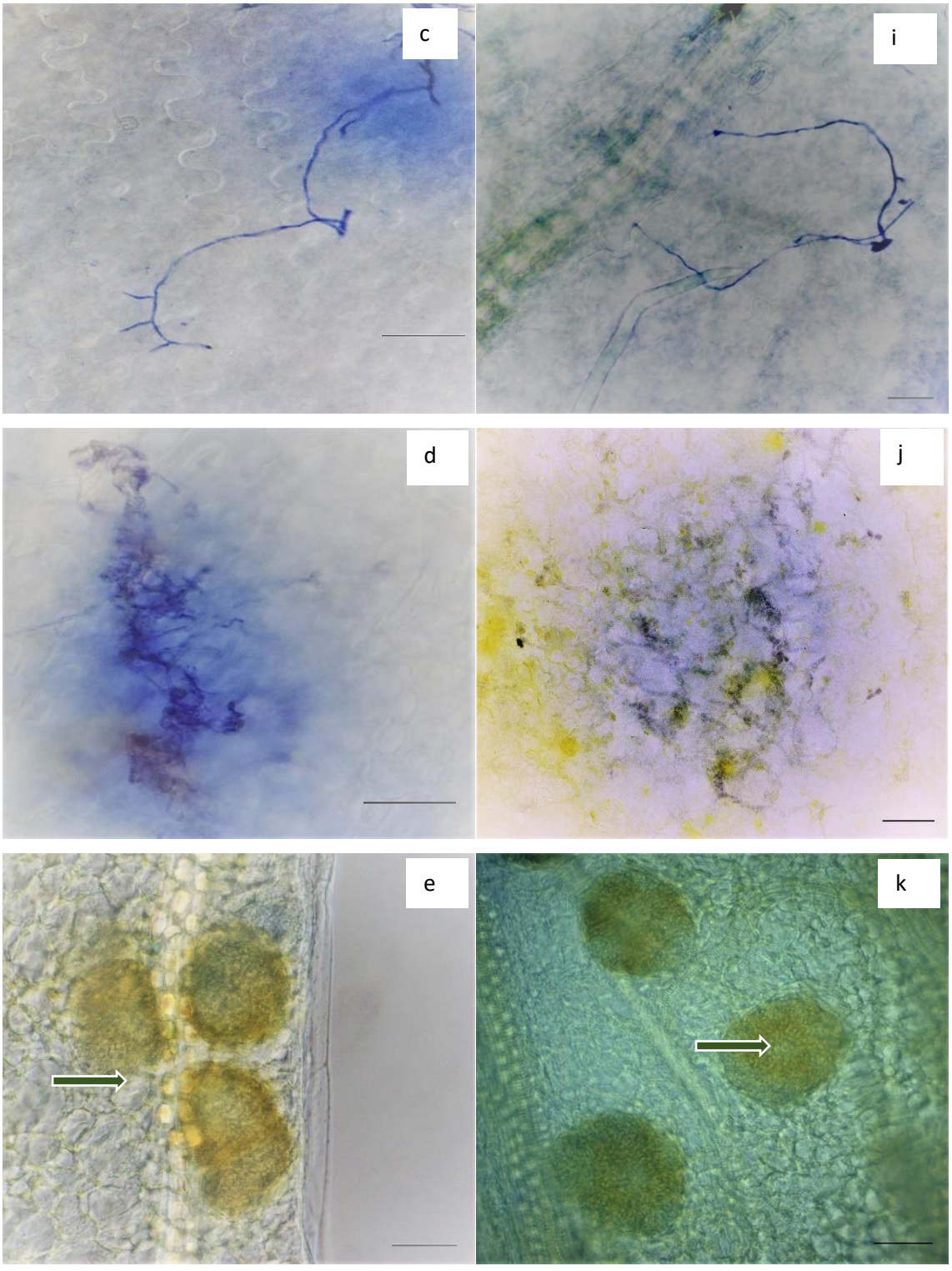
4.4 Results

4.4.1 Ascochyta blight

Conidial germination was observed in all genotypes by 6 hpi and became more apparent by 12 hpi (Fig 4.1 a, g). Germinated conidia had either one or two germ tubes. The length of germ tubes increased during the incubation period, and appressoria were observed by 24 hpi on all genotypes (Fig 4.1 b, h). Not all germ tubes ended in appressoria and some kept growing for longer periods without differentiating appressoria (Fig 4.1 c, i). Fungal growth in the host tissues became more apparent by 96 hpi as a result of penetrations (Fig 4.1 d, j). Pycnidia formation was observed by 144 hpi in all genotypes and continued to sporulate 192 and 240 hpi (Fig 4.1 e, f, k, l).

The proportion of conidial germination of *A. lentis* isolate AL-61 was recorded at 6, 12, 24 and 48 hpi on IG 72815, L01-827A, LR-66-629, LR-66-570 and the checks Eston and CDC Robin. Germination increased with incubation time for all genotype (Fig 4.2 a). Using regression analysis, it was determined that genotype ($P = 0.0014$), incubation time ($P < 0.0001$) and genotype by incubation time interaction ($P = 0.0026$) had significant effects on the proportion of conidial germination (Appendix 2, Table A2.1). The intercept for the regression graph of susceptible LR-66-570 was nominally higher than that of resistant LR-66-629 and was overall the highest in CDC Robin (Appendix 2, Table A2.3). In contrast, LR-66-629 had the highest rate of increase (slope) whereas LR-66-570 and CDC Robin had the lowest. Comparisons of means showed that on RIL LR-66-570, *A. lentis* had significantly higher conidial germination than on LR-66-629, IG 72815 and L01-827A from 6 to 24 hpi whereas there was no significant difference when compared with checks Eston (susceptible) and CDC Robin (partially resistant) from 6 to 48 hpi. There were no significant differences for conidial germination among LR-66-629, IG 72815 and L01-827A at any time points. Both Eston and CDC Robin had significantly higher conidial germination compared to LR-66-629, IG 72815 and L01-827A from 6 to 24 hpi. There were no significant differences for *A. lentis* conidial germination between Eston and CDC Robin at any time. (Appendix 2, Table A2.2).


a
g
b
h


c
i
d
j
e
k

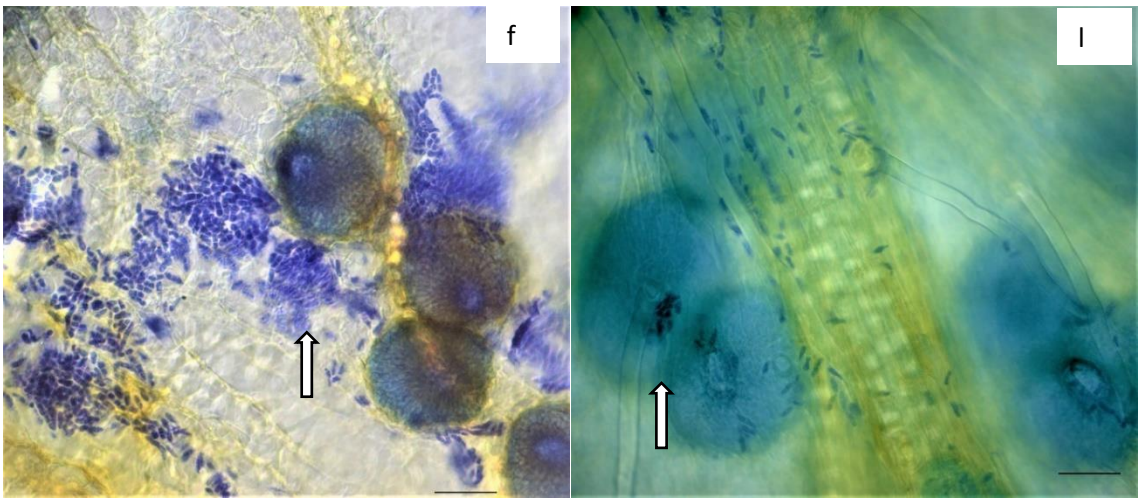


Figure 4.1 Stages of the infection process of *Ascochyta lentis* isolate AL-61 in Ascochyta blight-susceptible *Lens ervoides* RIL LR-66-570 (a-f) and Ascochyta blight-resistant LR-66-629 (g-l). a and g) Conidial germination at 12 hpi, ➞indicates one germtube, ⇩indicates two germtubes from the same conidia, b and h) ⇧indicates appressoria formation at 24 hpi, c and i) increase in germ tube length at 72 hpi, d and j) growth of fungal mass in the host tissues at 96 hpi, e and k) ➞ indicates pycnidia formation at 144 hpi, f and l) ⇧ indicates sporulating pycnidia next generation of conidia at 192 and 240 hpi. Scale bars represented in the bottom right corners represent 50 µm.

Germ tube length per 5 germinated conidia per leaflet increased over the period from 24 to 72 hpi on all six genotypes (Fig 4.2 c). Using regression analysis, it was determined that incubation time ($P < 0.0001$) had a significant effect on germ tube length whereas genotype ($P = 0.9389$) and the genotype by incubation time interaction ($P = 0.8952$) had no effects on germ tube length (Appendix 4, Table A4.1). The Intercept for the regression graph was nominally highest for susceptible LR-66-570 and lowest for IG 72815, whereas the rates of increase in germ tube length (slope) were the same for all genotypes (Appendix 4, Table A4.3). Comparisons of means showed that there were no significant differences among genotypes at any time point (Appendix 4, Table A4.2).

Appressoria formation of *A. lentis* isolate AL-61 increased with incubation time on all six genotypes included in the experiment (Fig 4.2 b). Using repeated measures analysis, it was determined that incubation time ($P = 0.0328$) had a significant effect on appressoria formation whereas genotype ($P = 0.1473$) and the genotype by incubation time interaction ($P = 0.3469$) had

no effects on appressoria formation (Appendix 3, Table A3.1). Comparisons of means revealed that the number of appressoria on resistant LR-66-629 and susceptible LR-66-570 were not significantly different at 24 and 48 hpi. The numbers of appressoria was similar on Eston and susceptible LR-66-570, but Eston had more appressoria than other genotypes at 24 hpi, but not at 48 hpi (Appendix 3, Table A3.2).

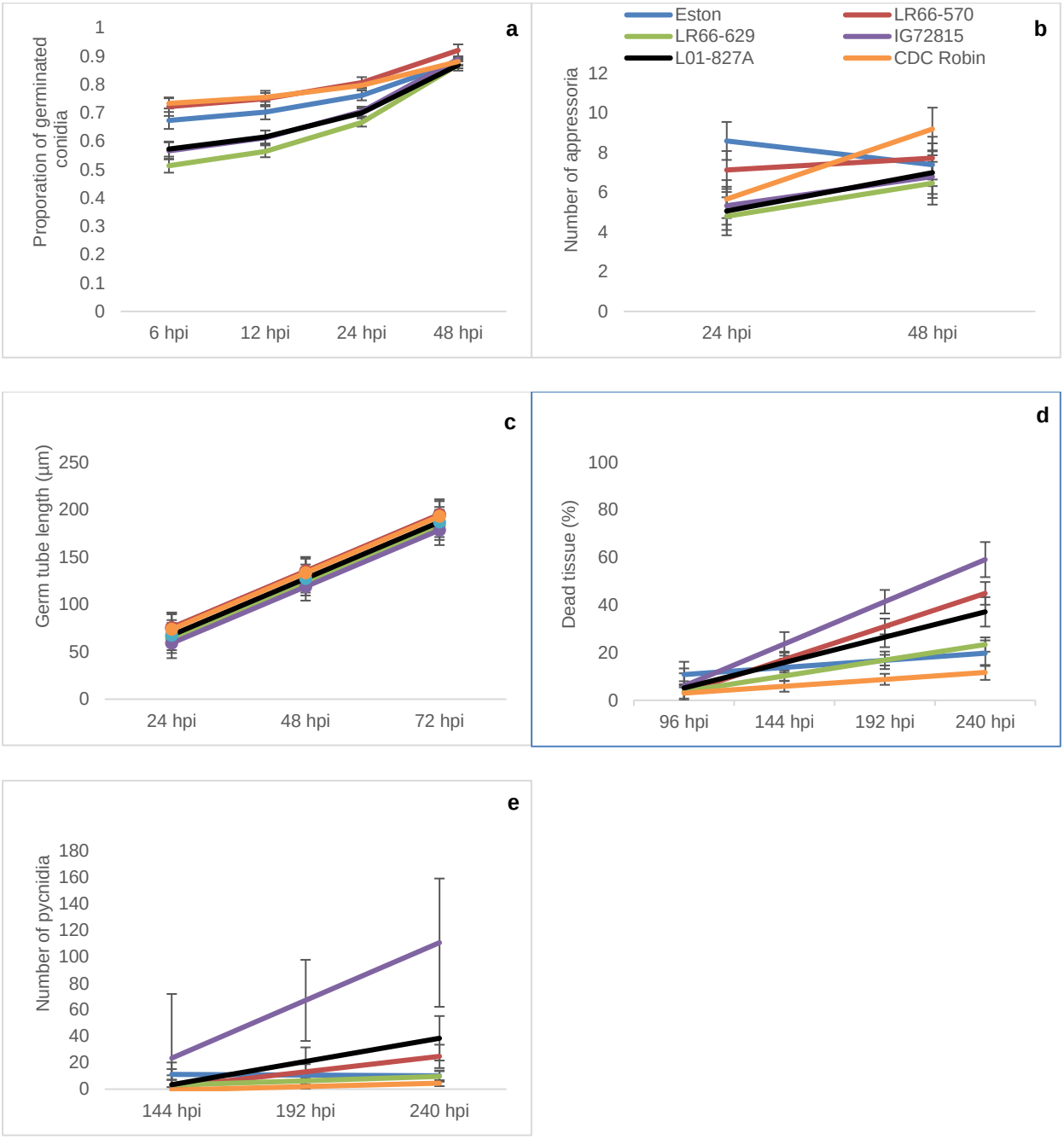


Figure 4.2 Quantitative parameters assessed during the infection process of *Ascochyta lentis* isolate AL-61 in *L. ervoides* and *L. culinaris* genotypes. a) Proportion of conidial germination per 50 conidia on genotypes up to 48 hours post-inoculation (hpi), b) number of appressoria formed per 25 germinated conidia at 24 and 48 hpi, c) germ tube length per 5 germinated conidia from 24 and 48 hpi, d) percentage of dead tissue per leaflet from 96 to 144 hpi, e) number of pycnidia per leaflet. Error bars represent standard errors of the means.

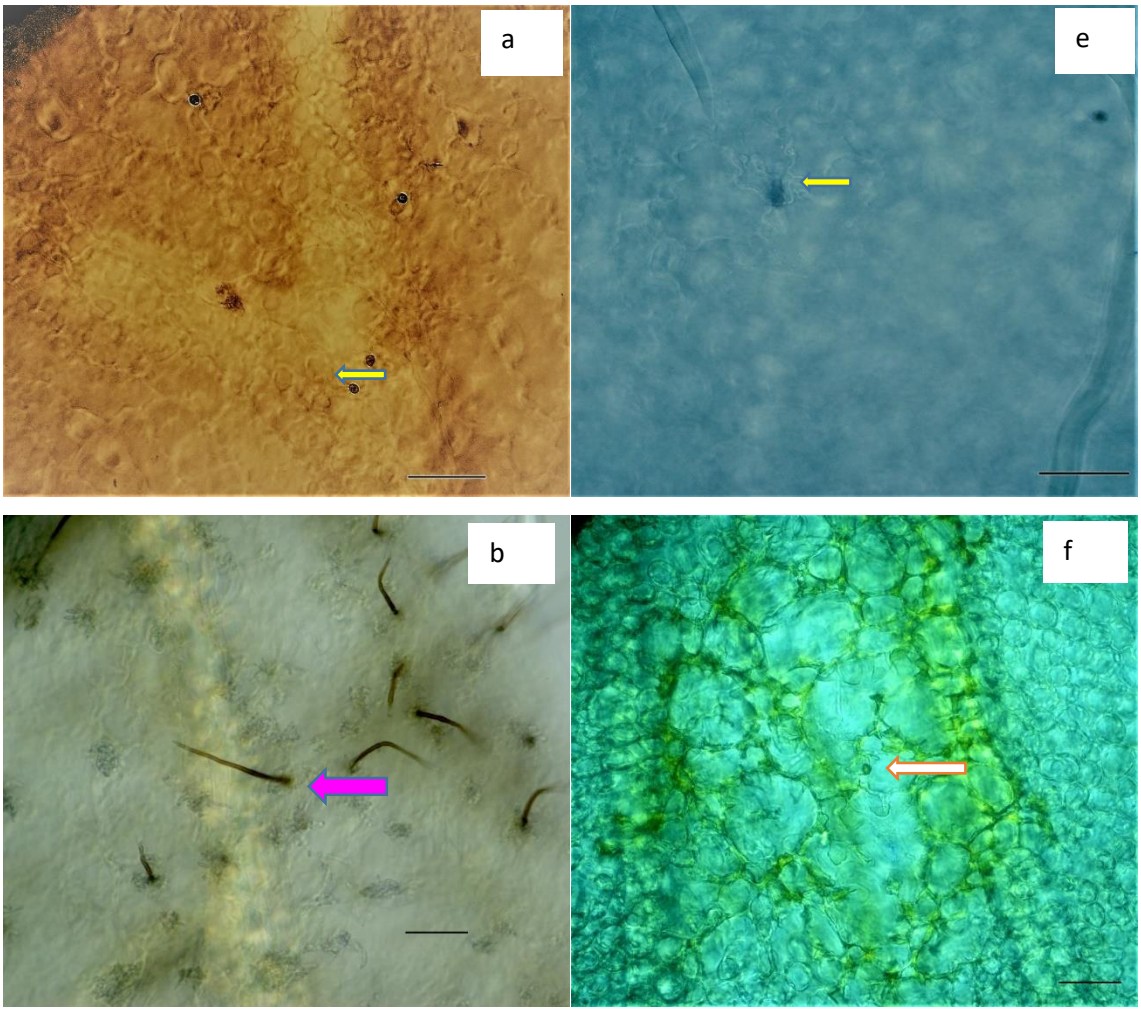
Percentage of dead tissue per leaflet due to *A. lentis* isolate AL-61 infection increased with incubation time for all six genotype (Fig 4.2 d) Regression analysis revealed that genotype ($P < 0.0001$), incubation time ($P < 0.0001$) and the genotype by incubation time interaction ($P = 0.0032$) had a significant effect on the percentage of dead tissue per leaflet (Appendix 5, Table A5.1). The intercept for the regression graph of LR-66-629 was nominally higher compared to that of IG 72815, but overall it was highest in Eston. The rate of increase in the percentage of dead tissue (slopes) was highest for IG 72815, lower for LR-66-629, and lowest for CDC Robin (Appendix 5, Table A5.3). There were no significant differences for percentage of dead tissue per leaflet among genotypes at 96 hpi based on means comparisons, but at 192 and 240 hpi susceptible LR-66-570 had a significantly higher percentage of dead tissue than LR-66-629 and Eston. There were no significant differences when Eston was compared with LR-66-629 at 144, 192 and 240 hpi (Appendix 5, Table A5.2).

Genotype ($P = 0.0324$) had a significant effect on the number of pycnidia of *A. lentis* isolate AL-61 per leaflet while incubation time ($P = 0.085$) and genotype by incubation time interaction ($P = 0.4298$) had no effects when regressing the number of pycnidia on incubation time (Appendix 6, Table A6.1). There were no significant differences among *L. ervoides* genotypes at any time points while Eston had a significantly higher number of pycnidia per leaflet compared to CDC Robin at 144 and 192 hpi (Appendix 6, Table A6.2, Fig 4.2 e).

4.4.2 Anthracnose

In very few instances, conidial germination was observed by 6 hpi, which did not yield sufficient data for further statistical analysis. Appressoria formation was observed by 6 hpi in all genotypes but at fewer instances. It seemed as if conidia were washed away during sample preparation, which is why no data were collected for conidial germination or number of appressoria formed per germinated conidia. Beneath some appressoria, infection vesicle / primary hyphae could be

identified in all genotypes except resistant LR-66-528 at 24 hpi, but they had formed in all genotypes by 48 hpi (Fig 4.3 a, e). Secondary hyphae were not visible as they did not stain well during sample preparation. At 120 hpi, cell death was observed in a few instances in resistant RIL LR-66-528, but this was not consistent (Fig 4.3 f), and was not the case for other genotypes. At 120 and 144 hpi, setae and acervuli were visible in all the genotypes (Fig 4.3 b, c, d, g, h).



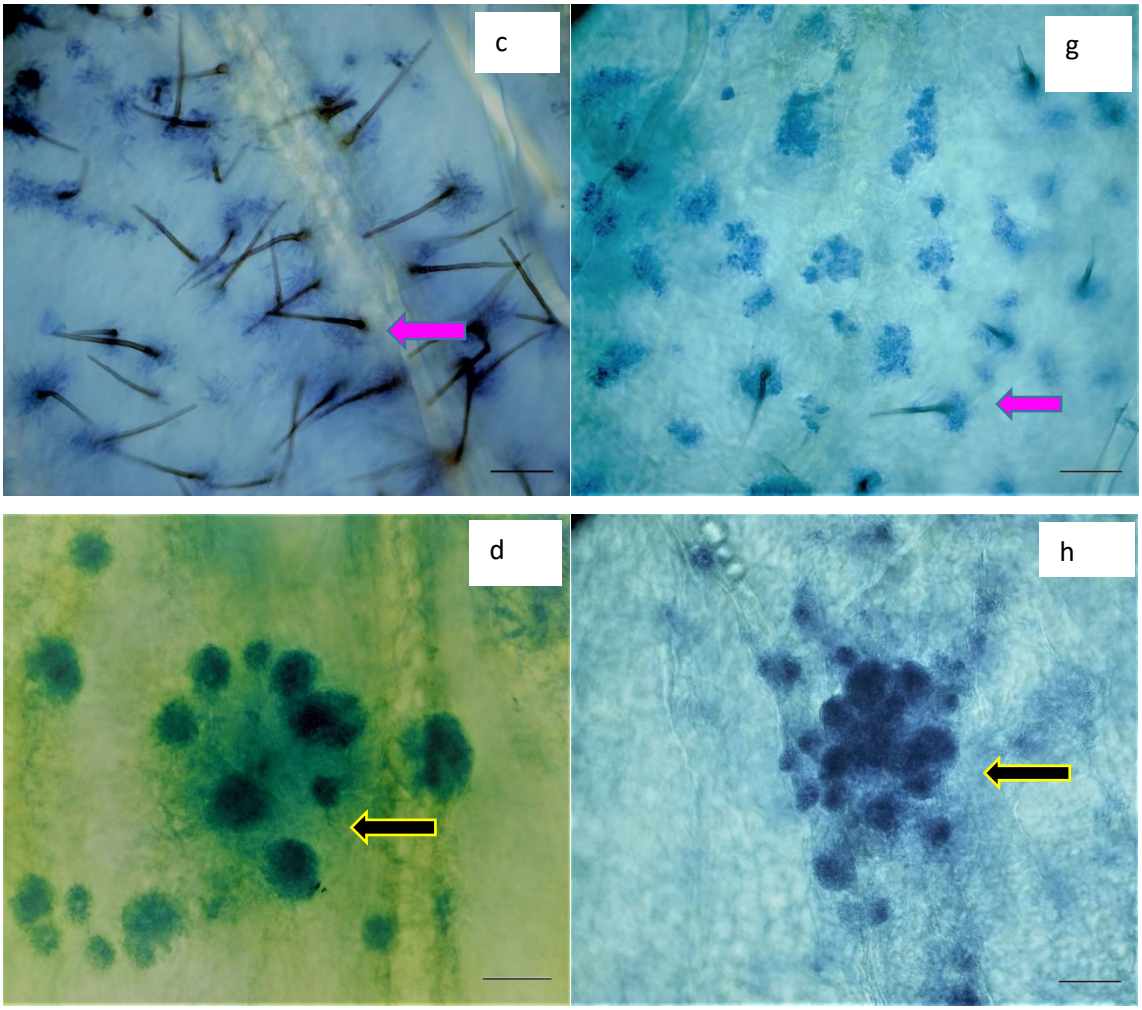


Figure 4.3 Stages of infection process of *Colletotrichum lentis* isolate CT-30 in anthracnose-susceptible *Lens ervoides* RIL LR-66-524 (a-d) and anthracnose-resistant LR-66-528 (e-h). a and e) ⇦ indicates primary hyphae formed beneath appressoria at 48 hours post-inoculation (hpi), b) ⇦ indicates setae formation in LR-66-524 at 120 hpi, f) ⇦ indicates appressoria surrounded by dead tissue at 120 hpi in LR-66-528 at many instances. c and g) ⇦ indicates setae formation at 120 and 144 hpi, d and h) ⇦ indicates acervuli formation at later time points, i.e. 120 and 144 hpi. Scale bars in bottom right corners indicate 50 µm.

The proportion of infection vesicle/primary hyphae formed by *C. lentis* isolate CT-30 increased with incubation time in IG 72815, L01-827A, Eston, CDC Robin, susceptible RIL LR-66-524 and resistant LR-66-528 (Fig 4.4 a). Using repeated measures analysis, it was determined that genotype ($P < 0.0001$), incubation time ($P < 0.0001$) and genotype by incubation time interaction ($P = 0.0005$) had significant effects on the proportion of infection vesicle/primary hyphae (Appendix 7,

Table A7.1). Fisher's least significant difference for comparison of means showed that the proportion of infection vesicle/primary hyphae formed in susceptible LR-66-524 was significantly higher than in the resistant RIL LR-66-528, whereas there were no significant differences between Eston and CDC Robin at 24 and 48 hpi (Appendix 7, Table A7.2).

The percentage of dead tissue per leaflet due to infection by *C. lentis* isolate CT-30 increased with incubation time among all six genotypes (Fig 4.4 b). Using regression analysis, it was determined that genotype ($P < 0.0001$), incubation time ($P < 0.0001$) and the genotype by incubation time interaction ($P = 0.0499$) had significant effects on the percentage of dead tissue per leaflet (Appendix 8, Table A8.1). The intercept for the regression graph was nominally highest in L01-827A and lowest in LR-66-528 among *L. ervoides* genotypes, while CDC Robin had the highest intercept among all genotypes (Appendix 8, Table A8.2). CDC Robin had the lowest rate of increases in the percentage of dead tissue (slope) whereas it was highest for IG 72815 followed by LR-66-528 (Appendix 8, Table A8.3). Comparisons of means showed that susceptible LR-66-524 had a significantly higher percentage of dead tissue compared to resistant LR-66-528 at 96, 120 and 144 hpi. Similarly, Eston had significantly higher percentage of dead tissue compared to CDC Robin at 120 and 144 hpi but not at 96 hpi. There was no difference between Eston and LR-66-524, but LR-66-528 had significantly less dead tissue at 96, 120 and 144 hpi compared to Eston.

Percentage of leaflet area covered by acervuli of *C. lentis* isolate CT-30 increased with incubation time for all six genotypes (Fig 4.4 c). Using regression analysis, it was determined that genotype ($P < 0.0001$), incubation time ($P = 0.0003$) and the genotype by incubation time interaction ($P = 0.0078$) had significant effects on the percentage of leaflet area covered by acervuli (Appendix 9, Table A9.1). The intercept for the regression graph was nominally highest for L01-827A and lowest for Eston, whereas the rate of increase in area covered by acervuli (slope) was highest for Eston and lowest for L01-827A (Appendix 9, Table A9.3). Comparisons of means showed no significant differences for a percentage of leaflet area covered by acervuli among genotypes at 96 hpi. At 120 and 144 hpi susceptible LR-66-524 had significantly more leaflet area covered by acervuli compared to resistant LR-66-528. Similarly, Eston had significantly a higher percentage of leaflet area covered by acervuli compared to CDC Robin at 120 and 144 hpi. There were no significant differences between LR-66-524 and Eston at 120 and 144 hpi (Appendix 9, Table A9.2).

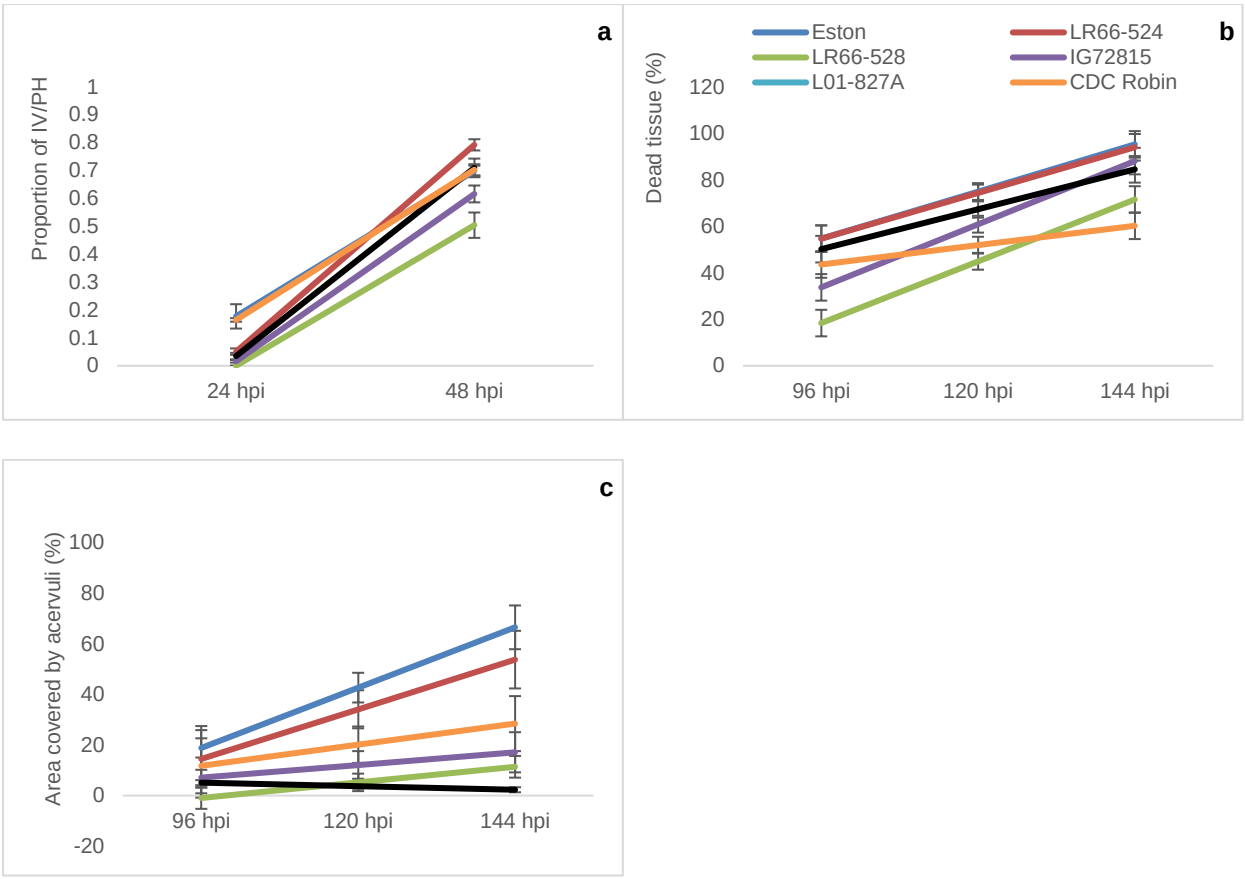
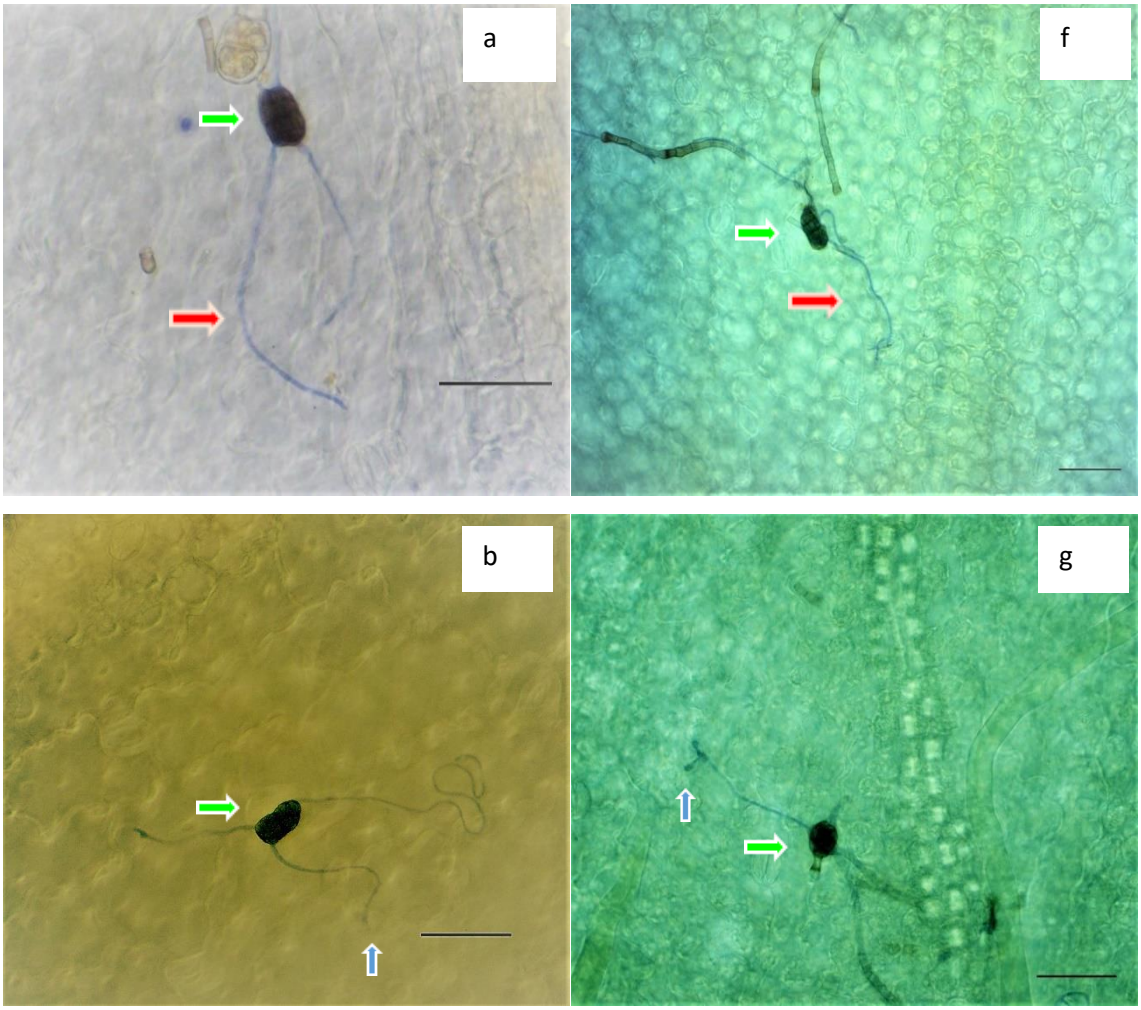


Figure 4.4 Quantitative parameters assessed for the infection process of *Colletotrichum lentis* race 0 isolate CT-30 in *L. ervoides* and *L. culinaris* genotypes. A) Proportion of infection vesicle (IV) / primary hyphae (PH) per 25 appressoria from 24 to 48 hpi, b) percentage of dead tissue per leaflet from 96 to 144 hpi, c) percentage of leaflet area covered with acervuli. Error bars represent standard errors of the means.

4.4.3 Stemphylium blight

Conidia of *S. botryosum* had germinated by 6 hpi and germ tube penetration was observed at 6 and 12 hpi in all genotypes (Fig 4.5 a, b, f, g). By 48 hpi, cell death seemed to start appearing at the point of penetration or around germinated conidia (Fig 4.5 c, h). At 72 and 96 hpi, hyphae started to emerge from the host leaf tissue (Fig 4.5 d, i). Conidiophores had developed by 120 hpi and started differentiating conidia (Fig 4.5 e, j).


a
f
b
g

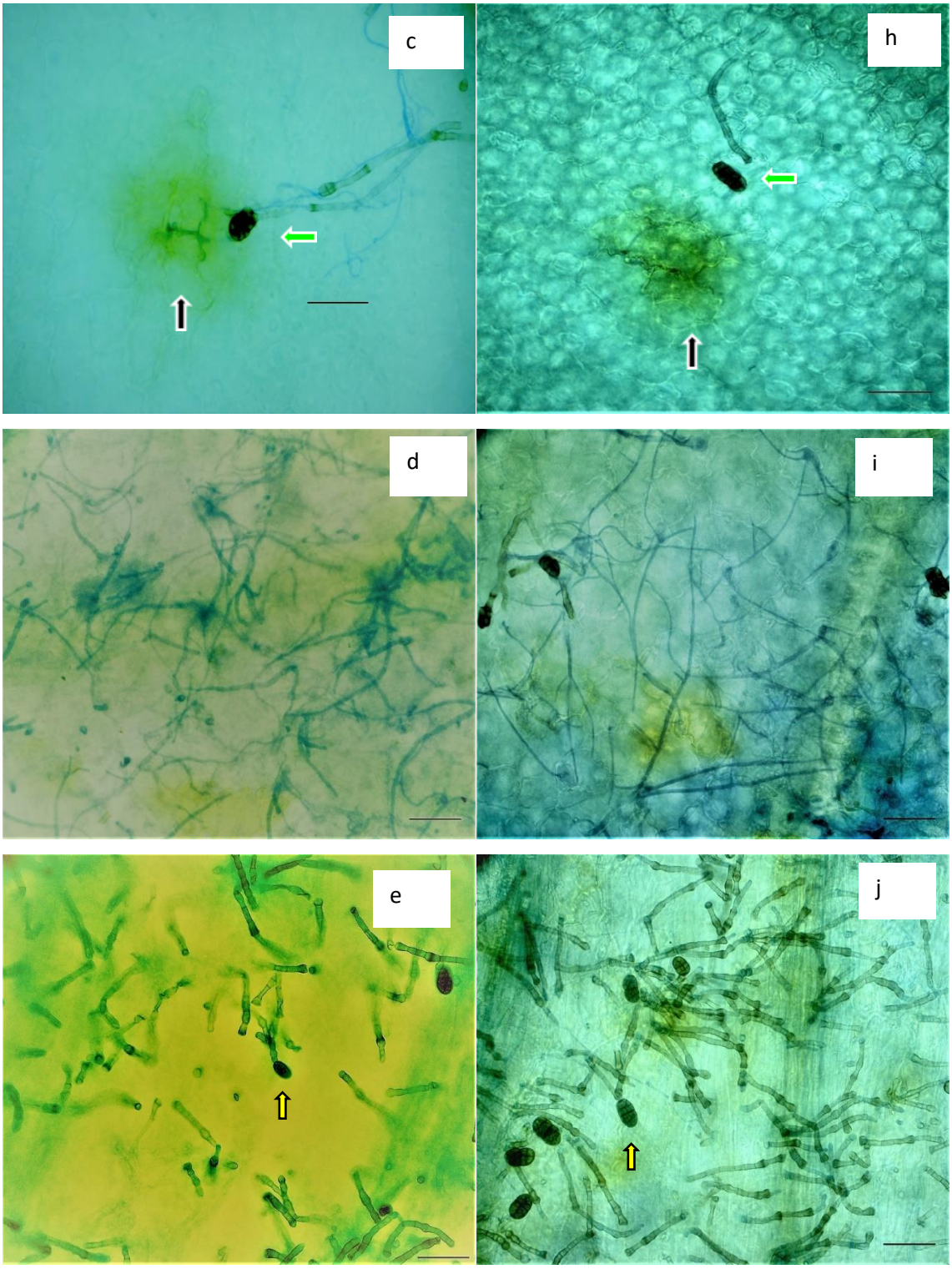


Figure 4.5 Stages of infection process *Stemphylium botryosum* isolate SB19 in Stemphylium blight-susceptible *Lens ervoides* RIL LR-66-577 (a-e) and Stemphylium blight-resistant LR-66-637 (f-j). a and f) ➞ indicates conidia and ➞ indicates germ tube at 6 hours post-inoculation

(hpi), b and g) → indicates conidia and ↑ indicates penetration by a germ tube at 12 hpi, c and h) → indicates conidia and ↑ indicates germ tube penetration and potentially inducing host tissue death (yellow colored area) at 48 hpi, d and i) hyphae coming out of the leaf tissue at 72 and 96 hpi, e and j) ⇧ indicates conidiophores with the next generation conidia at the tip at 120 and 144 hpi. Scale bars (50 µm) are indicated at the bottom right of each image.

The proportion of germinated conidia was recorded at 6, 12, 24 and 48 hpi on IG 72815, L01-827A, Eston, CDC Robin, resistant LR-66-637 and susceptible LR-66-577 (Fig 4.6 a). Using regression analysis, it was determined that genotype ($P = 0.0051$) and the genotype by incubation time interaction ($P = 0.0368$), but not incubation time ($P = 0.1636$), had significant effects on the proportion of germinated conidia of *S. botryosum* isolate SB-19 (Appendix 10, Table A10.1). The intercept for the regression graph was nominally highest for IG 72815 and lowest for Eston, whereas the rate of increase in conidial germination (slope) was lowest on IG 72815 and highest on susceptible LR-66-577 (Appendix 10, Table A10.3). There were no significant differences in conidial germination between susceptible LR-66-577 and resistant LR-66-637 at 6, 12, 24 and 48 hpi. Both, CDC Robin and LR-66-577 had significantly higher conidial germination compared to Eston at 6, 12 and 24 hpi but not at 48 hpi. LR-66-637 had significantly higher conidial germination compared to Eston at 12 and 24 hpi but not at 6 and 48 hpi. Both LR-66-637 and LR-66-577 had similar conidial germination compared to CDC Robin between 6 and 48 hpi (Appendix 10, Table A10.2).

Germ tube length was recorded at 12 and 24 hpi for all six genotypes and increased over time (Fig 4.6 b). Genotype ($P = 0.0053$) and incubation time ($P < 0.0001$) had significant effects on germ tube length whereas the genotype by incubation time interaction ($P = 0.1441$) did not (Appendix 11, Table A11.1). There were no significant differences in germ tube length between the susceptible RIL LR-66-577 and resistant LR-66-637. CDC Robin had significantly longer germ tubes than Eston but not compared to other genotypes. Eston had significantly shorter germ tubes compared to LR-66-577 but not to LR-66-637 (Appendix 11, Table A11.2).

The proportion of penetrations by germ tubes was recorded at 6 and 12 hpi and increased during this period (Fig 4.6 c). Using repeated measures analysis, it was determined that genotype ($P = 0.0155$) had a significant effect on penetration whereas incubation time ($P = 0.1636$) and the

genotype by incubation time interaction (P = 0.561) did not affected penetration (Appendix 12, Table A12.1). There was no significant difference in penetrations between susceptible LR-66-577 and resistant LR-66-637, but CDC Robin had significantly higher penetrations by germ tubes than all other genotypes (Appendix 12, Table A12.2).

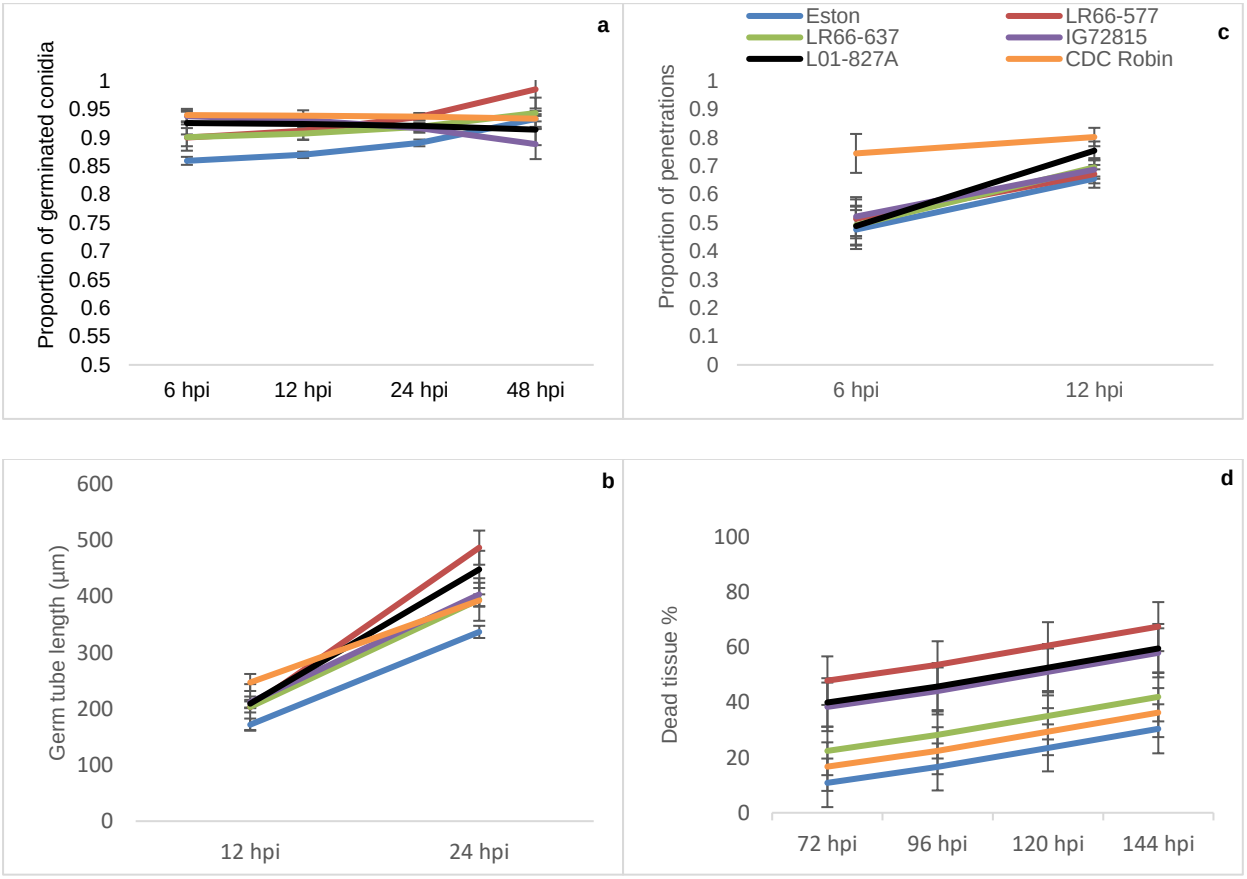


Figure 4.6 Quantitative parameters assessed for the infection process of *Stemphylium botryosum* isolate SB19 in *L. ervoides* and *L. culinaris* genotypes a) Proportion of germinated conidia per 50 conidia up to 48 hpi, b) proportion of penetrations by germ tubes per 25 germinated conidia at 6 and 12 hpi, c) germ tube length per 5 germinated conidia at 12 and 24 hpi, d) percentage of dead tissue per leaflet from 96 to 144 hpi. Error bars represent standard errors of the means.

The percentage of dead leaflet tissue was recorded from 72 to 144 hpi and increased for all six genotypes (Fig 4.6 d). Regression analysis revealed that genotype (P < 0.0001) and incubation time (P = 0.0003), but not the genotype by incubation time interaction (P = 0.5681) had significant effects on the percentage of dead leaflet tissue (Appendix 13, Table A13.1). The intercept for the regression graph was nominally highest for susceptible LR-66-577 and lowest for Eston, while the

rate of increase in dead tissue (slope) was the same for all the genotypes (Appendix 13, Table A13.3). Susceptible LR-66-577 had a significantly higher percentage of dead leaflet tissue compared to both Eston and LR-66-637 at 96, 120 and 144 hpi, but not at 72 hpi. Eston and the resistant LR-66-637 had similar levels of dead leaflet tissue. CDC Robin did not differ significantly from LR-66-637 at any time points but compared to LR-66-577 it had significantly lower percentage of dead leaflet tissue at 96, 120 and 144 hpi, but not at 72 hpi (Appendix 13, Table A13.2).

4.5 Discussion

The objective of these experiments were to study the progression of *A. lentis*, *C. lentis* and S. *botryosum* infection on selected *L. ervoides* LR-66 RILs, parents and *L. culinaris* checks at the cellular level at different time points to test the hypothesis that resistance to Stemphylium blight and anthracnose is quantitative whereas for Ascochyta blight it is qualitative.

4.5.1 Ascochyta blight

The success of *A. lentis* in infecting lentil depends on the germination of conidia and how soon the pathogen develops further infectious structures such as appressoria, which are required for the penetration into the host cells by certain fungal species. In all genotypes, once the infection process was initiated, the sequence of morphological changes and their timing was similar, which is in agreement with the observation made by Roundhill et al. (1995). Conidia were considered germinated once a germ tube became visible irrespective of the length of the germ tube, similar to a recent study conducted by Sambasivam et al. (2017) but unlike in a study reported by Sari (2017) where conidia were considered germinated only when the length of a germ tube was equal to either the length or width of the conidium. Conidia normally produced one germ tube, but two germ tubes were also observed from a single conidium in many instances, similar to observations reported on detached lentil leaflets by Roundhill et al. (1995). Conidia tended to accumulate at the tip of the leaflet due to inoculum run-off on the leaflet surfaces which is one reason why detached leaflet assays were preferred by other scientists (Roundhill et al. 1995, Sambasivam et al. 2017). Conidial germination had started on all genotypes by 6 hpi, increased with time and by 48 hpi there were no significant differences among the genotypes, which was similar to previous reports (Roundhill et

al. 1995, Sambasivam et al. 2017, Sari et al. 2017). Up to 24 hpi, however, significantly higher conidial germination was observed on susceptible LR-66-570 compared to resistant LR-66-629 and the parents of LR-66, IG 72815 and L01-827A. Sambasivam et al. (2017) reported that the low virulent *A. lentis* isolate Kewell had significantly lower germination on resistant lentil genotype ILL 7537 (64%) compared to that on moderately resistant genotype ILL 5588 (76%) and susceptible ILL 6002 (76%) at 2 hpi. In the same study, no such differences were when the same genotypes were inoculated with the virulent *A. lentis* isolate AL4. In contrast to the current study, Sari et al. (2017) did not find significant differences in conidial germination of isolate AL-57 after inoculation of susceptible lentil Eston, and the partially resistant genotypes CDC Robin, 964a-46, ILL 1704 and ILL 7537, and highly resistant *L. ervoides* L01-827A, probably because of differences in virulence of isolates in the experiments considering that AL-57 is more virulent than AL-61 (S. Banniza personal communication). Lack of differences in conidial germination of highly virulent isolate AL-57 agrees with the observation made by Sambasivam et al. (2017) with highly virulent isolate AL4. Apart from isolates, differences in results from previous studies could be also due to different lentil genotypes, experimental conditions and inoculation protocols, in particular the use of attached or detached leaves for histopathology.

The lower conidial germination in LR-66-629, IG 72815 and L01-827A compared to LR-66-570, CDC Robin and Eston might be the result of leaflet surface cuticle composition or some pre-penetration resistance mechanism which hinder conidial germination. One of reasons for similar levels of conidial germination on Eston and CDC Robin could be that both are *L. culinaris* and are closely related, as Eston is one of the parents of CDC Robin (Vandenberg 2002)..

Appressoria formation was observed in all genotypes. Germ tubes ending in a globular structure or with a pad that was wider than the germ tube was considered an appressorium, but these were not melanised (Sambasivam et al. 2017, Dita et al. 2007). Appressoria became visible at 24 hpi on *L. ervoides*, therefore, data were collected from this time point, unlike in previous studies where data on appressoria were collected from 6 to 24 hpi (Roundhill et al. 1995, Sambasivam et al. 2017, Sari et al. 2017). Appressoria formation was not observed at every germ tube tip, confirming that *A. lentis* does not necessarily require appressoria for infection (Roundhill 1995) unlike some other fungal pathogens such as *C. lentis* (Armstrong-Cho et al. 2012). In the current study, only incubation time had a significant effect on appressoria formation whereas neither genotype nor

genotype by incubation time interaction had any effect. Lack of a significant genotype by incubation time interaction effect on appressoria formation was also observed by Sambasivam et al. (2017). At early time points, no significant difference in appressoria formation was apparent between susceptible Eston and susceptible LR-66-570, but it was significantly higher compared to all other genotypes (resistant or partially resistant) at 24 hpi which is similar to the observations reported by Sambasivam et al. (2017) where susceptible ILL 6002 had a significantly higher percentage of appressoria formation by highly virulent isolate AL4 and low virulent isolate Kewell compared to resistant ILL 7537 at 12 hpi.

Germ tube length was significantly affected by incubation time only, whereas genotype and genotype by incubation time interaction had no effect on germ tube length. This is different from Sambasivam et al. (2017) who, although not analyzed in detail, presented data indicating that highly virulent isolate AL4 and low virulent isolate Kewell both had shorter germ tubes on resistant lentil genotype ILL 7537 compared to susceptible ILL 6002 after in at 6 and 12 hpi. Similarly, Sari et al. (2017) reported that genotype, incubation time and genotype by incubation time interaction had a significant effect on germ tube length, and that germ tube length appeared to be shorter in genotypes CDC Robin, ILL 7537 and L01-827A with higher levels of partial resistance compared to susceptible Eston. In the current study germ tube lengths of up to 300 µm were recorded among genotypes, which is longer than previously reported because in the current study germ tube length was measured until 72 hpi compared to 2 to 48 hpi in other studies (Roundhill et al. 1995, Sambasivam et al. 2017, Sari et al. 2017). Nominally, the intercept of the regression graph was highest in susceptible LR-66-570 and lowest in resistant parent IG 72815, but this trend was not confirmed through means comparisons.

Genotype, incubation time and genotype by incubation time interactions had a significant effect on the percentage of dead tissue per leaflet. Percentage of dead tissue increased over the incubation time; these findings are similar to previous reports where crops plants are phenotypically rated for the disease over days, which is sometimes summarized as the area under the disease progression curve (Vandenberg et al. 2006). Interestingly, there were no significant differences between resistant LR-66-629 and the susceptible check Eston whereas there were significant differences between susceptible LR-66-570 and Eston which is contrary to disease severity reported previously (Table 4.1, Bhadauria et al. 2017). LR-66-629 and CDC Robin had the lowest increase in the

percentage of dead tissue over time which would be expected with resistant reaction (Table 4.1, Bhadauria et al. 2017).

Pycnidia formation by *A. lentis* isolate AL-61 was observed for the first time at 144 hpi on all genotypes, though they possibily started to develop after 96 hpi. Overall, genotype had a significant effect on pycnidia formation whereas incubation time and genotype by incubation time interaction did not. No significant difference among *L. ervoides* genotypes were observed among means, despite differences in Ascochyta blight severity reported previously by Bhadauria et al. (2017, Table 4.1). Eston, however, had a significantly higher number of pycnidia per leaflet compared to CDC Robin at 144 and 192 hpi but not at 240 hpi. There were no differences in the rate of pycnidia formation among all genotypes, although Ascochyta blight severity significantly varied among them (Bhadauria et al. 2017). Pycnidia formation of *A. lentis* here confirmed that the pathogen completes its life cycle on both *L. ervoides* and *L. culinaris*, whereas Sambasivam et al. (2017) were not able to observe any pycnidia formation 21 day post inoculation on the resistant lentil accessions Indianhead, ILL 5588, Nipper, and ILL 7537, all of which had an Ascochyta blight rating of 5 or less on a scale of 1 – 9. In contrast, pycnidia were detected on susceptible lentil accessions Digger and ILL 6002, both of which were rated as 7 or more for Ascochyta blight severity.

4.5.2 Anthracnose

Conidial germination of *C. lentis* race 0 isolate CT-30 was observed by 6 hpi on *L. ervoides* and *L. culinaris* genotypes (Table 4.2), but only in a few cases. Chongo et al. (2002) observed germinated conidia from 3-6 hpi for isolate JPPTNL 882 with moderate virulence and more virulent isolate 95S29 on susceptible *L. culinaris* cultivar Eston and partially resistant lentil line PI 320937. Similarly, commencing observations 12 hpi, Armstrong-Cho et al. (2012) reported germinated conidia for three race 0 and three race 1 isolates on susceptible Eston and CDC Robin with partial resistance to race 1 isolates. In the current study, appressoria formation was observed as early as 6 hpi in a few cases on *L. ervoides* and *L. culinaris* genotypes, but appressoria became more evident by 12 hpi, which is similar to the observations reported by Chongo et al. (2002) and Armstrong-Cho et al. (2012) on cultivated lentil. The proportion of conidial germination and proportion of appressoria formation per germinated conidia was not recorded in the current study because a significant number of conidia appeared to have been washed away from samples collected at 6 and

12 hpi during the clearing process for preparation of histopathology samples. Armstrong-Cho et al. (2012) reported that sometimes no appressoria were observed above the infection hyphae on cultivated lentil leaflets, probably because they too, had been washed off during sample preparation, and indeed, infection vesicles and primary hyphae disconnected from appressoria were observed here as well.

In the current study, infection vesicles (IVs)/primary hyphae (PHs) were first observed at 24 hpi in both *L. ervoides* and *L. culinaris* genotypes, but it is possible that they developed between 12 and 24 hpi considering that Armstrong-Cho et al. (2012) observed IVs in detached leaflets of Eston and CDC Robin at 20 hpi. Genotypes, incubation time and genotype by incubation time interaction had a significant effect on the IVs/PHs formation which is similar to analyses by Armstrong-Cho et al. (2012). There was a significant increase in the formation of IVs/PHs over time in all the genotypes from 24 to 48 hpi, which is similar to results of Armstrong-Cho et al. (2012) indicating that the number of IVs/PHs of all isolates and both races increased by more than 50% from 20 to 30 hpi on detached leaflets of CDC Robin and Eston. In the current study, IVs/PHs were absent in LR-66-528, but present in all other five genotypes at 24 hpi. This indicates that penetration of *C. lentis* isolate CT-30 may have been delayed in LR-66-528 compared to other genotypes, which is similar to observations in the *Arabidopsis thaliana-Colletotrichum higginsianum* host-pathogen system (Birker et al. 2009). In susceptible *A. thaliana* accession Ler-0, more than 50% of appressoria *C. higginsianum* initiated successful penetrations through the leaf surface to form primary hyphae compared to 10% of appressoria on resistant accessions Ws-0, Gifu-2, Can-0 and Kondara. The shapes of IVs/PHs of *C. lentis* in the current study varied from single lobed or unbranched to several lobes in both *L. ervoides* and *L. culinaris* species at 48 hpi and PHs were present in single host epidermal cell only, as previously reported by Armstrong-Cho et al. (2012). There were no significant differences between Eston and CDC Robin for IVs/PHs formation which is in agreement with histopathology studied conducted by Armstrong-Cho et al. (2012). How early *C. lentis* develops IVs/PHs in a genotype after inoculation is an important indicator of disease reaction as it determines the pace of formation of subsequent stages of infection by the pathogen in the genotypes and determining the latent period of the pathogen. Development of IVs and PHs correlate with anthracnose resistant and susceptible reactions reported by Bhadauria et al. (2017, Table 4.1) indicating that LR-66-528 is the most resistant of all genotypes included in the current experiment.

Genotype, incubation time and genotype by incubation time interaction had a significant effect on the percentage of dead tissue per leaflet. Dead tissue started appearing by 96 hpi which is in agreement with the previous study reported by Chongo et al. (2002) where lesions became visible on cultivated lentil between 72 to 144 hpi. Chongo et al. (2002) proposed that *C. lentis* had switched from the biotrophic to the necrotrophic phase by this time, which was later confirmed by Bhadauria et al. (2013) who determined that the biotrophy-necrotrophy switch occurred between 48-56 hpi and by 68 hpi *C. lentis* had initiated necrotrophic growth in Eston. The dead tissue observed is the result of the necrotrophic phase of *C. lentis* in *L. culinaris* and *L. ervoides* genotypes. Further molecular investigations can confirm this by monitoring signaling pathways in *L. ervoides* and *L. culinaris*. LR-66-524 had a significantly higher percentage of dead tissue compared to LR-66-528 which correlates with the anthracnose severity ratings recorded by Bhadauria et al. (2017, Table 4.1). Eston had a significantly higher percentage of dead tissue per leaflet than CDC Robin, despite similar anthracnose severity ratings for both after *C. lentis* race 0 inoculations (Bhadauria et al. 2017, Table 4.1). However, differences in the amount of necrotic tissue were also reported by Chongo et al. (2002) showing that partially resistant *L. culinaris* PI 320937 had fewer and smaller lesions compared to susceptible Eston against moderately virulent *C. lentis* isolate JPPTNL 882, but not against more virulent isolate 95S29. CDC Robin had the lowest rate of increases in the percentage of dead tissue whereas it was highest for IG 72815 followed by LR-66-528 indicating that they delayed anthracnose development at earlier time points, but that the disease then increased abruptly at later time points possibly because the pathogen managed to overcome defence mechanisms in these genotypes. In comparison, CDC Robin probably had fewer or less effective defense responses to the pathogen from the start, therefore, the pace of infection was probably constant.

Acervuli formation accompanied with setae development were observed in all genotypes at the later time points, which signify that the pathogen was able to complete its life cycle in *L. ervoides* and *L. culinaris*. Percentage of area covered by acervuli increased significantly over time among all genotypes, and susceptible RIL LR-66-524 had more leaflet area covered by acervuli when compared to more resistant RIL LR-66-528 (Table 4.1, Bhadauria et al. 2017). Eston had a significantly higher percentage of leaflet area covered by acervuli compared to CDC Robin at 144 hpi, despite the fact that anthracnose severity was similar for both when assessed in a previous experiment at 168 hpi (Bhadauria et al. 2017). Chongo et al. (2002)) found that lesion number,

lesion size and colonization efficiency of moderately virulent *C. lentis* isolate JPPTNL 882 were higher on susceptible cultivar Eston compared to partially resistant PI 320937 at 72 to 144 hpi, whereas there were no differences for highly virulent isolate 95S29. From the results, it is clear that no apparent phenotypic differences for percentage of leaflet area covered by acervuli can be seen up to 96 hpi, indicating that a resistance mechanism may be triggered after 96 hpi as a result of which there were quantitative differences in rate of growth of *C. lentis* in the genotypes at later time points. In some instances, it seemed as if dead cells had developed around appressoria in LR-528 at 120 hpi, but this was not always the case. The Intercept for regression graph for percentage of leaflet area covered by acervuli was nominally highest for L01-827A and lowest for Eston, whereas the rate of increase in area covered by acervuli (slope) was highest for Eston and lowest for L01-827A which indicates that L01-827A initially may have delayed the infection process but later on the pathogen overcame the host defence mechanism.

4.5.3 Stemphylium Blight

Conidial germination was initiated by 6 hpi in all genotypes (Table 4.2). Conidia were considered germinated regardless the length of the germ tube. Due to the polyspermic nature of the conidium, several germ tubes were observed emerging from it which is in agreement with previous reports (Borges et al. 1976, Cowling and Gilchrist 1982b, Mwakutuya and Banniza 2010). The proportion of conidial germination increased nominally up to 100% by 48 hpi on all genotypes. Similarly, Borges et al. (1976) reported 100% conidial germination by *S. botryosum* on alfalfa leaves of resistant and susceptible cultivars by 12 hpi, whereas Cowling and Gilchrist (1982b) found up to 100% conidial germination on three alfalfa clones varying from highly resistant to susceptible against three high to low virulence *S. botryosum* isolates in all nine combinations. Similar to the current findings, Mwakutuya and Banniza (2010) observed more than 80% conidial germination after 20 hpi at 25 or 30^{o}C on lentil cultivar CDC Milestone for *S. botryosum* isolates SB19 and SB9. Overall genotype had a significant effect on the proportion of conidial germination, but not all genotypes differed significantly among each other when their means were compared. LR-66-577 which had significantly higher disease severity compared to LR-66-637 for Stemphylium blight (Bhadauria et al. 2017) had similar proportions of conidial germination in the current study. This observation is in agreement with the previous study conducted by Cowling and Gilchrist (1982b) on *S. botryosum* in alfalfa where no significant differences were observed in conidial

germination between resistant clone M9, moderately susceptible clone M3 and susceptible clone S2 of alfalfa against a high, a moderate and low virulent isolate of *S. botryosum*. In contrast, Eston had significantly lower conidial germination than CDC Robin, which correlates with Stemphylium blight severity reported earlier indicating Eston to be more resistant to *S. botryosum* compared to CDC Robin (Podder et al. 2013, Bhaduaria et al. 2017). The rate of increase in conidial germination was lowest in IG 72815 and highest in LR-66-577 which agrees with the previous disease severity ratings (Table 4.1, Bhaduaria et al. 2017).

Germ tube penetrations were observed at 6 and 12 hpi in all genotypes. No appressorium formation was observed for isolate SB-19, which is in agreement with the observation made by Mwakutuya and Banniza (2010) for *S. botryosum* on CDC Milestone, but in contrast the *S. vesicarium* infection process where appressoria formation is required for penetrating onion leaves (Aveling and Snyman 1993). Regardless of number and length of germ tubes, the first germ tube that penetrated was considered as penetration from a single conidium. Germ tubes mostly penetrated through stomata, but sometimes also through the epidermal surface as observed by Mwakutuya and Banniza (2010) in attached leaf assays in the lentil-*S. botryosum* host-pathogen system, and by Borges et al. (1976) in detached leaf assays in the alfalfa-*S. botryosum* host-pathogen system. Cowling and Gilchrist (1982b) only observed one out of 2,129 penetrations through epidermal tissue in attached leaf assays for the alfalfa-*S. botryosum* host-pathogen system whereas all others were through stomata. In the current study, it was noticed that one small germ tube from a single conidium would penetrate after growing a shorter distance while other germ tubes from the same conidium would continue growing for longer distances without penetration despite passing over a few stomata, which indicates lack of preference for stomatal proximity for germ tube penetration which is similar to previous findings by Cowling and Gilchrist (1982b) on alfalfa and Mwakutuya and Banniza (2010) on lentil. Genotype had a significant effect on the proportion of germ tube penetrations which is contrary to the results reported by Cowling and Gilchrist (1982b) in case of the alfalfa-*S. botryosum* host-pathogen system where there were no significant differences among resistant or susceptible alfalfa clones. However, differences were also reported by Borges et al. (1976) showing that resistant alfalfa PI 315457 had significantly fewer penetrations compared to susceptible genotypes at 12, 24 and 36 hpi. The main difference is that Borges et al. (1976) conducted histopathology on detached alfalfa leaves whereas Cowling and Gilchrist (1982b) conducted histopathology on attached leaves as in the current experiment. There was no significant increase in the proportion of

germ tube penetrations from 6 to 12 hpi in any genotype here, whereas Borges et al. (1976), reported significant increase from 20% at 12 hpi to 24% at 24 hpi and 80% at 36 hpi in germ tube penetrations in resistant alfalfa genotypes whereas on susceptible genotypes germ tube penetrations of 67% at 12 hpi increased to 73.9% at 24 hpi and 85.7% at 36 hpi. There were no significant differences between susceptible LR-66-577 and resistant LR-66-637 for penetrations which is in agreement with Cowling and Gilchrist (1982b) where phenotypically resistant, moderate and susceptible alfalfa clones did not differ in penetration success of *S. botryosum*, but is in contrast to Borges et al. (1976) where resistant alfalfa genotypes had significantly fewer penetrations compared to susceptible genotypes. Similar penetration success of *S. botryosum* in LR-66-577 and LR-66-637 does not correlate with Stemphylium blight severity, in contrast to susceptible check CDC Robin where significantly higher penetrations compared all other genotypes were associated with high Stemphylium blight severity ratings (Table 4.1, Bhaduaria et al. 2017, Podder et al. 2013).

Germ tube length was measured at 12 and 24 hpi in all six genotypes for the longest germ tube per conidium. There was an overall increase in germ tube length over time which reached a maximum of approximately 500 µm by 24 hpi. Though genotypes had a significant effect on germ tube length, means comparison revealed no significant differences between LR-66-577 and LR-66-637 which is contrary to Stemphylium blight severity ratings on these genotypes. In contrast, CDC Robin had significantly longer germ tubes than Eston and also had higher Stemphylium blight severity in previous assessments(Table 4.1, Bhaduaria et al. 2017). Cowling and Gilchrist (1982b) also found what on attached leaves of alfalfa against *S. botryosum*. Interestingly, susceptible check CDC Robin and *L. ervoides* genotypes did not differ significantly in germ tube length despite their differences in Stemphylium blight severity (Table 4.1, Bhaduaria et al. 2017).

At 48 hpi infection on the host tissue had increased and some dead tissue had developed around the germinated conidia, probably due to phytotoxins released by *S. botryosum* (Barash et al. 1982). At 72 hpi necrotic tissue formation became more apparent, probably due to the toxins released by fungus, as suggested by Borges et al. (1976) and Cowling and Gilchrist (1982b) in alfalfa. Hyphae emanating from the leaflet surface and stomata, and conidiophores started developing at 72 hpi in all the genotypes on some leaflets of at least in one of the three biological replications. Conidiophores became more apparent and started sporulating at 120 and 144 hpi. Genotype and

incubation time had a significant effect on the percentage dead leaflet tissue. Percentage of dead leaflet tissue increased from 72 to 144 hpi for all genotypes. At 72 hpi, there were no significant differences among genotypes probably because in *L. ervoides* the defence mechanisms were not active at a level where phenotypic differences became visible. In contrast, from 96 to 144 hpi host response probably became more pronounced considering that susceptible RIL LR-66-577 had a significantly higher percentage of dead tissue compared with resistant RIL LR-66-637 and CDC Robin at 96, 120 and 144 hpi. Similarly, when compared with Eston, LR-66-577 had more dead tissue at all time points. Overall, the development of cell death correlated with the previous Stemphylium blight severity data (Table 4.1, Bhaduaria et al. 2017), except for CDC Robin and Eston that had similar levels of dead tissue throughout, but CDC Robin was previously identified as susceptible and Eston as resistant (Table 4.1, Bhaduaria et al. 2017, Podder et al. 2013).

In conclusion, it is clear from the results of histopathology study that resistance to Ascochyta blight, anthracnose and Stemphylium blight is quantitative for all traits in *L. ervoides*. For Ascochyta blight, differences among the most resistant and susceptible RILs in conidial germination during the early infection period of 6 to 24 hpi correlates with differences in disease severity rating reported by Bhadauria et al. (2017), but considering that conidial germination at 48 hpi was the same, it is not clear whether those early time points are of any significance or not, which can be only resolved by further (molecular) investigations. Pycnidia of *A. lentis* isolate AL-61 developed on all *L. ervoides* genotypes (IG 72815, L01-827A, LR-66-629 and LR-66-570) confirming that *A. lentis* can complete its life cycle on the host plants and the nature of resistance in *L. ervoides* against Ascochyta blight is not based on non-host resistance. Based on the anthracnose study, it is clear that the biotrophic and necrotrophic phases of *C. lentis* infection were present on all host genotypes. LR-66-528 has shown most resistance among all genotypes by delaying the infectious stages of *C. lentis* at 24 hpi which probably resulted in a reduced frequency of the *C. lentis*-infected leaflet tissue at later time points. Twenty four and 48 hpi may warrant further molecular investigation because of significant differences among the most resistant and susceptible RILs for infection vesicle/primary hyphae formation of *C. lentis* which correlates with differences in disease severity rating reported by Bhadauria et al. (2017). These results could be further supplemented with molecular investigations in order to have a more clear understanding of lentil-*C. lentis* host-pathogen system. For Stemphylium blight, it was shown that conidial germination and germ tube penetration, though necessary steps for successful infection, did not differentiate between resistant

and susceptible *L. ervoides* RILs. Similarly, germ tube length does not seem to affect germ tube penetration, but it may be worthwhile exploring what host cues trigger direct or stomatal penetration. Dead tissue started appearing around 48 hpi and become more apparent necrotic at 72 hpi to 144 hpi, one of which could be a potential time point for further toxicological and molecular investigation.

Chapter 5

General discussion

The objective of this research was to contribute to an overall effort to understand the disease resistance mechanisms in *L. ervoides* to multiple fungal diseases. In the current study, an attempt was made to understand the resistance mechanisms against the infection of *A. lentis*, *S. botryosum* and *C. lentis* on *L. ervoides* at the macroscopic and / or microscopic level. This research is important as lentil has become a major source of income for Saskatchewan farmers and a major contributor to the economy of the province, very significantly evident in 2016 when Saskatchewan had one of the highest seeded areas with 5.8 million hectares since the start of lentil cropping in the 1970's (Statistics Canada 2016). To a large part, this was made possible by crop management and breeding efforts selecting for agronomic and acclimatization traits of lentil cultivars that had occurred during the past three decades.

These breeding efforts, however, resulted in a narrowing of the genetic base of the crop as a result of which lentil became more prone to both biotic and abiotic stress. Fungal diseases are one such constant biotic impediment to the production of lentil and can result in reduced yield and seed quality. Ascochyta blight, anthracnose and Stemphylium blight are three major diseases of lentil which are threats to the current and future lentil production in Canada. To counter this, scientists have started exploring the possibility of using wild relative species of lentil as a resource of novel resistance genes. In previous studies, it was reported that wild relatives are a depository for disease resistance genes for Ascochyta blight, anthracnose and Stemphylium blight (Bayaa et al. 1994, Ahmad et al. 1997, Tullu et al. 2006; 2010, Podder et al. 2013, Dadu et al. 2017). Despite the identification of resistance to different disease in wild accessions of lentil, integrating novel disease resistance genes from these species into lentil cultivars has been challenging. One of the reasons is the nature of the pathogens in lentils, including necrotrophs and hemi-biotrophs for which little research has been reported in very few crop species, whereas the majority of the research conducted so far in understanding host-pathogen interaction pathways are in biotrophic pathogens in model plant species such as *Arabidopsis thaliana* and *Medicago truncatula*.

Compared to biotrophs, research conducted for necrotrophs is still in its infancy and when it comes to lentil, it has just started. In many recent studies, scientists tried to apply previous findings in the biotrophic host-pathogen studies to observations on necrotrophic host-pathogens systems, and in some cases found potential overlap in resistance mechanisms.

Non-host resistance is another mechanism that has become increasingly of interest as in this, the pathogen is not able to complete its life cycle on the host which could offer durable resistance for crops, unlike host resistance. Based on the previous study by Sari (2014), it was hypothesized that *L. ervoides* had non-host resistance against *A. lentis*, therefore all *L. ervoides* accessions available at the CDC were screened against the standard *A. lentis* isolate AL-61 to test this hypothesis. AL-61 has moderate levels of virulence and differentiates between susceptible and partially resistant *L. culinaris* germplasm (Banniza, personal communication). Three different experiments were conducted, one under greenhouse and two under field conditions. Out of 166 *L. ervoides* accessions available at the CDC, 157 were identified phenotypically as *L. ervoides* and were multiplied from single plant per accessions for two generations to have enough seed for both field and greenhouse screening. Disease severity data were available only from greenhouse screening revealing a wider range of Ascochyta blight severity compared to *L. culinaris* checks, probably due to high genetic diversity among *L. ervoides accessions* and low diversity between the closely related *L. culinaris* checks Eston and CDC Robin (Vandenberg et al. 2002). Results from the pot experiment under field conditions showed that *A. lentis* was able to infect and complete its life cycle on all *L. ervoides* accessions, therefore, the null hypothesis that the nature of resistance in *L. ervoides* against Ascochyta blight is based on non-host resistance is rejected.

Exploiting non-host resistance was accomplished in case of lettuce, where it was transferred from *Lactuca saligna* (a wild relative of lettuce) to cultivated *Lactuca sativa* (Zhang et al., 2009). A total of 15 QTLs for non-host resistance to the downy mildew causing biotrophic pathogen *Bremia lactucae* were identified on the genetic map of a backcross inbred lines population. Examples for transfer of non-host resistance to necrotrophic pathogens are still lacking, so it remains to be seen whether transferable non-host resistance can work in case of necrotrophs, and the first step in doing so is to identify a source of non-host resistance in the crop species or a wild relative of interest. Recently Armstrong-Cho (2015) observed small dark flecks with limited expansion in chickpea leaflets of *C. oxyodon* (accession PI 561103) and *C. anatolicum* (accession PI 383626) infected

with *Ascochyta rabiei*, which authors predicted could be type II non-host resistance. Though having identified potential non-host Ascochyta blight resistance in perennial chickpea species, interspecific hybridization barriers are hurdle in introgression of this trait into cultivated chickpea.

The majority of accessions had partial resistance to Ascochyta blight probably because *L. ervoides* originated in damp and shady habitats in the middle-east that are conducive to disease development, which could have resulted in the evolution of higher resistance in *L. ervoides* compared to other *Lens* species (Bayaa et al. 1994). There is a possibility that *L. ervoides* accessions may have novel defence genes against *A. lentis* which could be highly valuable in the absence of non-host resistance in nature. Apart from resistance genes, hosts also possess susceptibility genes which help the pathogen in establishing on the host (Van Schie and Takken, 2014). If the current model is governed by susceptibility genes, then the highly resistant (HR) accessions will have fewer susceptibility genes or alleles as a result of loss of function mutation of some susceptibility genes (Eckardt 2002). *A. lentis* is still able to complete its life cycle but its development in general is restricted to that on highly susceptible (HS) accessions where the susceptibility genes are fully functional conferring higher susceptibility. One of the best studied example of a susceptible gene in agriculture is the MLO gene, which is a recessive mutant that confers resistant against all the races of the powdery mildew causing fungus *Erysiphe graminis* f. sp. *hordei* in barley (Freisleben et al. 1942, Jorgensen 1992, Van Schie and Takken 2014).

In order to understand the genetic control and mechanisms of resistance in lentil against multiple fungal pathogens, scientist have tried to develop interspecific populations using embryo rescue technique, but these populations were often subject of genetic distortion with each advancing generation, which resulted in biased results in genetic studies (Cohen et al. 1984, Ahmad et al. 1995, Fratini and Ruiz 2006, Fiala et al. 2009, Tullu et al. 2013, Saha et al. 2015). To avoid these problems, the intraspecific *L. ervoides* recombinant inbred line (RIL) population LR-66 was developed from a cross between *L. ervoides* accessions IG 72815 and L01-827A. LR-66 is of interest because IG 72815 is resistant to anthracnose and Stemphylium blight whereas L01-827A is susceptible to both but both parents are resistant to Ascochyta blight (Bhadauria et al. 2017). As a result, Bhadauria et al. (2017) was able to map disease resistance QTLs for anthracnose and Stemphylium blight, but not for Ascochyta blight on the genetic map of LR-66. Disease progression was studied through histopathology studies of Ascochyta blight, anthracnose and Stemphylium

blight on selected *L. ervoides* LR-66 RILs, parents and *L. culinaris* checks to understand the host responses against the causal pathogens to test the hypothesis that resistance to Stemphylium blight and anthracnose is quantitative whereas for Ascochyta blight is qualitative.

A. lentis isolate AL-61 was used to inoculate selected LR-66 RILs, parents and *L. culinaris* checks and fungal development was studied from 6 to 240 hpi. Conidial germination was recorded from 6 to 48 hpi. Genotype, incubation time and genotype by incubation time interaction had significant effects on proportional conidial germination. On susceptible RIL LR-66-570, *A. lentis* had significantly higher conidial germination compared to LR 66-629, IG 72815 and L01-827A from 6 to 24 hpi but was non-significant at 48 hpi. This trait might be important for further molecular investigations as it was previously reported that leaf surfaces could have a significant effect on the attachments of pathogen spores and their germination. For example, in maize, corn mutant *glossy 11* has an altered leaf cuticle structure in the form of decreased very-long-chain aldehyde levels (Hansjakob et al. 2011). These changes in the cuticle structure resulted in reduced germination of powdery mildew causing *Blumeria graminis* spores. In a recent study, gene silencing of a susceptibility gene an *Arabidopsis* orthologue *DND1* in potato and tomato showed that in well-silenced plants conidial germination of a necrotrophic pathogenic fungus *Botrytis cinerea* was reduced compared to the non-silenced checks. However, it was not clear in this case whether reduced conidial germination was the result of changes in the cuticle, but this possibility could not be ruled out (Sun et al. 2017). Similarly in *Arabidopsis*, cuticle mutants such as *Att1*, *Bdg*, *Bre1*/*Lacs2*/*Sma4*, *Lcr*, *Rwa2 Fdh*, which have resulted from loss of function mutation of enzymes, such as long-chain acyl CoA synthetase, fatty acid hydroxylase, fatty acid oxidase, have more permeable host cuticles resulting in enhanced perception of *B. cinerea* elicitors by the host resulting in more pronounced defence (Chassot et al. 2008, Van Schie and Takken 2014). Therefore, it is a possibility that either LR-66-629, IG 72815 and L01-827A have different chemical structures of their cuticle or they may be more permeable compared to LR-66-570 resulting in increased perception of pathogen elicitors and earlier activation of defence responses. *L. ervoides* genotypes did not differ significantly in appressoria formation, germ tube length and number of pycnidia in the current study, though LR-66-570 had a significantly higher percentage of dead tissue than LR 66-629 at 192 and 240 hpi. Presence of pycnidia in all genotypes proves that pathogen was able to complete its life cycle on the *L. ervoides* genotypes, further confirming that non-host resistance is not underlying resistance in this species.

The infection process of *C. lentis* isolate CT-30 (race 0) was studied on LR-66 RILs, parents and *L. culinaris* checks. Susceptible LR-66-524 had a significantly higher number of infection vesicles/primary hyphae than resistant LR-66-528 at 24 and 48 hpi. In fact, there were no infection vesicles/primary hyphae observed on LR-66-528 at 24 hpi whereas they were present in all other genotypes. These findings are in agreement with Birker et al. (2009) in case of the *A. thaliana–C. higginsianum* host-pathogen system, where more than 50% of appressoria successfully initiated penetrations, through penetration pegs, of the leaf surface to form primary hyphae in susceptible *A. thaliana* accession *Ler-0* compared to resistant *A. thaliana* accessions *Ws-0, Gifu-2, Can-0* and *Kondara* where penetration was 10% only. The authors identified a common *C. higginsianum* resistance locus in *MRC-J* locus in *Ws-0, Gifu-2, Can-0* and *Kondara* which was part of a major recognition gene complex MRC-J on chromosome 5 containing *RPS4* and *RRS1* genes (Toll-interleukin-1 receptor/nucleotide-binding site/leucine-rich repeat, TIR-NB-LRR) (Narusaka et al., 2009, Birker et al. 2009). When testing loss of function mutation of Ws-0 RPS4 (rps4-21), a higher penetration success and an increase in disease severity during the necrotic phase of infection was noted (Birker et al. 2009). In another study, Uppalapati et al. (2012) studied the infection process of *C. trifolii* on susceptibility gene mutant *irg1* of *M. truncatula* in which no abaxial epicuticular wax crystals were present thus had a reduced surface hydrophobicity. The *irg1* mutant was partially resistant to *C. trifolii* and had lower conidial germination, the formation of preinfection structures (appressoria) and disease symptoms compared to susceptible wild-type R108. Based on the example cited above, it is possible that LR-66-528 may have a combination of different cuticle structure and an earlier perception of pathogens effectors that could delay penetration and the formation of infection structures (IV/PH) compared to other genotypes in the experiment, hence a significantly higher percentage of dead tissue at 96 to 144 hpi and a significantly higher percentage of leaflet area covered by acervuli in susceptible LR-66-524 compared to resistant LR-66-528.

Stemphylium botryosum is a necrotrophic pathogen which has a broad host range. The pathogen produces non-host specific toxins such as stemphyloxin I, which is an Enolic β-Ketoaldehyde that can be extracted from *S. botryosum* culture. When was used in bioassays it induced necrotic symptoms in *Lycopersicon esculentum, Solanum tuberosum, S. melongena, Nicotiana tabacum, Capsicum annuum, M. sativa, L. sativa* at 250 ug/ml (Barash et al. 1982). In the current study, the infection process of *S. botryosum* isolate SB-19 was investigated from 6 to 144 hpi and susceptible LR-66-577 had a significantly higher percentage of dead leaflet tissue compared to resistant LR-

66-637 at 96 to 144 hpi. These differences could be due to phytotoxins produced by *S. botryosum* in the host plants. In a previous study, a *S. botryosum* toxin bioassay was conducted on susceptible *M. sativa* SW44 and resistant *M. cancellata* PI 315457, revealing that toxin application on the resistant and susceptible genotypes resulted in disease symptoms in the susceptible genotype but not in the resistant, similar to their response to infection (r = 0.73) (Borges et al. 1976). On the basis of these results, Borges et al. (1976) hypothesized that toxins present in the culture filtrate could be host specific, which suggests that a toxin model could also apply to the *S. botryosum*-lentil host-pathogen system. However, there are a few points that need clarification: 1) To date there is no clear race structure for this pathogen, though differences in virulence of isolates have been reported earlier (Cowling and Gilchrist 1982). 2) Characterisation of phytotoxins is incomplete, although some efforts have been made in the past. 3) Testing of a toxin model requires identification of a set of lentil differentials that interact with specific pathogen toxins. Current findings indicate that host resistance responses were visible only post-penetration, which requires more in-depth molecular and toxin assay investigations.

It is clear from the results of the histopathology study that resistance to Ascochyta blight, anthracnose, and Stemphylium blight are quantitative, and not qualitative as there were no discrete differences hence the hypothesis that resistance to Stemphylium blight and anthracnose is quantitative is accepted whereas the hypothesis of qualitative resistance against Ascochyta blight it is rejected. For Ascochyta blight, differences among the most resistant and susceptible RILs in conidial germination during the early infection period of 6 to 24 hpi correlates with differences in disease severity rating reported by Bhadauria et al. (2017), but considering that conidial germination at 48 hpi was the same, it is not clear whether those early time points are of any significance, so this needs further molecular investigations. Pycnidia of *A. lentis* developed on the *L. ervoides* parents and the two LR66-RILs confirming that *A. lentis* can complete its life cycle and the nature of resistance in *L. ervoides* against Ascochyta blight is not based on non-host resistance. A more complex resistance mechanism is probably involved which is acting at both the pre- and post-pathogen penetration phases into the host. For anthracnose, 24 and 48 hpi may warrant further molecular investigation because of significant differences among the most resistant and susceptible RILs for the formation of infection vesicles / primary hyphae which correlates with differences in disease severity rating reported by Bhadauria et al. (2017). In case of Stemphylium blight only post-pentration time points could be of interests for further molecular investigations.

Findings of this research study will contribute to future efforts of understanding the complex resistance mechanisms of *Lens* species to multiple fungal diseases. Based on the results of *L. ervoides* screening, it is possible now to select a susceptible parent for developing a bi-parental intra-specific *L. ervoides* RIL population that segregates for Ascochyta blight resistance to conduct QTL mapping, which was not possible on the LR-66 population. In the greenhouse experiment, 12 HR *L. ervoides* accessions were identified that could be used for testing allelsim for Ascochyta blight resistance genes. Results of the histopathology study could not give a clear picture of critical time points where the development of the three pathogens in resistant and susceptible genotypes diverged. Therefore, for future research, histopathology results should be supplemented with an assessment of fungal biomass through quantitative PCR in order to identify those time points most relevant for further transcriptomics studies, which would allow for host resistance genes expressed during those critical stages of infection by all three pathogens to be studied.

References

Abbo S, Ladizinsky G (1991). Anatomical aspects of hybrid embryo abortion in the genus *Lens* L. Botanical Gazette. 152: 316-320.

Abbo S, Ladizinsky G (1994). Genetical aspects of hybrid embryo abortion in the genus *Lens* L. Heredity. 72: 193-200.

Ahmad M, Russell AC, McNeil DL (1997). Identification and genetic characterization of different resistance sources to Ascochyta blight within the genus *Lens*. Euphytica. 97: 311-315.

Ahmad M, Fautrier AG, McNeil DL, Burritt DJ, Hill GD (1995). Attempts to overcome post fertilization barrier in interspecific crosses of the genus *Lens*. Plant Breeding. 114: 558–560.

Alo F, Furman BJ, Akhunov E, Dvorak J, Gepts P (2011). Leveraging genomic resources of model species for the assessment of diversity and phylogeny in wild and domesticated lentil. The Journal of. Heredity. 102: 315-329.

Andrahennadi CP (1994). Genetics and linkage of isozyme markers and resistance to seed borne Ascochyta infection in lentil. MSc Thesis, Univ. of Saskatchewan, Saskatoon, Canada.

Andrahennadi CP (1997). RAPD markers for Ascochyta resistance, phylogenetic studies, and cultivar identification in lentil. Ph.D. Thesis. University of Saskatchewan, Saskatoon

Armstrong-Cho CL, Banniza S (2006). *Glomerella truncate* sp. nov., the teleomorph of *Colletotrichum truncatum*. Mycological Research. 110: 951-956.

Armstrong-Cho CL, Wang J, Wei Y, Banniza S (2012). The infection process of two pathogenic races of *Colletotrichum truncatum* on lentil. Canadian Journal of Plant Pathology. 34: 58-67.

Arumuganathan K, Earle ED (1991). Nuclear DNA content of some important plant species. Plant Molecular Biology Reporter. 9: 208-218.

Aveling TAS, Snyman HG (1993). Infection studies of *Stemphylium vesicarium* on onion leaves. Mycological Research. 97: 984-988.

Bakr MA, Zahid MA (1987). Stemphylium blight: a new foliar disease of lentil in Bangladesh. Bangladesh Journal of Plant Pathology. 2: 69-70.

Barash I, Pupkin G, Netzer D, Kashman Y (1982). A Novel Enolic β-Ketoaldehyde Phytotoxin Produced by *Stemphylium botryosum* f. sp. *lycopersici*. The Plant Cell. 24: 353-370.

Barulina H (1930). Lentils of the USSR and other countries. Supplement to the Bulletien of Applied Botony, Genetics and Plant Breeding Leningrad. 40: 265-30 (English summary).

Bayaa B, Erskine W (1991). Legume program - annual report. ICARDA, Aleppo, Syria.

Bayaa B, Erskine W, Hamdi A (1994). Response of wild lentil to *Ascochyta fabae* f sp *lentis* from Syria. Genetic Resources and Crop Evolution. 41: 61-65.

Bayaa B, Erskine W, Hamdi A (1995). Evaluation of a wild lentil collection for resistance to vascular wilt. Genetic Resources and Crop Evolution. 42: 231-235.

Bernier CC, Buchwaldt L, Morrall RAA (1992). Screening for resistance to anthracnose in lentil (*Lens culinaris*). In: AEP (ed) Proc 1st European Conference on Grain Legumes, June 1-3, Recevil de Communications, Angers, France, pp 37-38, Poster

Bett K, Ramsay L, Sharpe A, Cook D, Penmetsa R V, Verma N, et al. (2014). "Lentil genome sequencing: establishing a comprehensive platform for molecular breeding," in Proceedings of International Food Legumes Research Conference (IFLRC-VI) and ICCLG-VII (Saskatoon, SK: Crop Development Center).

Bett K, Ramsay L, Chan C, Sharpe AG, Cook DR, R. Penmetsa V, Chang P, Coyne C, McGee R, Main D, Edwards D, Kaur S, Vandenberg A (2016). Lentil 1.0 and Beyond. Plant and Animal Genomics conference XXIV, January 8-15. https: //pag. confex.com/ pag/xxiv/webprogram/ Paper19117.html.

Bhadauria V, Bett KE, Zhou T, Vandenberg A, Wei Y, Banniza S (2013). Identification of *Lens culinaris* defense genes responsive to the anthracnose pathogen *Colletotrichum truncatum*. BMC Genetics. 14: 31.

Bhadauria V, Ramsay L, Bett KE, Banniza S (2017). QTL mapping reveals genetic determinants of fungal disease resistance in the wild lentil species *Lens ervoides*. Scientific Reports. 7: 3231.

Birker D, Heidrich K, Takahara H, Narusaka M, Deslandes L, Narusaka Y, Reymond M, Parker J E, O'Connell R (2009). A locus conferring resistance to *Colletotrichum higginsianum* is shared by four geographically distinct *Arabidopsis* accessions. The Plant Journal. 60: 602-613.

Booth C, Pirozynski KA (1967). *Pleospora herbarum*. Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria No. 150. Surrey, England.

Borges OL, Stanford EH, Webster RK (1976) Pathogenicity of different isolates of *Stemphylium botryosum* on alfalfa differentials. Phytopathology. 66: 715-716.

Buchwaldt L, Vandenberg A, Tullu A, Bernier CC (2001). Genetics of resistance to anthracnose (*Colletotrichum truncatum*) in lentil. In: AEP (ed) Proc 4th European Conference on Grain Legume Research, Cracow, Poland, pp 242, Poster

Buchwaldt L, Anderson KL, Morrall RAA, Bernier CC, Gossen BD (2004). Identification of lentil germplasm resistant to *Colletotrichum truncatum* and characterization of two pathogen races. Phytopathology. 94: 236-243.

Buchwaldt L, Shaikh R, Adam J, Tullu A, Slinkard A (2013). Recessive and dominant genes confer resistance to *Colletotrichum truncatum* in cultivated lentil. Canadian Journal of Plant Pathology. 35: 222-231.

Caudillo-Ruiz KB, Bhadauria V, Banniza S (2017). Aetiology of Stemphylium blight on lentil in Canada. Canadian Journal of Plant Pathology. 39, 4: 422-432.

Chassot C, Nawrath C, Metraux JP (2008). The cuticle: not only a barrier for plant defence: a novel defence syndrome in plants with cuticular defects. Plant signaling & behavior. 3: 142-44.

Chongo G, Bernier CC (1999). Field and growth chamber evaluation of components of partial resistance to *Colletotrichum truncatum* in lentil. Canadian Journal of Plant Pathology. 21: 58-63.

Chongo G, Gossen BD, Bernier CC (2002). Infection by *Colletotrichum truncatum* in resistant and susceptible lentil genotypes. Canadian Journal of Plant Pathology. 24: 81-85.

Chowdhury AM, Ahmed A, Zaman M (1997). Studies on the defence structural factors of some susceptible and resistant varieties of lentil plants. Journal of Mycopathological Research. 35: 35-39.

Chowdhury MA, Andrahennadi CP, Slinkard AE, Vandenberg A (2001). RAPD and SCAR markers for resistance to Ascochyta blight in lentil. Euphytica. 118: 331-337.

Cohen D, Ladizinsky G, Ziv M, Muehlbauer FJ (1984). Rescue of interspecific *Lens* hybrids by means of embryo culture. Plant Cell, Tissue and Organ Culture. 3: 343-347.

Cowling WA, Gilchrist DG (1982b). Expression of pathogen virulence and host resistance during infection of alfalfa with *Stemphylium botryosum*. Phytopathology. 72: 63-42.

Cubero J I, Pérez de la Vega M, Fratini R (2009). Origin, phylogeny, domestication and spread. In: Erskine W, Muehlbauer F, Sarker A and Sharma B (eds) The lentil: botany, production and uses. CABI, Wallingford

Dadu RHR, Ford R, Sambasivam P, Gupta D (2017). A Novel *Lens orientalis* Resistance Source to the Recently Evolved Highly Aggressive Australian *Ascochyta lentis* Isolates. Frontiers in Plant Science. 8: 1038.

Dita MA, Brommonschenkel SH, Matsuoka K, Mizubuti ESG. (2007). Histopathological study of the *Alternaria solani* infection process in potato cultivars with different levels of early blight resistance. Journal of Phytopathology.155: 462-469.

Duran Y, Fratini R, Garcia P, Pérez de la Vega, M (2004).An intersubspecific genetic map of *Lens*. Theoretical and Applied Genetics. 108: 1265-1273.

Eckardt NA. 2002. Plant disease susceptibility genes? Plant Cell. 14: 1983-86.

Eujayl I, Baum M, Powell W, Erskine W, Pehu E (1998a). A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theoretical and Applied Genetics. 97: 83-89.

Ferguson ME, Maxted N, Van Slageren M, Robertson LD (2000). A reassessment of the taxonomy of *Lens* Miller (*Leguminosae, Papilionoideae, Vicieae*). Botanical Journal of the Linnean Society. 133: 41-59.

Fiala JV, Tullu A, Banniza S, Séguin-Swartz G, Vandenberg A (2009). Interspecies transfer of resistance to anthracnose in lentil (*Lens culinaris* Medik.). Crop Science. 49: 825-830.

Food and Agriculture Organization of the United Nations (2014). FAOSTAT. http://faostat3.fao.org/home/E.

Ford R, Pang ECK, Taylor P (1999). Genetics of resistance to Ascochyta blight of lentil and the identification of closely linked RAPD markers. Theoretical and Applied Genetics. 98: 93-98.

Fratini R, Ruiz ML (2006). Interspecific hybridization in the genus *Lens* applying in vitro embryo rescue. Euphytica. 150: 271-280.

Fratini R, Ruiz ML (2008). Micropropagation of intra and interspecific *Lens* hybrids. Euphytica. 159: 199-206.

Fratini R, Ruiz ML and Pérez de la Vega M (2004). Intraspecific and inter-subspecific crossing in lentil (*Lens culinaris* Medik.). Canadian Journal of Plant Pathology. 84: 981-986.

Freisleben R, Lein A (1942). U ber die Auffindung einer Mehltauresistenten Mutante nachR¨ ontgenbestrahlung einer Anfalligen Reinen Linie von Sommergerste. *Naturwissenschaften* 30: 608.

Gibson RJ, Bernier CC, Morrall RAA (1991). Anthracnose of lentil in Manitoba in 1990. Canadian Plant Disease Survey. 71: 104.

Gossen BD, Morrall RAA (1983). Effect of Ascochyta blight on seed yield and quality of lentils. Canadian Journal of Plant Pathology. 5: 168-173.

Gujaria-Verma N, Vail SL, Carrasquilla-Garcia N, Penmetsa RV, Cook DR, Farmer AD, Vandenberg A, Bett KE (2014). Genetic mapping of legume orthologs reveals high conservation of synteny between lentil species and the sequenced genomes of *Medicago* and chickpea. Frontiers in Plant Science. 5: 676.

Gupta D (2003). Genetic and cytogenetic studies in populations derived from crosses between wild Lens species and cultivated lentils. PhD Thesis, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.

Gupta D, Sharma SK (2007). Widening the gene pool of cultivated lentils through introgression of alien chromatin from wild *Lens* subspecies. Plant Breeding. 126: 58—61.

Gupta D, Ford R, Taylor PWJ (2011). *Lens* Chittaranjan Kole (ed) Wild Crop Relatives: Genomic and Breeding Resources Legume Crops and Forages. pp 127-140.

Gupta D, Taylor PWJ, Inder P, Phan HTT, Ellwood SR, Mathur PN, Sarker A, Ford R (2012). Integration of EST-SSR markers of *Medicago truncatula* into intraspecific linkage map of lentil and identification of QTL conferring resistance to Ascochyta Blight at seedling and pod stages. Molecular Breeding. 30: 429-439.

Hamdi A, Erskine W (1994). Germplasm program legumes annual report. ICARDA Aleppo, Syria.

Hamwieh A, Udapa SM, Choumane W, Sarker A, Dreyer F, Jung C, Baum M (2005). A genetic linkage map of lentil based on microsatellite and AFLP markers and localization of fusarium vascular wilt resistance. Theoretical and Applied Genetics. 110: 669-677.

Hamwieh A, Udupa S M, Sarker A, Jung C, Baum M (2009). Development of new microsatellite markers and their application in the analysis of genetic diversity in lentils. Breeding Science. 59: 77-86.

Hansjakob A, Riederer M, Hildebrandt U (2011). Wax matters: absence of very-long-chain aldehydes from the leaf cuticular wax of the *glossy11* mutant of maize compromises the prepenetration processes of *Blumeria graminis*. Plant Pathology. 60: 1151-61.

Harlan JR and de Wet JMJ (1971). Toward rational classification of cultivated plants. Taxon. 20: 509-519.

Hashemi P A, Vandenberg, S Banniza (2005a). Stemphylium blight a potential limiting factor to the production of lentil in Saskatchewan. In: Proceedings of Pulse Days. January 10-11, 2005. Saskatoon, SK, Canada.

Havey MJ, Muehlbauer FJ (1989). Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theoretical and Applied Genetics. 77: 395-401.

Jorgensen JH (1992). Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica. 63: 141-52.

Kahraman A, Kusmenoglu I, Aydin N, Aydogan A, Erskine W, Muehlbauer FJ (2004b). QTL mapping of winter hardiness genes in lentil. Crop Science. 44: 13-22.

Kaiser WJ (1997). Inter- and international spread of Ascochyta pathogens, chickpeas, faba bean and lentils. Canadian Journal of Plant Pathology.19: 215-224.

Kant P, Materne M, Rodda MS, Slater AT (2017). Screening lentil germplasm for Stemphylium blight resistance. Australasian Plant Pathology. 46: 129-136.

Kaur S, Cogan NO, Pembleton LW, Shinozuka M, Savin KW, Materne M and Forster JW (2011). Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics. 12: 265.

Koike S T, Henderson DM, Butler EE (2001) Leaf spot disease of spinach in California caused by *Stemphylium botryosum*. Plant Disease. 85: 126-130.

Kumar P (2007). Genetics of resistance to Stemphylium leaf blight of lentil (*lens culinaris*). in the cross barimasur-4 × CDC milestone. M.Sc. Thesis, Department of Plant Sciences, Univ. of Saskatchewan, Saskatoon, Canada.

Ladizinsky G (1979). The origin of lentil and its wild gene pool. Euphytica. 28: 179-187.

Ladizinsky G (1993a). Wild lentils. Critical Reviews in Plant Sciences. 12: 169-184.

Ladizinsky G (1997). A new species of *Lens* from southeast Turkey. Botanical Journal of the Linnean Society. 123: 257-260.

Ladizinsky G, Abbo S (2015). The *lens* genus. The Search for Wild Relatives of Cool Season Legumes. Springer International Publishing Switzerland pp 1-28.

Ladizinsky G, Braun D, Goshen D, Muehlbauer FJ (1984). The biological species of the genus *Lens*. Botanical Gazette. 145: 253-261.

Ladizinsky G, Cohen D, Muehlbauer F J (1985). Hybridization in the genus *Lens* by means of embryo culture. Theoretical and Applied Genetics. 70: 97-101.

Lee S, Whitaker VM, Hutton SF (2016). Mini Review: Potential Applications of Non-host Resistance for Crop Improvement. Frontiers in Plant Science. 7: 997.

Morrall RAA (1988). A new disease of lentil induced by *Colletotrichum truncatum* in Manitoba. Plant Disease. 72: 994.

Morrall RAA, Sheppard JW (1981). Ascochyta blight of lentils in Western Canada: 1978 to 1980. Canadian Plant Disease Survey. 61: 7-13.

Morrall RAA, Pedersen EA (1991). Discovery of anthracnose in Saskatchewan in 1990. Canadian Plant Disease Survey. 71:105-106.

Muehlbauer FJ, Slinkard AE (1981). Genetics and breeding methodology. In: Lentils. (Eds.).: C. Webb & G. Hawtin, Commonwealth Agricultural Bureaux: 69-90.

Muehlbauer FJ, Weeden NF, Hoffman D L (1989). Inheritance and linkage relationships of morphological and isozyme loci in lentil (*Lens* Miller). The Journal of Heredity. 80: 298-303.

Muehlbauer FJ, Kaiser WJ, Clement SL, Summerfield R J (1995). Production and breeding of lentil. Advances in Agronomy. 54: 283-332.

Mwakutuya E, Banniza S (2010). Influence of temperature and wetness periods on the development of Stemphylium blight on lentil. Plant Disease. 94: 1219-1224.

Mysore KS and Ryu C (2004). Nonhost resistance: how much do we know? Trends in Plant Science. 9,2: 1360-1385.

Narusaka M, Shirasu K, Noutoshi Y, Kubo Y, Shiraishi T, Iwabuchi M, Narusaka, Y (2009). RRS1 and RPS4 provide a dual resistance-gene system against fungal and bacterial pathogens. The Plant Journal. 60,2: 218 -226.

Nasir M (1998). Improvement of drought and disease resistance in lentils in Nepal, Pakistan and Australia. Mid-Term Report 1995-1998. ACIAR Project PN-9436.

Parh DK (1998). Interspecific mapping of the Ascochyta gene(s) of lentil (*Lens culinaris* Medikus) using RAPD technology. MS Thesis, Lincoln University, Canterbury, New Zealand.

Phan HTT, Ellwood SR, Hane JK, Ford R, Materne M, Oliver R P (2007). Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. *culinaris*. Theoretical and Applied Genetics.114: 549-558.

Podder R (2012). The Potential for Breeding for Stemphylium Blight Resistance in the Genus Lens. M.Sc. Thesis, Department of Plant Sciences, Univ. of Saskatchewan, Saskatoon, Canada

Podder R, Banniza S, Vandenberg A (2013). Screening of wild and cultivated lentil germplasm for resistance to *Stemphylium blight*. Plant Genetic Resources.11,1: 26-35.

Richard MF, Marcelino PV, MaLuisa RS (2014). Lentil. Mohar Singh et al. (eds.), Broadening the Genetic Base of Grain Legumes. Springer publications pp 115-148

Rokaibah AA (1996). Leaf blight, a new bacterial disease of alfalfa associated with Stemphylium leaf spot. Alexandria Journal of Agricultural Sciences. 27: 47-55.

Roundhill SJ, Fineran BA, Cole AL, Ingerfeld M (1995). Structural aspects of lentil Ascochyta blight. Canadian Journal of Botany. 497: 485-497.

Rubeena A, Ford R, Taylor PWJ (2003). Construction of an intraspecific linkage map of lentil (*Lens culinaris* ssp. *culinaris*). Theoretical and Applied Genetics. 107: 910-916.

Rubeena A, Taylor PWJ, Ades PK, Ford R (2006). QTL mapping of resistance in lentil (*Lens culinaris* ssp. *culinaris*) to Ascochyta blight (*Ascochyta lentis*). Plant Breeding. 125: 506-512.

Saha GC, Sarker A, Chen W, Vandemark GJ, Muehlbauer FJ (2010). Inheritance and linkage map positions of genes conferring resistance to Stemphylium blight in lentil. Crop Science. 50: 1831-1839.

Saha S, Tullu A, Yuan HY, Lulsdorf MM, Vandenberg A (2015). Improvement of embryo rescue technique using 4-chloroindole-3 acetic acid in combination with in vivo grafting to overcome barriers in lentil interspecific crosses. Plant Cell, Tissue and Organ Culture. 120: 109-116.

Sakr B (1994). Inheritance and linkage of morphological markers and resistance to Ascochyta blight in lentil. PhD Thesis, Washington State Univ., Pullman, WA, USA.

Sambasivam P, Taylor PWJ, Ford R. (2017). Pathogenic variation and virulence related responses of Ascochyta lentis on lentil. European Journal of Plant Pathology. 147: 265-277.

Sari (2014). Genetics of resistance to Ascochyta blight in lentil. PhD thesis, Department of Plant Sciences, Univ. of Saskatchewan, Saskatoon, Canada.

Sari E, Bhadauria V, Vandenberg A, Banniza S (2017). Genotype-Dependent Interaction of Lentil Lines with Ascochyta lentis. Frontiers in Plant Science. 8: 764.

Shaikh R, Diederichsen A, Harrington M, Adam J, Conner RL, Buchwald TL (2012). New sources of resistance to *Colletotrichum truncatum* race Ct0 and Ct1 in *Lens culinaris* Medikus. subsp.

culinaris obtained by single plant selection in germplasm accessions. Genetic Resources and Crop Evolution. 60,1: 193-201.

Sharpe AG, Ramsay L, Sanderson LA, Fedoruk M, Clarke WE, Rong L, Kagale S, Vijayan P, Vandenberg A, Bett KE (2013). Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genomics. 14: 192.

Sillero JC, Rubiales D. (2002). Histological characterization of resistance to *Uromyces viciae-fabae* in faba bean. Phytopathology. 92: 294-299.

Singh M, Rana MK, Kumar K, Bisht IS, Dutta M, Gautam NK, Sarkar A, Bansal KC (2013). Broadening the genetic base of lentil cultivars through inter-sub-specific and interspecific crosses of *Lens* taxa. Plant Breeding. 132: 667-675.

Singh M, Bisht I S, Kumar S, Dutta M, Bansal KC, Karale M, Sarker A, Amri A, Kumar S, Datta S K (2014). Global Wild Annual *Lens* Collection: A Potential Resource for Lentil Genetic Base Broadening and Yield Enhancement. PLoS ONE. 9(9): e107781.

Singh M, Rana JC, Singh B, Kumar S, Saxena DR, Saxena A, Rizvi AH, Sarker A (2017). Comparative Agronomic Performance and Reaction to Fusarium wilt of *Lens culinaris* × *L. orientalis* and *L. culinaris* × *L. ervoides* derivatives. Frontiers in Plant Science. 8: 1162.

Sinha JN, Singh AP (1993). Effect of environment on the development and spread of Stemphylium blight of lentil. Indian Phytopathology. 46: 252 -253.

Statistics Canada (2016). website accessed on 22nd of February 2018 (http://www.statcan.gc.ca/daily-quotidien/160629/dq160629a-eng.htm)

Sudheesh S, Rodda MS, Davidson J, Javid M, Stephens A, Slater AT, Cogan NOI, Forster JW, Kaur S (2016). SNP-Based Linkage Mapping for Validation of QTLs for Resistance to Ascochyta Blight in Lentil. Frontiers in Plant Science.7: 1604.

Sun K, van Tuinen A, van Kan JAL, Wolters AA, Jacobsen E, Visser RGF, Bai Y (2017). Silencing of DND1 in potato and tomato impedes conidial germination, attachment and hyphal growth of *Botrytis cinerea*. BMC Plant Biology.17(1): 235.

Suvorova G (2014). Hybridization of Cultivated Lentil *Lens culinaris* Medik. and Wild Species *Lens tomentosus* Ladizinsky. Czech Journal of Genetics and Plant Breeding. 50: 130-134.

Tadmor Y, Zamir D, Ladizinsky G (1987). Genetic mapping of an ancient translocation in the genus *Lens*. Theoretical and Applied Genetics. 73: 883-892.

Tar'an B, Buchwaldt L, Tullu A, Banniza S, Warkentin TD, Vandenberg A (2003). Using molecular markers to pyramid genes for resistance to Ascochyta blight and anthracnose in lentil (*Lens culinaris* Medik). Euphytica. 134: 223-230.

Tay J, (1989). Inheritance of resistance to Ascochyta blight of lentil. MSc Thesis, Dept of Crop Science and Plant Ecology, Univ. of Saskatchewan, Saskatoon, Canada.

Taylor PWJ, K. Lindbeck, W. Chen, R. Ford, 2007. Lentil diseases. In: Lentil: an Ancient Crop for the Modern Times (S.S. Yadav, D. Mcneil, P.C. Stevenson, ed.), Springer, The Netherlands, 291-231.

Temel HY, Gol D, Kahriman A, Tanyolac MB (2014). Single nucleotide polymorphism discovery through Illumina-based transcriptome sequencing and mapping in lentil. Turkish Journal of Agriculture and Forestry. 38: 1-19.

Tullu A, Buchwaldt L, Warkentin TD, Taran B, Vandenberg A (2003). Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theoretical and Applied Genetics. 106: 428-434.

Tullu A, Tar'an B, Breitkreutz C, Buchwaidt L, Banniza S, Warkentin TD, Vandenberg A (2006). A quantitative-trait locus for resistance to Ascochyta blight *Ascochyta lentis* maps close to a gene for resistance to anthracnose *Colletotrichum truncatum* in lentil. Canadian Journal of Plant Pathology. 28: 588-595.

Tullu A, Buchwaldt L, Lulsdorf M, Banniza S, Barlow B, Slinkard AE, Sarker A, Tar'an B, Warkentin TD, Vandenberg A (2006). Sources of resistance to anthracnose (*Colletotrichum truncatum*) in wild Lens species. Genetic Resources and Crop Evolution. 53: 111-119.

Tullu A, Tar'an B, Warkentin TD, Vandenberg A (2008). Construction of an Intraspecific linkage map and QTL analysis for earliness and plant height in lentil. Crop Science. 48: 2254-2264.

Tullu A, Banniza S, Tar'an B, Warkentin TD, A Vandenberg A (2010). Sources of resistance to ascochyta blight in wild species of lentil (*Lens culinaris* Medik). Genetic Resources and Crop Evolution. 57: 1053-1063.

Tullu A, Bett KE, Banniza S, Vail S, Vandenberg A (2013). Widening the genetic base of cultivated lentil through hybridization of *Lens culinaris* 'Eston' and *L. ervoides* accession IG 72815. Canadian Journal of Plant Science. 93: 1037-1047.

Uppalapati SR, Ishiga Y, Doraiswamy V, Bedair M, Mittal S, et al. (2012). Loss of abaxial leaf epicuticular wax in *Medicago truncatula irg1/palm1* mutants results in reduced spore differentiation of anthracnose and nonhost rust pathogens. Plant Cell. 24: 353-70.

Vail S, Vandenberg A (2012). The Effect of Plant Age on Resistance to *Colletotrichum truncatum* in *Lens culinaris*. Plant Disease. 96, 8: 1118-1122.

Vail S, Strelioff JV, Tullu A, Vandenberg A (2012). Field evaluation of resistance to *Colletotrichum truncatum* in *Lens culinaris, Lens ervoides,* and *Lens ervoides* X *Lens culinaris* derivatives. Field Crops Research. 126: 145-151.

Vakulabharanam VR, Slinkard AE, Vandenberg A (1997). Inheritance of resistance to Ascochyta blight in lentil and linkage between isozyme. In: Proceedings of the Third International Food Legume Research Conference 22-26 September, Adelaide, Australia 162.

Van Oss H, Aron Y, Ladizinsky G (1997). Chloroplast DNA variation and evolution in the genus *Lens* Mill. Theoretical and Applied Genetics. 94: 452-457.

Van Schie CC, Takken FL (2014). Susceptibility genes 101: how to be a good host. Annual Review of Phytopathology. 52: 551-581.

Vandenberg B, Slinkard AE (1989). Inheritance of four new quantitative genes in lentil. The Journal of Heredity. 80: 320-322.

Vandenberg A, Kiehn FA, Vera C, Gaudiel R, Buchwaldt L, Dueck S, Wahab J, Slinkard AE (2002). Cultivar description CDC Robin lentil. Canadian Journal of Plant Science. 82: 111-112.

Vandenberg A, Banniza S, Warkentin TD, Ife S, Barlow B, McHale S, et al. (2006). CDC Redberry lentil. Canadian Journal of Plant Science. 86: 497 - 498.

Verma P, Sharma T R, Srivastava P S, Abdin M Z, Bhatia S (2014). Exploring genetic variability within lentil (*Lens culinaris* Medik.) and across related legumes using a newly developed set of microsatellite markers. Molecular Biology Reports. 41: 5607-5625.

Verma P, Goyal R, Chahota RK, Sharma TR, Abdin MZ, Bhatia S (2015). Construction of a Genetic Linkage Map and Identification of QTLs for Seed Weight and Seed Size Traits in Lentil (*Lens culinaris* Medik.). PLoS ONE. 10: e0139666.

Vleeshouwers V, van Dooijeweert W, Govers F, Kamoun S, Colon LT (2000). The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta. 210: 853-864.

Weeden NF, Muehlbauer FJ, Ladizinsky G (1992). Extensive Conservation of Linkage Relationships between Pea and Lentil Genetic Maps. The Journal of Heredity. 83 (2): 123-129.

Wong MML, Gujaria-Verma N, Ramsay L, Yuan HY, Caron C, Diapari M, Vandenberg A, Bett KE (2015). Classification and Characterization of Species within the Genus *Lens* Using Genotyping-by-Sequencing (GBS). PLoS ONE. 10(3): e0122025.

Xi K, Burnett PA, Tewari JP, Chen MH, Turkington TK, Helm JH (2000). Histopathological study of barley cultivars resistant and susceptible to *Rhynchosporium secalis*. Phytopathology. 90: 94-102.

Ye G, McNeil DL, Hill GD (2000). Two major genes confer Ascochyta blight resistance in *Lens orientalis*. New Zealand Plant Protection. 53: 109-113.

Ye G, McNeil DL, Hill GD (2001). Inheritance of resistance to Ascochyta blight in lentil. New Zealand Plant Protection. 54: 198-201.

Ye G, Mcneil DL, Hill GD (2003). Inheritance of foliar resistance to Ascochyta blight in lentil (*Lens culinaris*). New Zealand Journal of Crop and Horticultural Science. 31: 2, 77-83.

Zamir D, Ladizinsky G (1984). Genetics of allozyme variants and linkage groups in lentil. Euphytica. 33: 329-336.

Zhang NW, Lindhout P, Niks RE, Jeuken MJW (2009). Genetic dissection *of Lactuca saligna* nonhost resistance to downy mildew at various lettuce developmental stages. Plant Pathology. 58: 923-932.

Zimniak-Przybylska Z, Przybylska J, Krajewski P (2001). Genetic resources, *Lens*, lentil, SDS-PAGE, seed globulins. Journal of Applied Genetics. 42: 435-447

Appendix 1 Categorisation of *Lens ervoides* accessions in previous disease screening reports

Accessions No.	Disease reaction	Publication
IG 72661	Resistant to fusarium wilt	Bayaa et al. 1995
IG 72582	Susceptible to Fusarium	Bayaa et al. 1995
IG 72731, IG 72652, IG 72564, IG 72755, IG 72709, IG 72756, IG 72665, IG 72661, IG 72573, IG 72656, IG 72574, IG 72657, IG 72716, IG 72586, IG 72664, IG 72681, IG 72730, IG 72653, IG 72659, IG 72708, IG 72651, IG 72817, IG 72826, IG 72646, IG 72568, IG 72662, IG 72707	Resistant to Ascochyta blight	Bayaa et al. 1994
IG 136613, IG 136616, IG 72567	Resistant to Ascochyta blight	Ahmad et al. 1997
IG 72582, IG 136632	Susceptible to Ascochyta blight	Ahmad et al. 1997
IG 72664	Susceptible to Ascochyta blight under field conditions only, no data recorded/available under greenhouse conditions	Tullu et al. 2010
IG 72731, IG 72564, IG 72784, IG 72566, IG 72567, IG 72575, IG 72792 IG 72577, IG 72924, IG 72921 IG 72826	Resistant to Ascochyta blight under field conditions only, no data recorded/available under greenhouse conditions	Tullu et al. 2010
IG 72579, IG 72565, IG 72665, IG 72859, IG 72815, IG 72590, IG 72571, IG 107445, IG 72846, IG 72646, IG 72707, IG 72910	Resistant to Ascochyta blight under both greenhouse and field conditions	Tullu et al. 2010
IG 72570, IG 72652, IG 72578, IG 72576, IG 72914, IG 72729, IG 72582, IG 72862, IG 72654, IG 72841, IG 72783, IG 72657, IG 72730, IG 72708, IG 72799	Susceptible to Ascochyta blight under greenhouse conditions but resistant under field condition	Tullu et al. 2010
IG 72659	Susceptible to Ascochyta blight under field conditions but resistant under greenhouse conditions	Tullu et al. 2010
IG 72573	Resistant to anthracnose under field conditions (race 1 and 0 mixture), no data was recorded/available under greenhouse conditions	Tullu et al. 2006
IG 72589, IG 72587, IG 72922, IG 72582, IG 72590, IG 72861, IG 72571, IG 72730, IG 72842, IG 72663	Resistant to anthracnose race 1, susceptible to race 0 under greenhouse conditions and resistant under field conditions (race 1 and 0 mixture)	Tullu et al. 2006

IG 72652, IG 107435, IG 72579, IG 72710, IG 72564, IG 72808, IG 72803, IG 107442, IG 72814IG 107441, IG 72665, IG 72567d1, IG 72679, IG 72815, IG 116023, IG 72913, IG 72655, IG 107437, IG 116022, IG 72716, IG 107445, IG 72664, IG 107440, IG 72588, IG 72792, IG 72793, IG 72653, IG 72659, IG 72846, IG 107444, IG 72708, IG 72799, IG 72646, G 72662, IG 72707, IG 107439	Resistant to anthracnose (race 1 and race 0) under both green house and field conditions (race 1 and 0 mixture)	Tullu et al. 2006
IG 72918	Resistant to anthracnose race 1, susceptible to Race 0 under greenhouse conditions and susceptible under field conditions (race 1 and 0 mixture)	Tullu et al. 2006
IG 72585, IG 72575, IG 72577, IG 107438	Resistant to anthracnose race 1 under greenhouses and field conditions (race 1 and 0 mixture)	Tullu et al. 2006
IG 72681, IG 72651	Resistant to anthracnose race 0 under greenhouse and field conditions (race 1 and 0 mixture)	Tullu et al. 2006
IG 72570, IG 72583, IG 72565, IG 72576, IG 72566, IG 107436	Resistant to anthracnose (race1 and race 0 mixture) under field conditions but susceptible both race 0 and 1 under greenhouses conditions	Tullu et al. 2006
IG 72803, L01-827A	Very resistant to stemphylium blight	Podder et al. 2013
IG 107441, IG 72654, IG 72815, IG 72651, IG 72799, IG 72646	Resistant to stemphylium blight	Podder et al. 2013
IG 107435	Intermediate resistant	Podder et al. 2013
IG 72563, IG 72564, IG 72581, IG 72577	Resistant to rust	Singh et al. 2014
IG 72817	Resistant to powdery mildew	Singh et al. 2014
IG 136630	Moderately resistant to both isolates of *Ascochyta lentis*	Dadu et al. 2017
IG 72583, IG 136614, IG 72578, IG 72565, IG 136627	Susceptible to both isolates of *Ascochyta lentis*	Dadu et al. 2017
IG 136631	Moderately resistant one isolate and susceptible another isolate of *Ascochyta lentis*	Dadu et al. 2017

Appendix 2. Conidial germination of *Ascochyta lentis* isolate AL 61

Table A2.1 Type 3 tests of fixed effects of the regression analysis for conidial germination of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 6 to 48 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	6.64	15.72	0.0014
Time	1	25.1	364.65	<.0001
Time*Genotype	5	8.74	9.26	0.0026

Table A2.2 Comparison of means of conidial germination of *Ascochyta lentis* over time on *Lens ervoides* and *Lens culinaris* genotypes

Effect	Genotype	_Genotype	Time	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	6	0.1071	0.04235	8.46	2.53	0.0338
Genotype	Eston	L01-827A	6	0.1007	0.03998	5.81	2.52	0.0466
Genotype	Eston	LR-66-570	6	-0.04872	0.04424	6.99	-1.1	0.3073
Genotype	Eston	LR-66-629	6	0.1596	0.03861	6.25	4.13	0.0056
Genotype	Eston	Robin	6	-0.06038	0.03489	5.24	-1.73	0.1414
Genotype	IG 72815	L01-827A	6	-0.00639	0.04006	7.5	-0.16	0.8774
Genotype	IG 72815	LR-66-570	6	-0.1558	0.04432	8.53	-3.52	0.0071
Genotype	IG 72815	LR-66-629	6	0.05244	0.0387	8.64	1.35	0.2098
Genotype	IG 72815	Robin	6	-0.1675	0.03499	8.02	-4.79	0.0014
Genotype	L01-827A	LR-66-570	6	-0.1494	0.04206	6.12	-3.55	0.0116
Genotype	L01-827A	LR-66-629	6	0.05884	0.03609	5.32	1.63	0.1605
Genotype	L01-827A	Robin	6	-0.1611	0.03208	4.3	-5.02	0.0061
Genotype	LR-66-570	LR-66-629	6	0.2083	0.04076	6.45	5.11	0.0018
Genotype	LR-66-570	Robin	6	-0.01166	0.03726	5.43	-0.31	0.766
Genotype	LR-66-629	Robin	6	-0.2199	0.03036	5.23	-7.24	0.0006
Genotype	Eston	IG 72815	12	0.09034	0.03619	8.4	2.5	0.0358
Genotype	Eston	L01-827A	12	0.08786	0.03403	5.7	2.58	0.0437
Genotype	Eston	LR-66-570	12	-0.04765	0.03751	6.84	-1.27	0.2455
Genotype	Eston	LR-66-629	12	0.1382	0.03295	6.17	4.19	0.0054
Genotype	Eston	Robin	12	-0.05198	0.02976	5.17	-1.75	0.1392
Genotype	IG 72815	L01-827A	12	-0.00249	0.03417	7.4	-0.07	0.9439
Genotype	IG 72815	LR-66-570	12	-0.138	0.03764	8.41	-3.67	0.0058
Genotype	IG 72815	LR-66-629	12	0.04781	0.03309	8.57	1.44	0.184
Genotype	IG 72815	Robin	12	-0.1423	0.02992	7.96	-4.76	0.0015
Genotype	L01-827A	LR-66-570	12	-0.1355	0.03556	5.93	-3.81	0.0091
Genotype	L01-827A	LR-66-629	12	0.0503	0.03071	5.2	1.64	0.1601
Genotype	L01-827A	Robin	12	-0.1398	0.02726	4.19	-5.13	0.006
Genotype	LR-66-570	LR-66-629	12	0.1858	0.03453	6.31	5.38	0.0014
Genotype	LR-66-570	Robin	12	-0.00433	0.0315	5.29	-0.14	0.8958
Genotype	LR-66-629	Robin	12	-0.1901	0.02589	5.15	-7.34	0.0006
Genotype	Eston	IG 72815	24	0.0568	0.02449	9.03	2.32	0.0454

Genotype	Eston	L01-827A	24	0.06212	0.02386	7.03	2.6	0.0351
Genotype	Eston	LR-66-570	24	-0.04551	0.02579	8.15	-1.76	0.1149
Genotype	Eston	LR-66-629	24	0.09535	0.02265	7.08	4.21	0.0039
Genotype	Eston	Robin	24	-0.03518	0.0205	6	-1.72	0.137
Genotype	IG 72815	L01-827A	24	0.005324	0.02367	8.62	0.22	0.8273
Genotype	IG 72815	LR-66-570	24	-0.1023	0.02562	9.65	-3.99	0.0027
Genotype	IG 72815	LR-66-629	24	0.03855	0.02245	9.23	1.72	0.1192
Genotype	IG 72815	Robin	24	-0.09198	0.02028	8.54	-4.54	0.0016
Genotype	L01-827A	LR-66-570	24	-0.1076	0.02502	7.7	-4.3	0.0029
Genotype	L01-827A	LR-66-629	24	0.03323	0.02176	6.64	1.53	0.1729
Genotype	L01-827A	Robin	24	-0.0973	0.01952	5.56	-4.99	0.0031
Genotype	LR-66-570	LR-66-629	24	0.1409	0.02387	7.72	5.9	0.0004
Genotype	LR-66-570	Robin	24	0.01033	0.02184	6.64	0.47	0.6513
Genotype	LR-66-629	Robin	24	-0.1305	0.01802	6.14	-7.24	0.0003
Genotype	Eston	IG 72815	48	-0.0103	0.01439	2.73	-0.72	0.5305
Genotype	Eston	L01-827A	48	0.01065	0.02367	3.47	0.45	0.6794
Genotype	Eston	LR-66-570	48	-0.04124	0.02474	3.64	-1.67	0.1779
Genotype	Eston	LR-66-629	48	0.00974	0.01797	3.95	0.54	0.617
Genotype	Eston	Robin	48	-0.00159	0.01688	3.8	-0.09	0.9299
Genotype	IG 72815	L01-827A	48	0.02095	0.02051	2.33	1.02	0.4012
Genotype	IG 72815	LR-66-570	48	-0.03094	0.02175	2.53	-1.42	0.2656
Genotype	IG 72815	LR-66-629	48	0.02004	0.01355	2.84	1.48	0.2408
Genotype	IG 72815	Robin	48	0.008712	0.01207	3.11	0.72	0.5209
Genotype	L01-827A	LR-66-570	48	-0.05189	0.02874	4.18	-1.81	0.1422
Genotype	L01-827A	LR-66-629	48	-0.00091	0.02317	3.33	-0.04	0.9709
Genotype	L01-827A	Robin	48	-0.01224	0.02234	3.05	-0.55	0.6214
Genotype	LR-66-570	LR-66-629	48	0.05098	0.02427	3.5	2.1	0.1134
Genotype	LR-66-570	Robin	48	0.03965	0.02347	3.23	1.69	0.1832
Genotype	LR-66-629	Robin	48	-0.01132	0.01618	3.9	-0.7	0.5234

Table A2.3 Parameter estimates of the regression analysis for conidial germination of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 6 to 48 h post-inoculations

	Effect	Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Intercepts	Genotype	Eston	0.6441	0.0344	3.42	18.73	0.0001
	Genotype	IG 72815	0.5202	0.03435	5.73	15.15	<.0001
	Genotype	L01-827A	0.5305	0.03087	2.62	17.19	0.0009
	Genotype	LR-66-570	0.6939	0.03799	3.82	18.27	<.0001
	Genotype	LR-66-629	0.4632	0.02816	3.12	16.45	0.0004
	Genotype	Robin	0.7129	0.0208	2.44	34.27	0.0002
Slopes	Time(Genotype)	Eston	0.004907	0.000806	4.64	6.09	0.0022
	Time(Genotype)	IG 72815	0.007703	0.000735	6.28	10.48	<.0001
	Time(Genotype)	L01-827A	0.007052	0.000839	4.21	8.41	0.0009
	Time(Genotype)	LR-66-570	0.004729	0.001004	4.95	4.71	0.0054
	Time(Genotype)	LR-66-629	0.008474	0.000675	4.53	12.55	0.0001
	Time(Genotype)	Robin	0.003507	0.000521	3.89	6.73	0.0028

Appendix 3. Appressoria formation of *Ascochyta lentis* isolate AL 61

Table A3.1 Type 3 tests of fixed effects for appressoria formation of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 and 48 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	24	1.82	0.1473
Time	1	24	5.13	0.0328
Genotype*Time	5	24	1.18	0.3469

Table A3.2 Comparison of means for appressoria formation of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 and 48 h post-inoculations

Effect	Genotype	Time	_Genotype	_Time	Estimate	**STDERR**	DF	t Value	Pr > |t|
Genotype*Time	Eston	24	Eston	48	1.2	1.4414	24	0.83	0.4133
Genotype*Time	Eston	24	IG 72815	24	3.2667	1.3537	24	2.41	0.0238
Genotype*Time	Eston	24	IG 72815	48	1.8	1.4414	24	1.25	0.2238
Genotype*Time	Eston	24	L01-827A	24	3.5333	1.3537	24	2.61	0.0154
Genotype*Time	Eston	24	L01-827A	48	1.6	1.4414	24	1.11	0.278
Genotype*Time	Eston	24	LR-66-570	24	1.4667	1.3537	24	1.08	0.2894
Genotype*Time	Eston	24	LR-66-570	48	0.8667	1.4414	24	0.6	0.5533
Genotype*Time	Eston	24	LR-66-629	24	3.8	1.3537	24	2.81	0.0098
Genotype*Time	Eston	24	LR-66-629	48	2.1333	1.4414	24	1.48	0.1519
Genotype*Time	Eston	24	Robin	24	2.9333	1.3537	24	2.17	0.0404
Genotype*Time	Eston	24	Robin	48	-0.6	1.4414	24	-0.42	0.6809
Genotype*Time	Eston	48	IG 72815	24	2.0667	1.4414	24	1.43	0.1645
Genotype*Time	Eston	48	IG 72815	48	0.6	1.5241	24	0.39	0.6973
Genotype*Time	Eston	48	L01-827A	24	2.3333	1.4414	24	1.62	0.1186
Genotype*Time	Eston	48	L01-827A	48	0.4	1.5241	24	0.26	0.7952
Genotype*Time	Eston	48	LR-66-570	24	0.2667	1.4414	24	0.18	0.8548
Genotype*Time	Eston	48	LR-66-570	48	-0.3333	1.5241	24	-0.22	0.8287
Genotype*Time	Eston	48	LR-66-629	24	2.6	1.4414	24	1.8	0.0838
Genotype*Time	Eston	48	LR-66-629	48	0.9333	1.5241	24	0.61	0.546
Genotype*Time	Eston	48	Robin	24	1.7333	1.4414	24	1.2	0.2409
Genotype*Time	Eston	48	Robin	48	-1.8	1.5241	24	-1.18	0.2492
Genotype*Time	IG 72815	24	IG 72815	48	-1.4667	1.4414	24	-1.02	0.3191
Genotype*Time	IG 72815	24	L01-827A	24	0.2667	1.3537	24	0.2	0.8455
Genotype*Time	IG 72815	24	L01-827A	48	-1.6667	1.4414	24	-1.16	0.259
Genotype*Time	IG 72815	24	LR-66-570	24	-1.8	1.3537	24	-1.33	0.1961
Genotype*Time	IG 72815	24	LR-66-570	48	-2.4	1.4414	24	-1.66	0.1089
Genotype*Time	IG 72815	24	LR-66-629	24	0.5333	1.3537	24	0.39	0.6971
Genotype*Time	IG 72815	24	LR-66-629	48	-1.1333	1.4414	24	-0.79	0.4394
Genotype*Time	IG 72815	24	Robin	24	-0.3333	1.3537	24	-0.25	0.8076
Genotype*Time	IG 72815	24	Robin	48	-3.8667	1.4414	24	-2.68	0.013
Genotype*Time	IG 72815	48	L01-827A	24	1.7333	1.4414	24	1.2	0.2409
Genotype*Time	IG 72815	48	L01-827A	48	-0.2	1.5241	24	-0.13	0.8967

Genotype*Time	IG 72815	48	LR-66-570	24	-0.3333	1.4414	24	-0.23	0.8191
Genotype*Time	IG 72815	48	LR-66-570	48	-0.9333	1.5241	24	-0.61	0.546
Genotype*Time	IG 72815	48	LR-66-629	24	2	1.4414	24	1.39	0.178
Genotype*Time	IG 72815	48	LR-66-629	48	0.3333	1.5241	24	0.22	0.8287
Genotype*Time	IG 72815	48	Robin	24	1.1333	1.4414	24	0.79	0.4394
Genotype*Time	IG 72815	48	Robin	48	-2.4	1.5241	24	-1.57	0.1284
Genotype*Time	L01-827A	24	L01-827A	48	-1.9333	1.4414	24	-1.34	0.1924
Genotype*Time	L01-827A	24	LR-66-570	24	-2.0667	1.3537	24	-1.53	0.1399
Genotype*Time	L01-827A	24	LR-66-570	48	-2.6667	1.4414	24	-1.85	0.0767
Genotype*Time	L01-827A	24	LR-66-629	24	0.2667	1.3537	24	0.2	0.8455
Genotype*Time	L01-827A	24	LR-66-629	48	-1.4	1.4414	24	-0.97	0.3411
Genotype*Time	L01-827A	24	Robin	24	-0.6	1.3537	24	-0.44	0.6616
Genotype*Time	L01-827A	24	Robin	48	-4.1333	1.4414	24	-2.87	0.0085
Genotype*Time	L01-827A	48	LR-66-570	24	-0.1333	1.4414	24	-0.09	0.9271
Genotype*Time	L01-827A	48	LR-66-570	48	-0.7333	1.5241	24	-0.48	0.6348
Genotype*Time	L01-827A	48	LR-66-629	24	2.2	1.4414	24	1.53	0.14
Genotype*Time	L01-827A	48	LR-66-629	48	0.5333	1.5241	24	0.35	0.7294
Genotype*Time	L01-827A	48	Robin	24	1.3333	1.4414	24	0.92	0.3642
Genotype*Time	L01-827A	48	Robin	48	-2.2	1.5241	24	-1.44	0.1618
Genotype*Time	LR-66-570	24	LR-66-570	48	-0.6	1.4414	24	-0.42	0.6809
Genotype*Time	LR-66-570	24	LR-66-629	24	2.3333	1.3537	24	1.72	0.0976
Genotype*Time	LR-66-570	24	LR-66-629	48	0.6667	1.4414	24	0.46	0.6479
Genotype*Time	LR-66-570	24	Robin	24	1.4667	1.3537	24	1.08	0.2894
Genotype*Time	LR-66-570	24	Robin	48	-2.0667	1.4414	24	-1.43	0.1645
Genotype*Time	LR-66-570	48	LR-66-629	24	2.9333	1.4414	24	2.03	0.053
Genotype*Time	LR-66-570	48	LR-66-629	48	1.2667	1.5241	24	0.83	0.4141
Genotype*Time	LR-66-570	48	Robin	24	2.0667	1.4414	24	1.43	0.1645
Genotype*Time	LR-66-570	48	Robin	48	-1.4667	1.5241	24	-0.96	0.3455
Genotype*Time	LR-66-629	24	LR-66-629	48	-1.6667	1.4414	24	-1.16	0.259
Genotype*Time	LR-66-629	24	Robin	24	-0.8667	1.3537	24	-0.64	0.5281
Genotype*Time	LR-66-629	24	Robin	48	-4.4	1.4414	24	-3.05	0.0055
Genotype*Time	LR-66-629	48	Robin	24	0.8	1.4414	24	0.55	0.584
Genotype*Time	LR-66-629	48	Robin	48	-2.7333	1.5241	24	-1.79	0.0855
Genotype*Time	Robin	24	Robin	48	-3.5333	1.4414	24	-2.45	0.0219

Appendix 4. Germ tube length of *Ascochyta lentis* isolate AL 61

Table A4.1 Type 3 tests of fixed effects of the regression analysis for germ tube length of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 to 72 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	41	0.25	0.9389
Time	1	41	101.46	<.0001
Time*Genotype	5	41	0.32	0.8952

Table A4.2 Comparison of means for germ tube length of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 to 72 h post-inoculations

Effect	Genotype	_Genotype	Time	rep	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	24	2	16.233	16.133	46	1.01	0.3196
Genotype	Eston	L01-827A	24	2	7.7473	16.133	46	0.48	0.6333
Genotype	Eston	LR-66-570	24	2	-0.2471	16.133	46	-0.02	0.9878
Genotype	Eston	LR-66-629	24	2	10.8238	16.133	46	0.67	0.5056
Genotype	Eston	Robin	24	2	1.6413	16.133	46	0.1	0.9194
Genotype	IG 72815	L01-827A	24	2	-8.4857	16.133	46	-0.53	0.6014
Genotype	IG 72815	LR-66-570	24	2	-16.4801	16.133	46	-1.02	0.3124
Genotype	IG 72815	LR-66-629	24	2	-5.4092	16.133	46	-0.34	0.7389
Genotype	IG 72815	Robin	24	2	-14.5917	16.133	46	-0.9	0.3705
Genotype	L01-827A	LR-66-570	24	2	-7.9944	16.133	46	-0.5	0.6226
Genotype	L01-827A	LR-66-629	24	2	3.0764	16.133	46	0.19	0.8496
Genotype	L01-827A	Robin	24	2	-6.106	16.133	46	-0.38	0.7068
Genotype	LR-66-570	LR-66-629	24	2	11.0708	16.133	46	0.69	0.496
Genotype	LR-66-570	Robin	24	2	1.8884	16.133	46	0.12	0.9073
Genotype	LR-66-629	Robin	24	2	-9.1825	16.133	46	-0.57	0.572
Genotype	Eston	IG 72815	48	2	16.233	16.133	46	1.01	0.3196
Genotype	Eston	L01-827A	48	2	7.7473	16.133	46	0.48	0.6333
Genotype	Eston	LR-66-570	48	2	-0.2471	16.133	46	-0.02	0.9878
Genotype	Eston	LR-66-629	48	2	10.8238	16.133	46	0.67	0.5056
Genotype	Eston	Robin	48	2	1.6413	16.133	46	0.1	0.9194
Genotype	IG 72815	L01-827A	48	2	-8.4857	16.133	46	-0.53	0.6014
Genotype	IG 72815	LR-66-570	48	2	-16.4801	16.133	46	-1.02	0.3124
Genotype	IG 72815	LR-66-629	48	2	-5.4092	16.133	46	-0.34	0.7389
Genotype	IG 72815	Robin	48	2	-14.5917	16.133	46	-0.9	0.3705
Genotype	L01-827A	LR-66-570	48	2	-7.9944	16.133	46	-0.5	0.6226
Genotype	L01-827A	LR-66-629	48	2	3.0764	16.133	46	0.19	0.8496
Genotype	L01-827A	Robin	48	2	-6.106	16.133	46	-0.38	0.7068
Genotype	LR-66-570	LR-66-629	48	2	11.0708	16.133	46	0.69	0.496
Genotype	LR-66-570	Robin	48	2	1.8884	16.133	46	0.12	0.9073
Genotype	LR-66-629	Robin	48	2	-9.1825	16.133	46	-0.57	0.572
Genotype	Eston	IG 72815	72	2	16.233	16.133	46	1.01	0.3196
Genotype	Eston	L01-827A	72	2	7.7473	16.133	46	0.48	0.6333
Genotype	Eston	LR-66-570	72	2	-0.2471	16.133	46	-0.02	0.9878
Genotype	Eston	LR-66-629	72	2	10.8238	16.133	46	0.67	0.5056
Genotype	Eston	Robin	72	2	1.6413	16.133	46	0.1	0.9194
Genotype	IG 72815	L01-827A	72	2	-8.4857	16.133	46	-0.53	0.6014
Genotype	IG 72815	LR-66-570	72	2	-16.4801	16.133	46	-1.02	0.3124
Genotype	IG 72815	LR-66-629	72	2	-5.4092	16.133	46	-0.34	0.7389
Genotype	IG 72815	Robin	72	2	-14.5917	16.133	46	-0.9	0.3705
Genotype	L01-827A	LR-66-570	72	2	-7.9944	16.133	46	-0.5	0.6226
Genotype	L01-827A	LR-66-629	72	2	3.0764	16.133	46	0.19	0.8496

Genotype	L01-827A	Robin	72	2	-6.106	16.133	46	-0.38	0.7068
Genotype	LR-66-570	LR-66-629	72	2	11.0708	16.133	46	0.69	0.496
Genotype	LR-66-570	Robin	72	2	1.8884	16.133	46	0.12	0.9073
Genotype	LR-66-629	Robin	72	2	-9.1825	16.133	46	-0.57	0.572

Table A4.3 Parameter estimates of the regression analysis for germ tube length of *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 to 72 h post-inoculations

	Effect	Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Intercept	Genotype	Eston	15.7073	18.6908	32.5	0.84	0.4068
	Genotype	IG 72815	-0.5257	18.6908	32.5	-0.03	0.9777
	Genotype	L01-827A	7.96	18.6908	32.5	0.43	0.673
	Genotype	LR-66-570	15.9544	18.6908	32.5	0.85	0.3996
	Genotype	LR-66-629	4.8835	18.6908	32.5	0.26	0.7955
	Genotype	Robin	14.066	18.6908	32.5	0.75	0.4571
Slopes	Time		2.4869	0.2377	46	10.46	<.0001

Appendix 5. Percentage of dead tissue per leaflet of *Ascochyta lentis* isolate AL 61

Table A5.1 Type 3 tests of fixed effects of the regression analysis for percentage of dead tissue per leaflet caused by *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 96 to 240 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	18.7	10.4	<.0001
Time	1	40.4	69.49	<.0001
Time*Genotype	5	18.7	5.34	0.0032

Table A5.2 Comparison of means for percentage of dead tissue per leaflet caused by *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 96 to 240 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	Standard	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	96	4.786	8.899	17.6	0.54	0.5974
Genotype	Eston	L01-827A	96	5.6526	7.9308	19.4	0.71	0.4845
Genotype	Eston	LR-66-570	96	7.586	6.902	19.6	1.1	0.2851
Genotype	Eston	LR-66-629	96	7.086	5.8226	14.6	1.22	0.2429
Genotype	Eston	Robin	96	7.7855	5.8853	15.1	1.32	0.2056
Genotype	IG 72815	L01-827A	96	0.8667	9.4	17.7	0.09	0.9276
Genotype	IG 72815	LR-66-570	96	2.8	8.5499	15.8	0.33	0.7476
Genotype	IG 72815	LR-66-629	96	2.3	7.7049	11.9	0.3	0.7705
Genotype	IG 72815	Robin	96	2.9995	7.7524	12.2	0.39	0.7055
Genotype	L01-827A	LR-66-570	96	1.9333	7.5369	17.7	0.26	0.8005
Genotype	L01-827A	LR-66-629	96	1.4333	6.5628	13.3	0.22	0.8304
Genotype	L01-827A	Robin	96	2.1328	6.6185	13.6	0.32	0.7522
Genotype	LR-66-570	LR-66-629	96	-0.5	5.2736	15.9	-0.09	0.9256

Genotype	LR-66-570	Robin	96	0.1995	5.3427	16.2	0.04	0.9707
Genotype	LR-66-629	Robin	96	0.6995	3.8484	19.4	0.18	0.8577
Genotype	Eston	IG 72815	144	-9.9215	5.8258	17.6	-1.7	0.1062
Genotype	Eston	L01-827A	144	-2.0215	5.1919	19.4	-0.39	0.7013
Genotype	Eston	LR-66-570	144	-3.3215	4.5184	19.6	-0.74	0.471
Genotype	Eston	LR-66-629	144	3.5119	3.8118	14.6	0.92	0.3718
Genotype	Eston	Robin	144	7.8843	3.8528	15.1	2.05	0.0586
Genotype	IG 72815	L01-827A	144	7.9	6.1537	17.7	1.28	0.2158
Genotype	IG 72815	LR-66-570	144	6.6	5.5972	15.8	1.18	0.2558
Genotype	IG 72815	LR-66-629	144	13.4333	5.044	11.9	2.66	0.0208
Genotype	IG 72815	Robin	144	17.8057	5.0751	12.2	3.51	0.0042
Genotype	L01-827A	LR-66-570	144	-1.3	4.9341	17.7	-0.26	0.7952
Genotype	L01-827A	LR-66-629	144	5.5333	4.2964	13.3	1.29	0.2197
Genotype	L01-827A	Robin	144	9.9057	4.3328	13.6	2.29	0.0389
Genotype	LR-66-570	LR-66-629	144	6.8333	3.4524	15.9	1.98	0.0654
Genotype	LR-66-570	Robin	144	11.2057	3.4976	16.2	3.2	0.0055
Genotype	LR-66-629	Robin	144	4.3724	2.5194	19.4	1.74	0.0985
Genotype	Eston	IG 72815	192	-24.6289	5.8258	17.6	-4.23	0.0005
Genotype	Eston	L01-827A	192	-9.6956	5.1919	19.4	-1.87	0.077
Genotype	Eston	LR-66-570	192	-14.2289	4.5184	19.6	-3.15	0.0051
Genotype	Eston	LR-66-629	192	-0.06224	3.8118	14.6	-0.02	0.9872
Genotype	Eston	Robin	192	7.983	3.8528	15.1	2.07	0.0558
Genotype	IG 72815	L01-827A	192	14.9333	6.1537	17.7	2.43	0.0262
Genotype	IG 72815	LR-66-570	192	10.4	5.5972	15.8	1.86	0.0819
Genotype	IG 72815	LR-66-629	192	24.5667	5.044	11.9	4.87	0.0004
Genotype	IG 72815	Robin	192	32.6119	5.0751	12.2	6.43	<.0001
Genotype	L01-827A	LR-66-570	192	-4.5333	4.9341	17.7	-0.92	0.3705
Genotype	L01-827A	LR-66-629	192	9.6333	4.2964	13.3	2.24	0.0426
Genotype	L01-827A	Robin	192	17.6786	4.3328	13.6	4.08	0.0012
Genotype	LR-66-570	LR-66-629	192	14.1667	3.4524	15.9	4.1	0.0008
Genotype	LR-66-570	Robin	192	22.2119	3.4976	16.2	6.35	<.0001
Genotype	LR-66-629	Robin	192	8.0453	2.5194	19.4	3.19	0.0047
Genotype	Eston	IG 72815	240	-39.3363	8.899	17.6	-4.42	0.0003
Genotype	Eston	L01-827A	240	-17.3697	7.9308	19.4	-2.19	0.0409
Genotype	Eston	LR-66-570	240	-25.1363	6.902	19.6	-3.64	0.0017
Genotype	Eston	LR-66-629	240	-3.6363	5.8226	14.6	-0.62	0.5419
Genotype	Eston	Robin	240	8.0818	5.8853	15.1	1.37	0.1898
Genotype	IG 72815	L01-827A	240	21.9667	9.4	17.7	2.34	0.0314
Genotype	IG 72815	LR-66-570	240	14.2	8.5499	15.8	1.66	0.1165
Genotype	IG 72815	LR-66-629	240	35.7	7.7049	11.9	4.63	0.0006
Genotype	IG 72815	Robin	240	47.4182	7.7524	12.2	6.12	<.0001
Genotype	L01-827A	LR-66-570	240	-7.7667	7.5369	17.7	-1.03	0.3166
Genotype	L01-827A	LR-66-629	240	13.7333	6.5628	13.3	2.09	0.0561
Genotype	L01-827A	Robin	240	25.4515	6.6185	13.6	3.85	0.0019
Genotype	LR-66-570	LR-66-629	240	21.5	5.2736	15.9	4.08	0.0009

Genotype	LR-66-570	Robin	240	33.2182	5.3427	16.2	6.22	<.0001
Genotype	LR-66-629	Robin	240	11.7182	3.8484	19.4	3.04	0.0066

Table A5.3 Parameter estimates of the regression analysis for percentage of dead tissue per leaflet caused by *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 96 to 240 h post-inoculations

		Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Intercepts	Genotype	Eston	4.8342	10.2782	10.1	0.47	0.6481
	Genotype	IG 72815	-29.3667	14.2778	9.68	-2.06	0.0677
	Genotype	L01-827A	-16.1667	11.8745	10	-1.36	0.2032
	Genotype	LR-66-570	-24.5667	9.0633	10.4	-2.71	0.0212
	Genotype	LR-66-629	-9.4	5.4007	10.3	-1.74	0.1116
	Genotype	Robin	-2.7537	5.6567	10.5	-0.49	0.6364
Slopes	Time(Genotype)	Eston	0.06235	0.05772	9.86	1.08	0.3058
	Time(Genotype)	IG 72815	0.3688	0.08055	9.41	4.58	0.0012
	Time(Genotype)	L01-827A	0.2222	0.06685	9.62	3.32	0.0081
	Time(Genotype)	LR-66-570	0.2896	0.05075	9.85	5.71	0.0002
	Time(Genotype)	LR-66-629	0.1368	0.02954	9.76	4.63	0.001
	Time(Genotype)	Robin	0.06029	0.03104	9.74	1.94	0.0816

Appendix 6. Number of pycnidia per leaflet of *Ascochyta lentis* isolate AL 61

Table A6.1 Type 3 tests of fixed effects of the regression analysis for number of pycnidia per leaflet formed by *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotype incubated for 144 to 240 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	8.66	4.18	0.0324
Time	1	9.31	3.71	0.085
time*geno	5	8.66	1.09	0.4298

Table A6.2 Comparison of means for number of pycnidia per leaflet formed by *Ascochyta lentis* on *Lens ervoides* and *Lens culinaris* genotype incubated for 144 to 240 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	Standard	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	144	-12.3204	48.6531	7.07	-0.25	0.8073
Genotype	Eston	L01-827A	144	7.8018	17.2047	7.63	0.45	0.6628
Genotype	Eston	LR-66-570	144	9.4907	9.5489	9.38	0.99	0.3452
Genotype	Eston	LR-66-629	144	8.1129	5.353	13.9	1.52	0.1521
Genotype	Eston	Robin	144	11.8549	4.2471	7.96	2.79	0.0236
Genotype	IG 72815	L01-827A	144	20.1222	51.3229	8.61	0.39	0.7045
Genotype	IG 72815	LR-66-570	144	21.8111	49.2872	7.43	0.44	0.6707
Genotype	IG 72815	LR-66-629	144	20.4333	48.6487	7.06	0.42	0.687
Genotype	IG 72815	Robin	144	24.1753	48.5395	7	0.5	0.6337
Genotype	L01-827A	LR-66-570	144	1.6889	18.9234	10.2	0.09	0.9306

Genotype	L01-827A	LR-66-629	144	0.3111	17.1921	7.5	0.02	0.986
Genotype	L01-827A	Robin	144	4.0531	16.8805	7.04	0.24	0.8171
Genotype	LR-66-570	LR-66-629	144	-1.3778	9.5263	8.51	-0.14	0.8884
Genotype	LR-66-570	Robin	144	2.3642	8.9517	7.18	0.26	0.7991
Genotype	LR-66-629	Robin	144	3.7419	4.196	7.85	0.89	0.399
Genotype	Eston	IG 72815	192	-56.5178	30.7709	7.07	-1.84	0.1085
Genotype	Eston	L01-827A	192	-10.2955	10.8812	7.63	-0.95	0.3731
Genotype	Eston	LR-66-570	192	-2.6066	6.0393	9.38	-0.43	0.6758
Genotype	Eston	LR-66-629	192	4.2822	3.3855	13.9	1.26	0.2268
Genotype	Eston	Robin	192	8.7108	2.6861	7.96	3.24	0.0119
Genotype	IG 72815	L01-827A	192	46.2222	32.4595	8.61	1.42	0.1897
Genotype	IG 72815	LR-66-570	192	53.9111	31.1719	7.43	1.73	0.1249
Genotype	IG 72815	LR-66-629	192	60.8	30.7681	7.06	1.98	0.0883
Genotype	IG 72815	Robin	192	65.2285	30.6991	7	2.12	0.0712
Genotype	L01-827A	LR-66-570	192	7.6889	11.9682	10.2	0.64	0.5348
Genotype	L01-827A	LR-66-629	192	14.5778	10.8733	7.5	1.34	0.2192
Genotype	L01-827A	Robin	192	19.0063	10.6762	7.04	1.78	0.118
Genotype	LR-66-570	LR-66-629	192	6.8889	6.025	8.51	1.14	0.284
Genotype	LR-66-570	Robin	192	11.3174	5.6616	7.18	2	0.0847
Genotype	LR-66-629	Robin	192	4.4285	2.6538	7.85	1.67	0.1344
Genotype	Eston	IG 72815	240	-100.72	48.6531	7.07	-2.07	0.0768
Genotype	Eston	L01-827A	240	-28.3929	17.2047	7.63	-1.65	0.1393
Genotype	Eston	LR-66-570	240	-14.704	9.5489	9.38	-1.54	0.1566
Genotype	Eston	LR-66-629	240	0.4516	5.353	13.9	0.08	0.934
Genotype	Eston	Robin	240	5.5666	4.2471	7.96	1.31	0.2265
Genotype	IG 72815	L01-827A	240	72.3222	51.3229	8.61	1.41	0.1939
Genotype	IG 72815	LR-66-570	240	86.0111	49.2872	7.43	1.75	0.122
Genotype	IG 72815	LR-66-629	240	101.17	48.6487	7.06	2.08	0.0758
Genotype	IG 72815	Robin	240	106.28	48.5395	7	2.19	0.0647
Genotype	L01-827A	LR-66-570	240	13.6889	18.9234	10.2	0.72	0.4858
Genotype	L01-827A	LR-66-629	240	28.8444	17.1921	7.5	1.68	0.1344
Genotype	L01-827A	Robin	240	33.9595	16.8805	7.04	2.01	0.0839
Genotype	LR-66-570	LR-66-629	240	15.1556	9.5263	8.51	1.59	0.148
Genotype	LR-66-570	Robin	240	20.2706	8.9517	7.18	2.26	0.057
Genotype	LR-66-629	Robin	240	5.1151	4.196	7.85	1.22	0.2582

Appendix 7. Proportion of Infection pegs of *Colletotrichum lentis* race 0 isolate CT-30

Table A7.1 Type 3 tests of fixed effects for proportion of infection pegs formed by *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 and 48 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	22	26.51	<.0001
Time	1	22	1685.38	<.0001
Genotype*Time	5	22	7.05	0.0005

Table A7.2 Comparison of means for proportion of infection pegs formed by *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 24 and 48 h post-inoculations

Effect	Genotype	Time	_Genotype	_Time	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype*Time	Eston	24	Eston	48	-0.5216	0.0356	22	-14.65	<.0001
Genotype*Time	Eston	24	IG 72815	24	0.1611	0.02405	22	6.7	<.0001
Genotype*Time	Eston	24	IG 72815	48	-0.4389	0.03252	22	-13.5	<.0001
Genotype*Time	Eston	24	L01-827A	24	0.1424	0.02405	22	5.92	<.0001
Genotype*Time	Eston	24	L01-827A	48	-0.5322	0.03252	22	-16.37	<.0001
Genotype*Time	Eston	24	LR-66-524	24	0.1264	0.02405	22	5.26	<.0001
Genotype*Time	Eston	24	LR-66-524	48	-0.6149	0.03252	22	-18.91	<.0001
Genotype*Time	Eston	24	LR-66-528	24	0.1771	0.02405	22	7.36	<.0001
Genotype*Time	Eston	24	LR-66-528	48	-0.3269	0.03252	22	-10.05	<.0001
Genotype*Time	Eston	24	Robin	24	0.01274	0.02405	22	0.53	0.6017
Genotype*Time	Eston	24	Robin	48	-0.5256	0.03252	22	-16.16	<.0001
Genotype*Time	Eston	48	IG 72815	24	0.6827	0.03252	22	20.99	<.0001
Genotype*Time	Eston	48	IG 72815	48	0.08267	0.0392	22	2.11	0.0466
Genotype*Time	Eston	48	L01-827A	24	0.664	0.03252	22	20.42	<.0001
Genotype*Time	Eston	48	L01-827A	48	-0.01067	0.0392	22	-0.27	0.7881
Genotype*Time	Eston	48	LR-66-524	24	0.648	0.03252	22	19.93	<.0001
Genotype*Time	Eston	48	LR-66-524	48	-0.09333	0.0392	22	-2.38	0.0264
Genotype*Time	Eston	48	LR-66-528	24	0.6987	0.03252	22	21.48	<.0001
Genotype*Time	Eston	48	LR-66-528	48	0.1947	0.0392	22	4.97	<.0001
Genotype*Time	Eston	48	Robin	24	0.5343	0.03252	22	16.43	<.0001
Genotype*Time	Eston	48	Robin	48	-0.004	0.0392	22	-0.1	0.9197
Genotype*Time	IG 72815	24	IG 72815	48	-0.6	0.0356	22	-16.85	<.0001
Genotype*Time	IG 72815	24	L01-827A	24	-0.01867	0.02405	22	-0.78	0.4459
Genotype*Time	IG 72815	24	L01-827A	48	-0.6933	0.03252	22	-21.32	<.0001
Genotype*Time	IG 72815	24	LR-66-524	24	-0.03467	0.02405	22	-1.44	0.1636
Genotype*Time	IG 72815	24	LR-66-524	48	-0.776	0.03252	22	-23.86	<.0001
Genotype*Time	IG 72815	24	LR-66-528	24	0.016	0.02405	22	0.67	0.5128
Genotype*Time	IG 72815	24	LR-66-528	48	-0.488	0.03252	22	-15.01	<.0001
Genotype*Time	IG 72815	24	Robin	24	-0.1484	0.02405	22	-6.17	<.0001
Genotype*Time	IG 72815	24	Robin	48	-0.6867	0.03252	22	-21.11	<.0001
Genotype*Time	IG 72815	48	L01-827A	24	0.5813	0.03252	22	17.88	<.0001
Genotype*Time	IG 72815	48	L01-827A	48	-0.09333	0.0392	22	-2.38	0.0264
Genotype*Time	IG 72815	48	LR-66-524	24	0.5653	0.03252	22	17.38	<.0001
Genotype*Time	IG 72815	48	LR-66-524	48	-0.176	0.0392	22	-4.49	0.0002
Genotype*Time	IG 72815	48	LR-66-528	24	0.616	0.03252	22	18.94	<.0001
Genotype*Time	IG 72815	48	LR-66-528	48	0.112	0.0392	22	2.86	0.0092
Genotype*Time	IG 72815	48	Robin	24	0.4516	0.03252	22	13.89	<.0001
Genotype*Time	IG 72815	48	Robin	48	-0.08667	0.0392	22	-2.21	0.0377
Genotype*Time	L01-827A	24	L01-827A	48	-0.6747	0.0356	22	-18.95	<.0001
Genotype*Time	L01-827A	24	LR-66-524	24	-0.016	0.02405	22	-0.67	0.5128
Genotype*Time	L01-827A	24	LR-66-524	48	-0.7573	0.03252	22	-23.29	<.0001
Genotype*Time	L01-827A	24	LR-66-528	24	0.03467	0.02405	22	1.44	0.1636

Genotype*Time	L01-827A	24	LR-66-528	48	-0.4693	0.03252	22	-14.43	<.0001
Genotype*Time	L01-827A	24	Robin	24	-0.1297	0.02405	22	-5.39	<.0001
Genotype*Time	L01-827A	24	Robin	48	-0.668	0.03252	22	-20.54	<.0001
Genotype*Time	L01-827A	48	LR-66-524	24	0.6587	0.03252	22	20.25	<.0001
Genotype*Time	L01-827A	48	LR-66-524	48	-0.08267	0.0392	22	-2.11	0.0466
Genotype*Time	L01-827A	48	LR-66-528	24	0.7093	0.03252	22	21.81	<.0001
Genotype*Time	L01-827A	48	LR-66-528	48	0.2053	0.0392	22	5.24	<.0001
Genotype*Time	L01-827A	48	Robin	24	0.545	0.03252	22	16.76	<.0001
Genotype*Time	L01-827A	48	Robin	48	0.006667	0.0392	22	0.17	0.8665
Genotype*Time	LR-66-524	24	LR-66-524	48	-0.7413	0.0356	22	-20.82	<.0001
Genotype*Time	LR-66-524	24	LR-66-528	24	0.05067	0.02405	22	2.11	0.0468
Genotype*Time	LR-66-524	24	LR-66-528	48	-0.4533	0.03252	22	-13.94	<.0001
Genotype*Time	LR-66-524	24	Robin	24	-0.1137	0.02405	22	-4.73	0.0001
Genotype*Time	LR-66-524	24	Robin	48	-0.652	0.03252	22	-20.05	<.0001
Genotype*Time	LR-66-524	48	LR-66-528	24	0.792	0.03252	22	24.35	<.0001
Genotype*Time	LR-66-524	48	LR-66-528	48	0.288	0.0392	22	7.35	<.0001
Genotype*Time	LR-66-524	48	Robin	24	0.6276	0.03252	22	19.3	<.0001
Genotype*Time	LR-66-524	48	Robin	48	0.08933	0.0392	22	2.28	0.0327
Genotype*Time	LR-66-528	24	LR-66-528	48	-0.504	0.0356	22	-14.16	<.0001
Genotype*Time	LR-66-528	24	Robin	24	-0.1644	0.02405	22	-6.83	<.0001
Genotype*Time	LR-66-528	24	Robin	48	-0.7027	0.03252	22	-21.61	<.0001
Genotype*Time	LR-66-528	48	Robin	24	0.3396	0.03252	22	10.44	<.0001
Genotype*Time	LR-66-528	48	Robin	48	-0.1987	0.0392	22	-5.07	<.0001
Genotype*Time	Robin	24	Robin	48	-0.5383	0.0356	22	-15.12	<.0001

Appendix 8. Percentage of dead tissue per leaflet of *Colletotrichum lentis* race 0 isolate CT-30

Table A8.1 Type 3 tests of fixed effects of the regression analysis for percentage of dead tissue per leaflet caused by *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 96 to 144 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	42	11.36	<.0001
Time	1	42	120.87	<.0001
Time*Genotype	5	42	2.44	0.0499

Table A8.2 Comparison of means for percentage of dead tissue per leaflet caused by *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 96 to 144 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	96	20.963	8.0808	42	2.59	0.013
Genotype	Eston	L01-827A	96	4.5185	8.0808	42	0.56	0.579
Genotype	Eston	LR-66-524	96	0.01852	8.0808	42	0	0.9982
Genotype	Eston	LR-66-528	96	36.3241	8.0808	42	4.5	<.0001
Genotype	Eston	Robin	96	11.1296	8.0808	42	1.38	0.1757
Genotype	IG 72815	L01-827A	96	-16.4444	8.0808	42	-2.04	0.0482

Genotype	IG 72815	LR-66-524	96	-20.9444	8.0808	42	-2.59	0.0131
Genotype	IG 72815	LR-66-528	96	15.3611	8.0808	42	1.9	0.0642
Genotype	IG 72815	Robin	96	-9.8333	8.0808	42	-1.22	0.2304
Genotype	L01-827A	LR-66-524	96	-4.5	8.0808	42	-0.56	0.5806
Genotype	L01-827A	LR-66-528	96	31.8056	8.0808	42	3.94	0.0003
Genotype	L01-827A	Robin	96	6.6111	8.0808	42	0.82	0.4179
Genotype	LR-66-524	LR-66-528	96	36.3056	8.0808	42	4.49	<.0001
Genotype	LR-66-524	Robin	96	11.1111	8.0808	42	1.38	0.1764
Genotype	LR-66-528	Robin	96	-25.1944	8.0808	42	-3.12	0.0033
Genotype	Eston	IG 72815	120	14.0741	5.1107	42	2.75	0.0087
Genotype	Eston	L01-827A	120	7.6296	5.1107	42	1.49	0.1429
Genotype	Eston	LR-66-524	120	0.6296	5.1107	42	0.12	0.9025
Genotype	Eston	LR-66-528	120	30.0185	5.1107	42	5.87	<.0001
Genotype	Eston	Robin	120	23.0741	5.1107	42	4.51	<.0001
Genotype	IG 72815	L01-827A	120	-6.4444	5.1107	42	-1.26	0.2143
Genotype	IG 72815	LR-66-524	120	-13.4444	5.1107	42	-2.63	0.0119
Genotype	IG 72815	LR-66-528	120	15.9444	5.1107	42	3.12	0.0033
Genotype	IG 72815	Robin	120	9	5.1107	42	1.76	0.0855
Genotype	L01-827A	LR-66-524	120	-7	5.1107	42	-1.37	0.1781
Genotype	L01-827A	LR-66-528	120	22.3889	5.1107	42	4.38	<.0001
Genotype	L01-827A	Robin	120	15.4444	5.1107	42	3.02	0.0043
Genotype	LR-66-524	LR-66-528	120	29.3889	5.1107	42	5.75	<.0001
Genotype	LR-66-524	Robin	120	22.4444	5.1107	42	4.39	<.0001
Genotype	LR-66-528	Robin	120	-6.9444	5.1107	42	-1.36	0.1815
Genotype	Eston	IG 72815	144	7.1852	8.0808	42	0.89	0.379
Genotype	Eston	L01-827A	144	10.7407	8.0808	42	1.33	0.191
Genotype	Eston	LR-66-524	144	1.2407	8.0808	42	0.15	0.8787
Genotype	Eston	LR-66-528	144	23.713	8.0808	42	2.93	0.0054
Genotype	Eston	Robin	144	35.0185	8.0808	42	4.33	<.0001
Genotype	IG 72815	L01-827A	144	3.5556	8.0808	42	0.44	0.6622
Genotype	IG 72815	LR-66-524	144	-5.9444	8.0808	42	-0.74	0.466
Genotype	IG 72815	LR-66-528	144	16.5278	8.0808	42	2.05	0.0471
Genotype	IG 72815	Robin	144	27.8333	8.0808	42	3.44	0.0013
Genotype	L01-827A	LR-66-524	144	-9.5	8.0808	42	-1.18	0.2464
Genotype	L01-827A	LR-66-528	144	12.9722	8.0808	42	1.61	0.1159
Genotype	L01-827A	Robin	144	24.2778	8.0808	42	3	0.0045
Genotype	LR-66-524	LR-66-528	144	22.4722	8.0808	42	2.78	0.0081
Genotype	LR-66-524	Robin	144	33.7778	8.0808	42	4.18	0.0001
Genotype	LR-66-528	Robin	144	11.3056	8.0808	42	1.4	0.1691

Table A8.3 Parameter estimates of the regression analysis for percentage of dead tissue per leaflet caused by *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 96 to 144 h post-inoculations

	Effect	Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Intercepts	Genotype	Eston	-26.4259	22.4232	42	-1.18	0.2452
	Genotype	IG 72815	-74.9444	22.4232	42	-3.34	0.0018
	Genotype	L01-827A	-18.5	22.4232	42	-0.83	0.414
	Genotype	LR-66-524	-24	22.4232	42	-1.07	0.2906
	Genotype	LR-66-528	-87.9722	22.4232	42	-3.92	0.0003
	Genotype	Robin	10.2222	22.4232	42	0.46	0.6508
Slopes	Time(Genotype)	Eston	0.8449	0.1844	42	4.58	<.0001
	Time(Genotype)	IG 72815	1.1319	0.1844	42	6.14	<.0001
	Time(Genotype)	L01-827A	0.7153	0.1844	42	3.88	0.0004
	Time(Genotype)	LR-66-524	0.8194	0.1844	42	4.44	<.0001
	Time(Genotype)	LR-66-528	1.1076	0.1844	42	6.01	<.0001
	Time(Genotype)	Robin	0.3472	0.1844	42	1.88	0.0667

Appendix 9. Percentage of leaflet area covered by acervuli per leaflet of *Colletotrichum lentis* race 0 isolate CT-30

Table A9.1 Type 3 tests of fixed effects of the regression analysis for percentage of leaflet area covered by acervuli per leaflet of *Colletotrichum lentis* on *Lens ervoides* and *Lens culinaris* genotype incubated from 96 to 144 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	14	15.14	<.0001
Time	1	25.7	17.11	0.0003
Time*Genotype	5	14	5	0.0078

Table A9.2 Comparison of means for percentage of leaflet area covered by acervuli of *Colletotrichum lentis* per leaflet on *Lens ervoides* and *Lens culinaris* genotype incubated from 96 to 144 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	96	11.7222	11.0599	12.9	1.06	0.3086
Genotype	Eston	L01-827A	96	13.7222	8.4055	7.55	1.63	0.1434
Genotype	Eston	LR-66-524	96	4.3472	13.7472	12.6	0.32	0.757
Genotype	Eston	LR-66-528	96	19.7778	8.8175	9.01	2.24	0.0516
Genotype	Eston	Robin	96	7.0556	13.3218	12.9	0.53	0.6054
Genotype	IG 72815	L01-827A	96	2	7.6756	7.33	0.26	0.8016
Genotype	IG 72815	LR-66-524	96	-7.375	13.3135	11.6	-0.55	0.5902
Genotype	IG 72815	LR-66-528	96	8.0556	8.1247	8.81	0.99	0.3479
Genotype	IG 72815	Robin	96	-4.6667	12.8737	11.6	-0.36	0.7235
Genotype	L01-827A	LR-66-524	96	-9.375	11.2063	7.34	-0.84	0.4292
Genotype	L01-827A	LR-66-528	96	6.0556	3.7872	8.92	1.6	0.1446

Genotype	L01-827A	Robin	96	-6.6667	10.6801	7.3	-0.62	0.5515
Genotype	LR-66-524	LR-66-528	96	15.4306	11.5185	8.13	1.34	0.2166
Genotype	LR-66-524	Robin	96	2.7083	15.2446	13.5	0.18	0.8616
Genotype	LR-66-528	Robin	96	-12.7222	11.0073	8.13	-1.16	0.2806
Genotype	Eston	IG 72815	120	30.5556	6.9949	12.9	4.37	0.0008
Genotype	Eston	L01-827A	120	38.9556	5.3161	7.55	7.33	0.0001
Genotype	Eston	LR-66-524	120	8.5556	8.6945	12.6	0.98	0.3437
Genotype	Eston	LR-66-528	120	37.4444	5.5767	9.01	6.71	<.0001
Genotype	Eston	Robin	120	22.5556	8.4255	12.9	2.68	0.0191
Genotype	IG 72815	L01-827A	120	8.4	4.8545	7.33	1.73	0.1252
Genotype	IG 72815	LR-66-524	120	-22	8.4202	11.6	-2.61	0.0233
Genotype	IG 72815	LR-66-528	120	6.8889	5.1385	8.81	1.34	0.2136
Genotype	IG 72815	Robin	120	-8	8.1421	11.6	-0.98	0.3458
Genotype	L01-827A	LR-66-524	120	-30.4	7.0875	7.34	-4.29	0.0032
Genotype	L01-827A	LR-66-528	120	-1.5111	2.3953	8.92	-0.63	0.5439
Genotype	L01-827A	Robin	120	-16.4	6.7547	7.3	-2.43	0.0441
Genotype	LR-66-524	LR-66-528	120	28.8889	7.285	8.13	3.97	0.004
Genotype	LR-66-524	Robin	120	14	9.6415	13.5	1.45	0.1694
Genotype	LR-66-528	Robin	120	-14.8889	6.9616	8.13	-2.14	0.0644
Genotype	Eston	IG 72815	144	49.3889	11.0599	12.9	4.47	0.0006
Genotype	Eston	L01-827A	144	64.1889	8.4055	7.55	7.64	<.0001
Genotype	Eston	LR-66-524	144	12.7639	13.7472	12.6	0.93	0.3706
Genotype	Eston	LR-66-528	144	55.1111	8.8175	9.01	6.25	0.0001
Genotype	Eston	Robin	144	38.0556	13.3218	12.9	2.86	0.0136
Genotype	IG 72815	L01-827A	144	14.8	7.6756	7.33	1.93	0.0933
Genotype	IG 72815	LR-66-524	144	-36.625	13.3135	11.6	-2.75	0.0181
Genotype	IG 72815	LR-66-528	144	5.7222	8.1247	8.81	0.7	0.4994
Genotype	IG 72815	Robin	144	-11.3333	12.8737	11.6	-0.88	0.3965
Genotype	L01-827A	LR-66-524	144	-51.425	11.2063	7.34	-4.59	0.0022
Genotype	L01-827A	LR-66-528	144	-9.0778	3.7872	8.92	-2.4	0.0403
Genotype	L01-827A	Robin	144	-26.1333	10.6801	7.3	-2.45	0.0429
Genotype	LR-66-524	LR-66-528	144	42.3472	11.5185	8.13	3.68	0.0061
Genotype	LR-66-524	Robin	144	25.2917	15.2446	13.5	1.66	0.1202
Genotype	LR-66-528	Robin	144	-17.0556	11.0073	8.13	-1.55	0.1592

Table A9.3 Parameter estimates of the regression analysis for percentage of leaflet area covered by acervuli of *Colletotrichum lentis* per leaflet of *Lens ervoides* and *Lens culinaris* genotypes incubated from 96 to 144 h post-inoculations

Intercepts	Effect	Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
	Genotype	Eston	-76.5	32.248	6.87	-2.37	0.0501
	Genotype	IG 72815	-12.8889	29.3117	6.41	-0.44	0.6746
	Genotype	L01-827A	10.7111	7.9677	5.4	1.34	0.2326
	Genotype	LR-66-524	-64.0139	43.426	6.95	-1.47	0.1843
	Genotype	LR-66-528	-25.6111	13.1438	7.17	-1.95	0.0914

	Genotype	Robin	-21.5556	41.3337	6.87	-0.52	0.6184
Slopes	Time(Genotype)	Eston	0.9931	0.2642	6.79	3.76	0.0075
	Time(Genotype)	IG 72815	0.2083	0.24	6.32	0.87	0.4171
	Time(Genotype)	L01-827A	-0.05833	0.06144	4.05	-0.95	0.3955
	Time(Genotype)	LR-66-524	0.8177	0.3564	6.9	2.29	0.056
	Time(Genotype)	LR-66-528	0.2569	0.1057	6.47	2.43	0.0481
	Time(Genotype)	Robin	0.3472	0.3392	6.82	1.02	0.3409

Appendix 10. Proportion of conidial germination of *Stemphylium botryosum* isolate SB-19

Table A10.1 Type 3 tests of fixed effects of the regression analysis for proportion of conidial germination of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 6 to 48 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	7.51	8.78	0.0051
Time	1	21.5	2.08	0.1636
Time*Genotype	5	8.82	3.95	0.0368

Table A10.2 Comparison of means for proportion of conidial germination of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 6 to 48 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	6	-0.07861	0.01256	5.43	-6.26	0.0011
Genotype	Eston	L01-827A	6	-0.06653	0.02124	7.13	-3.13	0.0162
Genotype	Eston	LR-66-577	6	-0.04092	0.02418	4.66	-1.69	0.1556
Genotype	Eston	LR-66-637	6	-0.0417	0.01744	6.6	-2.39	0.0502
Genotype	Eston	Robin	6	-0.08061	0.01304	5.41	-6.18	0.0012
Genotype	IG 72815	L01-827A	6	0.01208	0.02258	8.31	0.54	0.6066
Genotype	IG 72815	LR-66-577	6	0.03769	0.02537	5.44	1.49	0.1928
Genotype	IG 72815	LR-66-637	6	0.03691	0.01905	7.86	1.94	0.0894
Genotype	IG 72815	Robin	6	-0.00199	0.01513	6.47	-0.13	0.8992
Genotype	L01-827A	LR-66-577	6	0.02561	0.03061	8.77	0.84	0.425
Genotype	L01-827A	LR-66-637	6	0.02483	0.02562	10.6	0.97	0.3541
Genotype	L01-827A	Robin	6	-0.01407	0.02285	8.5	-0.62	0.5541
Genotype	LR-66-577	LR-66-637	6	-0.00078	0.0281	7.29	-0.03	0.9786
Genotype	LR-66-577	Robin	6	-0.03968	0.02561	5.59	-1.55	0.1757
Genotype	LR-66-637	Robin	6	-0.0389	0.01937	8.04	-2.01	0.0793
Genotype	Eston	IG 72815	12	-0.06106	0.008723	4.48	-7	0.0014
Genotype	Eston	L01-827A	12	-0.05434	0.01464	7.68	-3.71	0.0064
Genotype	Eston	LR-66-577	12	-0.04254	0.01653	4.82	-2.57	0.0516
Genotype	Eston	LR-66-637	12	-0.03723	0.01237	6.6	-3.01	0.0211
Genotype	Eston	Robin	12	-0.06917	0.01096	5.04	-6.31	0.0014
Genotype	IG 72815	L01-827A	12	0.006721	0.01503	8.1	0.45	0.6666
Genotype	IG 72815	LR-66-577	12	0.01853	0.01688	5.13	1.1	0.3212
Genotype	IG 72815	LR-66-637	12	0.02383	0.01284	7	1.86	0.1058

Genotype	IG 72815	Robin	12	-0.00811	0.01148	5.44	-0.71	0.5092
Genotype	L01-827A	LR-66-577	12	0.0118	0.02057	8.73	0.57	0.5805
Genotype	L01-827A	LR-66-637	12	0.01711	0.01741	10.7	0.98	0.3474
Genotype	L01-827A	Robin	12	-0.01483	0.01643	9.25	-0.9	0.3898
Genotype	LR-66-577	LR-66-637	12	0.005304	0.01902	7.25	0.28	0.7882
Genotype	LR-66-577	Robin	12	-0.02663	0.01813	6.22	-1.47	0.1906
Genotype	LR-66-637	Robin	12	-0.03194	0.01445	8	-2.21	0.0581
Genotype	Eston	IG 72815	24	-0.02596	0.009983	5.55	-2.6	0.0437
Genotype	Eston	L01-827A	24	-0.02996	0.006886	5.89	-4.35	0.005
Genotype	Eston	LR-66-577	24	-0.04576	0.008283	5.6	-5.53	0.0019
Genotype	Eston	LR-66-637	24	-0.02829	0.009581	5.64	-2.95	0.0275
Genotype	Eston	Robin	24	-0.04629	0.008758	7.52	-5.29	0.0009
Genotype	IG 72815	L01-827A	24	-0.004	0.008719	3.76	-0.46	0.6715
Genotype	IG 72815	LR-66-577	24	-0.0198	0.009859	4.73	-2.01	0.104
Genotype	IG 72815	LR-66-637	24	-0.00233	0.01097	5.47	-0.21	0.8396
Genotype	IG 72815	Robin	24	-0.02033	0.01026	5.72	-1.98	0.0972
Genotype	L01-827A	LR-66-577	24	-0.0158	0.006705	3.44	-2.36	0.0886
Genotype	L01-827A	LR-66-637	24	0.001672	0.008255	3.74	0.2	0.85
Genotype	L01-827A	Robin	24	-0.01633	0.007284	5.32	-2.24	0.0718
Genotype	LR-66-577	LR-66-637	24	0.01747	0.009452	4.7	1.85	0.1275
Genotype	LR-66-577	Robin	24	-0.00053	0.008616	5.58	-0.06	0.9533
Genotype	LR-66-637	Robin	24	-0.018	0.009871	5.78	-1.82	0.1198
Genotype	Eston	IG 72815	48	0.04424	0.02992	6.24	1.48	0.1878
Genotype	Eston	L01-827A	48	0.01879	0.03068	8.24	0.61	0.5566
Genotype	Eston	LR-66-577	48	-0.05222	0.03653	7.64	-1.43	0.1924
Genotype	Eston	LR-66-637	48	-0.01041	0.03055	9.89	-0.34	0.7405
Genotype	Eston	Robin	48	-0.00054	0.01476	2.44	-0.04	0.9735
Genotype	IG 72815	L01-827A	48	-0.02545	0.03803	10.6	-0.67	0.5178
Genotype	IG 72815	LR-66-577	48	-0.09646	0.04289	10.5	-2.25	0.0471
Genotype	IG 72815	LR-66-637	48	-0.05465	0.03793	11.6	-1.44	0.1761
Genotype	IG 72815	Robin	48	-0.04478	0.02689	4.78	-1.67	0.1595
Genotype	L01-827A	LR-66-577	48	-0.07101	0.04342	11.8	-1.64	0.1284
Genotype	L01-827A	LR-66-637	48	-0.0292	0.03853	14	-0.76	0.4611
Genotype	L01-827A	Robin	48	-0.01934	0.02773	6.68	-0.7	0.5092
Genotype	LR-66-577	LR-66-637	48	0.04181	0.04334	12.5	0.96	0.3529
Genotype	LR-66-577	Robin	48	0.05168	0.03409	6.31	1.52	0.1779
Genotype	LR-66-637	Robin	48	0.009865	0.0276	8.39	0.36	0.7296

Table A10.3 Parameter estimates of the regression analysis for proportion of conidial germination of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 6 to 48 h post-inoculations

	Effect	Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Intercepts	Genotype	Eston	0.8489	0.008925	3.49	95.11	<.0001
	Genotype	IG 72815	0.9451	0.01491	4.23	63.39	<.0001

	Genotype	L01-827A	0.9276	0.02666	5.79	34.8	<.0001
	Genotype	LR-66-577	0.8882	0.03092	4.08	28.73	<.0001
	Genotype	LR-66-637	0.8951	0.02136	5.35	41.91	<.0001
	Genotype	Robin	0.941	0.01263	3.36	74.49	<.0001
Slopes	Time(Genotype)	Eston	0.001755	0.00042	2.61	4.18	0.0327
	Time(Genotype)	IG 72815	-0.00117	0.00083	5.72	-1.41	0.2108
	Time(Genotype)	L01-827A	-0.00028	0.001115	5.89	-0.25	0.8127
	Time(Genotype)	LR-66-577	0.002024	0.001327	4.87	1.53	0.1893
	Time(Genotype)	LR-66-637	0.00101	0.000969	7.56	1.04	0.3296
	Time(Genotype)	Robin	-0.00015	0.000295	4.59	-0.51	0.6320

Appendix 11. Germ tube length per 5 germinated conidia of *Stemphylium botryosum* isolate SB-19

Table A11.1 Type 3 tests of fixed effects for germ tube length per 5 germinated conidia of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 12 and 24 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	7.43	8.77	0.0053
Time	1	11.3	231.82	<.0001
Genotype*Time	5	7.43	2.33	0.1441

Table A11.2 Comparison of means for germ tube length per 5 germinated conidia of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 12 to 24 h post-inoculations

Effect	Genotype	_Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	-53.1722	13.9042	5.34	-3.82	0.0109
Genotype	Eston	L01-827A	-74.2561	18.5794	3.08	-4	0.0267
Genotype	Eston	LR-66-577	-90.6473	26.567	4.37	-3.41	0.0235
Genotype	Eston	LR-66-637	-44.5781	21.018	2.98	-2.12	0.1247
Genotype	Eston	Robin	-65.4885	12.0951	7.22	-5.41	0.0009
Genotype	IG 72815	L01-827A	-21.0839	20.513	4.03	-1.03	0.3617
Genotype	IG 72815	LR-66-577	-37.4751	27.9535	5.12	-1.34	0.2365
Genotype	IG 72815	LR-66-637	8.5941	22.7452	3.78	0.38	0.7258
Genotype	IG 72815	Robin	-12.3163	14.8957	5.98	-0.83	0.4401
Genotype	L01-827A	LR-66-577	-16.3912	30.5494	5.77	-0.54	0.6116
Genotype	L01-827A	LR-66-637	29.6779	25.8692	4.37	1.15	0.3102
Genotype	L01-827A	Robin	8.7676	19.3326	3.5	0.45	0.6768
Genotype	LR-66-577	LR-66-637	46.0692	32.0909	5.96	1.44	0.2014
Genotype	LR-66-577	Robin	25.1588	27.0991	4.68	0.93	0.3986
Genotype	LR-66-637	Robin	-20.9103	21.6867	3.32	-0.96	0.3999

Appendix 12. Proportion of germ tube penetrations of *Stemphylium botryosum* isolate SB-19

Table A12.1 Type 3 tests of fixed effects for proportion of germ tube penetrations of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 6 and 12 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	17.1	3.89	0.0155
Time	1	17.1	30.74	<.0001
Genotype*Time	5	17.1	0.81	0.561

Table A12.2 Comparison of means for proportion of germ tube penetrations of *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated for 6 and 12 h post-inoculations

Effect	Genotype	_Genotype	Estimate	STDERR	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	-0.03867	0.05362	17.1	-0.72	0.4805
Genotype	Eston	L01-827A	-0.05524	0.05362	17.1	-1.03	0.3173
Genotype	Eston	LR-66-577	-0.02664	0.05362	17.1	-0.5	0.6257
Genotype	Eston	LR-66-637	-0.02754	0.05362	17.1	-0.51	0.6141
Genotype	Eston	Robin	-0.2073	0.05362	17.1	-3.87	0.0012
Genotype	IG 72815	L01-827A	-0.01657	0.05362	17.1	-0.31	0.7611
Genotype	IG 72815	LR-66-577	0.01203	0.05362	17.1	0.22	0.8251
Genotype	IG 72815	LR-66-637	0.01113	0.05362	17.1	0.21	0.838
Genotype	IG 72815	Robin	-0.1686	0.05362	17.1	-3.14	0.0059
Genotype	L01-827A	LR-66-577	0.0286	0.05362	17.1	0.53	0.6006
Genotype	L01-827A	LR-66-637	0.0277	0.05362	17.1	0.52	0.6121
Genotype	L01-827A	Robin	-0.152	0.05362	17.1	-2.84	0.0114
Genotype	LR-66-577	LR-66-637	-0.0009	0.05362	17.1	-0.02	0.9867
Genotype	LR-66-577	Robin	-0.1806	0.05362	17.1	-3.37	0.0036
Genotype	LR-66-637	Robin	-0.1797	0.05362	17.1	-3.35	0.0038

Appendix 13. Percentage of dead leaflet tissue of *Stemphylium botryosum* isolate SB-19

Table A13.1 Type 3 tests of fixed effects of the regression analysis for percentage of dead leaflet tissue caused by *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 72 to 144 h post-inoculations

Effect	Num DF	Den DF	F Value	Pr > F
Genotype	5	58	8.99	<.0001
Time	1	58	14.8	0.0003
Time*Genotype	5	58	0.78	0.5681

Table A13.2 Comparison of means for percentage of dead leaflet tissue caused by *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 72 to 144 h post-inoculations

Effect	Genotype	_Genotype	Time	Estimate	Standard	DF	t Value	Pr > |t|
Genotype	Eston	IG 72815	72	-21.6698	10.8235	58	-2	0.05
Genotype	Eston	L01-827A	72	-26.9421	10.8235	58	-2.49	0.0157
Genotype	Eston	LR-66-577	72	-28.4143	10.8235	58	-2.63	0.0111
Genotype	Eston	LR-66-637	72	-10.0267	10.8235	58	-0.93	0.3581
Genotype	Eston	Robin	72	-12.3208	10.8235	58	-1.14	0.2597
Genotype	IG 72815	L01-827A	72	-5.2722	10.8235	58	-0.49	0.628
Genotype	IG 72815	LR-66-577	72	-6.7444	10.8235	58	-0.62	0.5356
Genotype	IG 72815	LR-66-637	72	11.6431	10.8235	58	1.08	0.2865
Genotype	IG 72815	Robin	72	9.3491	10.8235	58	0.86	0.3913
Genotype	L01-827A	LR-66-577	72	-1.4722	10.8235	58	-0.14	0.8923
Genotype	L01-827A	LR-66-637	72	16.9154	10.8235	58	1.56	0.1235
Genotype	L01-827A	Robin	72	14.6213	10.8235	58	1.35	0.182
Genotype	LR-66-577	LR-66-637	72	18.3872	10.8235	58	1.7	0.0947
Genotype	LR-66-577	Robin	72	16.0935	10.8235	58	1.49	0.1425
Genotype	LR-66-637	Robin	72	-2.2941	10.8235	58	-0.21	0.8329
Genotype	Eston	IG 72815	96	-25.3286	7.6182	58	-3.32	0.0015
Genotype	Eston	L01-827A	96	-28.2952	7.6182	58	-3.71	0.0005
Genotype	Eston	LR-66-577	96	-33.7952	7.6182	58	-4.44	<.0001
Genotype	Eston	LR-66-637	96	-10.9919	7.6182	58	-1.44	0.1544
Genotype	Eston	Robin	96	-8.2897	7.6182	58	-1.09	0.281
Genotype	IG 72815	L01-827A	96	-2.9667	7.6182	58	-0.39	0.6984
Genotype	IG 72815	LR-66-577	96	-8.4667	7.6182	58	-1.11	0.271
Genotype	IG 72815	LR-66-637	96	14.3367	7.6182	58	1.88	0.0649
Genotype	IG 72815	Robin	96	17.0389	7.6182	58	2.24	0.0292
Genotype	L01-827A	LR-66-577	96	-5.5	7.6182	58	-0.72	0.4732
Genotype	L01-827A	LR-66-637	96	17.3033	7.6182	58	2.27	0.0269
Genotype	L01-827A	Robin	96	20.0056	7.6182	58	2.63	0.011
Genotype	LR-66-577	LR-66-637	96	22.8033	7.6182	58	2.99	0.0041
Genotype	LR-66-577	Robin	96	25.5056	7.6182	58	3.35	0.0014
Genotype	LR-66-637	Robin	96	2.7022	7.6182	58	0.35	0.7241
Genotype	Eston	IG 72815	120	-29.719	7.6182	58	-3.9	0.0003
Genotype	Eston	L01-827A	120	-29.919	7.6182	58	-3.93	0.0002
Genotype	Eston	LR-66-577	120	-40.2524	7.6182	58	-5.28	<.0001
Genotype	Eston	LR-66-637	120	-12.1502	7.6182	58	-1.59	0.1162
Genotype	Eston	Robin	120	-3.4524	7.6182	58	-0.45	0.6521
Genotype	IG 72815	L01-827A	120	-0.2	7.6182	58	-0.03	0.9791
Genotype	IG 72815	LR-66-577	120	-10.5333	7.6182	58	-1.38	0.1721
Genotype	IG 72815	LR-66-637	120	17.5689	7.6182	58	2.31	0.0247
Genotype	IG 72815	Robin	120	26.2667	7.6182	58	3.45	0.0011
Genotype	L01-827A	LR-66-577	120	-10.3333	7.6182	58	-1.36	0.1802
Genotype	L01-827A	LR-66-637	120	17.7289	7.6182	58	2.33	0.0232

Genotype	L01-827A	Robin	120	26.4667	7.6182	58	3.47	0.001
Genotype	LR-66-577	LR-66-637	120	28.1022	7.6182	58	3.69	0.0005
Genotype	LR-66-577	Robin	120	36.8	7.6182	58	4.83	<.0001
Genotype	LR-66-637	Robin	120	8.6978	7.6182	58	1.14	0.2583
Genotype	Eston	IG 72815	144	-34.1095	11.637	58	-2.93	0.0048
Genotype	Eston	L01-827A	144	-31.5429	11.637	58	-2.71	0.0088
Genotype	Eston	LR-66-577	144	-46.7095	11.637	58	-4.01	0.0002
Genotype	Eston	LR-66-637	144	-13.3084	11.637	58	-1.14	0.2575
Genotype	Eston	Robin	144	1.3849	11.637	58	0.12	0.9057
Genotype	IG 72815	L01-827A	144	2.5667	11.637	58	0.22	0.8262
Genotype	IG 72815	LR-66-577	144	-12.6	11.637	58	-1.08	0.2834
Genotype	IG 72815	LR-66-637	144	20.8011	11.637	58	1.79	0.0791
Genotype	IG 72815	Robin	144	35.4944	11.637	58	3.05	0.0034
Genotype	L01-827A	LR-66-577	144	-15.1667	11.637	58	-1.3	0.1972
Genotype	L01-827A	LR-66-637	144	18.2344	11.637	58	1.57	0.1226
Genotype	L01-827A	Robin	144	32.9278	11.637	58	2.83	0.0064
Genotype	LR-66-577	LR-66-637	144	33.4011	11.637	58	2.87	0.0057
Genotype	LR-66-577	Robin	144	48.0944	11.637	58	4.13	0.0001
Genotype	LR-66-637	Robin	144	14.6933	11.637	58	1.26	0.2118

Table A13.3 Parameter estimates of the regression analysis for percentage of dead leaflet tissue caused by *Stemphylium botryosum* on *Lens ervoides* and *Lens culinaris* genotypes incubated from 72 to 144 h post-inoculations

	Effect	Genotype	Estimate	Error	DF	t Value	Pr>|t|
Intercepts	Genotype	Eston	-11.0276	11.6571	12.6	-0.95	0.3619
	Genotype	IG 72815	16.4962	11.6571	12.6	1.42	0.1812
	Genotype	L01-827A	18.0795	11.6571	12.6	1.55	0.1456
	Genotype	LR-66-577	25.9962	11.6571	12.6	2.23	0.0445
	Genotype	LR-66-637	0.5434	11.6571	12.6	0.05	0.9635
	Genotype	Robin	-5.1566	11.6571	12.6	-0.44	0.6657
slope		time	0.2878	0.07416	63	3.88	0.0003