# Investigating the potential use of unsaturated fatty acids as antifungal crop protective agents

by

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## ABSTRACT

Pathogenic fungi cause significant yield losses and quality reductions to major crops including wheat, canola, and barley. Toxic metabolites produced by some phytopathogenic fungi also pose significant risks to animal and human health. Extensive application of synthetic fungicides is not a sustainable solution since it poses risks to human, animal and environmental health. Unsaturated fatty acids may provide such an alternative because of their possible direct antifungal activity against phytopathogens as well as through the stimulation of plant defense pathways. The present study assessed the *in vitro* and *in vivo* efficacy of two hydroxy fatty acids; coriolic acid and ricinoleic acid, against the phytopathogens *Fusarium graminearum*, *Pyrenophora tritici-repentis, Pyrenophora teres* f. *teres, Sclerotinia sclerotiorum*, *Leptosphaeria maculans*, and *Aspergillus niger*. Antifungal activity was evaluated using the broth micro-dilution method to determine the minimum inhibitory concentration (MIC). Results indicated that both coriolic acid and ricinoleic acid possessed the strongest inhibitory activity against *L. maculans* with MIC of 0.73 g/L and 0.83 g/L, respectively, followed by *A. niger* with MIC of 0.78 g/L for coriolic and MIC of 0.88 g/L for ricinoleic acid. A weaker inhibitory activity of coriolic acid and ricinoleic acid was observed in terms of other fungal pathogens with MICs which varied greatly between species. An antifungal effect was observed for coriolic acid *in vivo* against pathogenic fungi of wheat and barley. This effect was not correlated to the *in vitro* activity because ricinoleic acid with equivalent *in vitro* antifungal activity showed no protective effect *in vivo*. Moreover, neither coriolic acid nor ricinoleic acid controlled fungal pathogens of canola. In conclusion, coriolic acid inhibits some phytopathogens *in vivo* and may have the potential to be an effective crop protection agent.

## DEDICATION

*This work is dedicated to my parents, Mr. Mohammadreza Yasari and Mrs. Farah Rasoulpour Hedayati for their love, sacrifice, and tolerance. It is also dedicated to my beloved husband, Mr. Aidin Foroutan Naddafi, for his support in every single moment of my life.*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AOS | Allene oxide synthase |
| BLAST | Basic Local Alignment Search Tool |
| DAB | 3,3'-Diaminobenzidine |
| FHB | Fusarium head blight |
| H2O2 | Hydrogen peroxide |
| HSTs | Host-selective toxins |
| HUFAs | Hydroxy unsaturated fatty acids |
| ICS1 | Isochorismate synthase 1 |
| JA | Jasmonic acid |
| LPCB | Lacto Phenol Cotton Blue |
| MBSU | Molecular Biology Service Unit |
| MIC | Minimum Inhibitory Concentration |
| NBT | Nitroblue Tetrazolium |
| NFNB | Net form of net blotch |
| O2 – | Superoxide |
| PAD4 | Protein arginine deiminase 4 |
| PCR | Polymerase chain reaction |
| PDA | Potato dextrose agar |
| PDB | Potato Dextrose Broth |
| PR | Pathogenesis-related |
| PR1 | Pathogenesis-related protein 1 |
| RCBD | Randomized complete block design |

| | |
|---|---|
| ROS | Reactive Oxygen Species |
| RT | Room temperature |
| SA | Salicylic acid |
| SDW | Sterile distilled water |
| SEM | Standard error of the mean |
| SFNB | Spot form of net blotch |
| TMV | Tobacco mosaic virus |
| UFAs | Unsaturated fatty acids |
| VSP2 | Vegetative storage protein 2 |
| WRKY70 | WRKY transcription factor 70 |

# 1. GENERAL INTRODUCTION

Environmental factors (i.e. abiotic stresses) and challenges from disease causing organisms (i.e. biotic stresses) pose significant challenges to the growth and development of plants. Biotic stresses include diseases to plants caused by living organisms such as bacteria, fungi, viruses, nematodes, weeds and herbivorous insects (Fujita et al. 2006; Atkinson and Urwin, 2012) which negatively impress survival, crop productivity and food production worldwide (Agarwal et al. 2006). Amongst biotic stresses, pathogenic fungi pose some of the more serious threats for plants to cope with and are of major economic concerns to the agriculture industry (Montesinos et al. 2002; Strange and Scott 2005). For instance, pathogenic fungi cause severe yield losses and quality reductions to major crops including wheat (*Triticum aestivum* L.) (Shabeer and Bockus 1988; Savary et al. 2012), canola (*Brassica napus* L.) (Pageau et al. 2006; del Río et al. 2007; Hwang et al. 2016), and barley (*Hordeum vulgare* L.) (Jayasena et al. 2007; Murray and Brennan 2010). Therefore, continued research into the development of effective disease control strategies to combat this threat posed by fungal pathogens is of utmost relevance.

The importance of the aforementioned crops for the Canadian economy cannot be understated (Statistics Canada 2017a,b) as they collectively contribute around $38.7 billion towards the economy per annum (Canola Council of Canada 2017a; The Canadian Wheat Alliance 2017; Barley Council of Canada 2017). In addition, their nutritional value for human health and wellness including the notable role of canola oil ingredients e.g. α-linolenic acid (11% of oil content by weight) and linoleic acid (21% of the oil content by weight) (Przybylski et al. 2005; Gunstone 2011, Canola Council of Canada 2017b) in the prevention of cardiovascular

diseases further increase the value of some of the major Canadian crops (Ascherio et al. 1996; Hu et al. 1999; Djousse et al. 2001). Similarly, various health benefits of arabinoxylan oligosaccharides, which are the main component of dietary fiber in cereal grains such as wheat and barley, have also been demonstrated (Lu et al. 2000; Aune et al. 2011; Jonnalagadda et al. 2011; Broekaert et al. 2011; François et al. 2012). Therefore, the major Canadian crops have significant value from both economic and nutritional / health standpoints.

As alluded to earlier, severe diseases caused by pathogenic fungi have led to extensive yield losses in the production of wheat, canola and barley resulting in negative effects on the economy. In addition to crop and associated economic losses, toxic metabolites produced by some phytopathogenic fungi pose significant risks to animal and human health (Fajardo et al. 1995; Bottalico and Perrone 2002; Yoshida et al. 2008). Excessive application of synthetic fungicides also is not an ideal solution since it leads to insensitivity in pathogen populations (Brown et al. 2004; Gossen et al. 2014) and poses significant risks to human, animal, and environmental health (Alavanja et al. 2014). Therefore, the development of alternate crop protection strategies to mitigate the negative effects of fungal plant pathogens is of utmost necessity.

Studies have shown that unsaturated fatty acids (UFAs) and their derivatives (methylated or hydroxy) could have potential as environmentally-friendly fungicides, due to their direct antifungal activity against phytopathogens as well as through the stimulation of defense pathways in different plant species (Graner et al. 2003; Prost et al. 2005; Pohl et al. 2011). Numerous studies have been conducted on the application of UFAs and their derivatives against phytopathogens demonstrating their antifungal activity and potential as environmentally-friendly fungicides because of their molecular configuration and structure (Sjögren et al. 2003; Pohl et al.

2011; Black et al. 2013). However, the precise mechanism behind this antimicrobial activity and compound-target specificity is still unknown. Moreover, their potential as effective antifungal agents in the protection of important crops for the Canadian economy has not been investigated.

# 2. LITERATURE REVIEW

## 2.1. Wheat

Wheat is an important monocotyledonous crop belonging to the family Poaceae (Gramineae) and genus *Triticum* (NCBI 2017a). History showed that wheat was the first domesticated crop (Gill et al. 2004; Li et al. 2007); however, Canada has a relatively short history in terms of wheat cultivation (Agriculture and Agri-food Canada 2017). *T. aestivum* L. (bread or common wheat) and *T. turgidum* L. (durum or pasta wheat) are two important species in the *Triticum* genus, which are considered to be important food staples for human and animals (Paux et al. 2008; Shewry 2009; Zohary and Hopf 2012; Cooper 2015). Wheat grain is primarily composed of macronutrients including carbohydrates, proteins, lipids, fibre, as well as micronutrients (minerals and vitamins) (Šramková et al. 2009), supplying more than 20% of the world total dietary calories and proteins alone and making it one of the most important crops for global food security (Hawkesford et al. 2013; Shiferaw 2013). Industrial usage of wheat includes application of its fiber/gluten/starch in food additives, aquaculture feeding, adhesives and making plastics, and even production of ethanol (Day et al. 2006; FAOSTAT 2017).

Wheat is the second largest grain crop after corn, produced worldwide about 755 million tons in 2017 (USDA 2017a). Among all the wheat species, common wheat is the most widely grown wheat globally. The European Union, China, India, Russia, and the United States are the top five wheat producing countries around the world (USDA 2017b). Canada is the sixth largest producer of wheat with the seeded area of 9.12 million hectares and total production of 29.9 million tons in 2017 (Statistics 2017c). More than 90% of Canadian wheat crops are grown in the Prairie provinces Alberta, Saskatchewan and Manitoba (Aboukhaddour et al. 2013) with a

production of 9.98, 12.9, and 4.36 million tons in 2017, respectively (Statistics 2017c). Currently, Canada is the fourth largest exporter of wheat (21 million tons in 2017) in the world (USDA 2017b).

### 2.1.1. Important fungal diseases of wheat

Fungal pathogens impact the development and production of the wheat crop. *Fusarium graminearum* Schwabe is the primary fungal agent of the devastating disease called fusarium head blight (FHB) or scab infecting wheat and other small grains worldwide (Parry et al. 1995). In 1982, scab reduced wheat production in the United States by about 4% in total, representing more than 2.72 million metric tons (Boosalis et al. 1982). Severe outbreaks of FHB also have been reported in Canada (Martin and Johnston 1982; Seaman 1982; Tekauz et al. 1986). However, other *Fusarium* species including *F. avenaceum*, *F. culmorum*, and *F. poae* can cause FHB as well as seedling blight and foot rot diseases in wheat and other cereals (Wong et al. 1992; Parry et al. 1995; McMullen et al. 1997; Schmale III and Bergstrom 2003).

*Puccinia graminis* f. sp. *tritici* Eriks & E. Henn. (causal agent of stem rust), *Puccinia triticina* Eriks. (= *Puccinia recondita* Rob. ex Desmaz. F. sp. *tritici*) (causal agent of leaf rust), and *Puccinia striiformis* Westend. f. sp. *tritici* (Pst) (causal agent of stripe or yellow rust) are also three obligate fungal parasites causing major losses (up to 100%) on susceptible cultivars of wheat around the world (Leonard and Szabo 2005; Chen 2005; Wegulo 2012a; Sharma et al. 2016). Powdery mildew, caused by biotrophic fungi *Blumeria graminis* f. sp. *tritici* Em., (Mwale et al. 2014) and Septoria tritici blotch caused by *Mycosphaerella graminicola* (anamorph: *Septoria tritici*) (Ponomarenko et al. 2011) are other important foliar pathogen of wheat worldwide. Tan spot caused by the fungus *Pyrenophora tritici-repentis* is another foliar disease which has the potential to reduce yield up to 50 % in all major wheat growing regions around the

world (Hosford 1982; Shabeer and Bockus 1988; Lamari and Strelkov 2010). In the subsequent section, the causal agent of tan spot disease in wheat, which was used for investigations in the research described in this thesis, is outlined.

### 2.1.2. Pyrenophora tritici-repentis

#### 2.1.2.1. Taxonomy, epidemiology, and symptoms

*Pyrenophora tritici-repentis* (Died.) Drechs. (Anamorph: *Drechslera tritici-repentis* (Died.) Shoem.) is an ascomycete fungus belonging to Pleosporaceae family. The pathogen can infect all classes of cereals such as wheat, rye, wild barley, oat as well as other grasses (Hosford 1971; Krupinsky 1992; Strelkov and Lamari et al. 2003; Wegulo et al. 2012b).

The disease caused by this fungus is recognized by two distinct symptoms on the leaves of susceptible wheat cultivars; this includes necrosis (tan color) and extensive chlorosis (yellow color). Initially, the lesions on leaves appear as tan/brown flecks, then they develop into oval- or diamond-shaped lesions with dark brown center and/or yellow circular border. In severe infection, the lesions may coalesce and cover most or the entire of the leaf surface resulting in their death (Kader 2010). These symptoms develop specifically and result from an interaction between the pathogen secreted host-selective toxins (HSTs) and the target receptors of a toxin-sensitive host plant (Strelkov and Lamari 2003; Singh et al. 2010; Aboukhaddour et al. 2011). HSTs lead to toxicity and disease induction in certain susceptible hosts (Scheffer and Briggs 1981). These compounds have no or little effect on resistant host genotypes (Scheffer and Livingston, 1984).

#### 2.1.2.2. Importance of tan spot on wheat

Tan spot is one of the major foliar diseases caused by *P. tritici-repentis* affecting wheat crops worldwide (Hosford 1982). The first severe outbreak of tan spot dates to 1974 in Canada

(Tekauz 1976). Severe disease epidemics have been also reported in South America, USA, and Australia (Kohli et al. 1992; Murray and Brown 1987; Schilder and Bergstrom 1995) as well as in some of the European countries (Cook and Yarham 1989; Leisova et al. 2008). Yield reductions due to tan spot may vary depending on the host growth stage at the time of infection. Yield loss of 13% during the seedling stage of infection, 35% during late growth stage and 48% from the presence of disease throughout the season were reported (Rees and Platz 1983). Not only *P. tritici-repentis* decrease wheat productivity, but it also affects the seed quality by causing red smudge (reddish discoloration of grain) and/or black smudge (blackening of the germ end) (Francl and Jordahl 1992; Fernandez et al. 2001). Such a reduction in grain yield associated with tan spot was reported in Germany ranging from 10-36% (Wolf and Hoffmann 1993). In Canada, the susceptibility of all registered durum wheat cultivars to red smudge has also been reported (Fernandez et al. 1997).

**2.1.2.3. Management of tan spot in wheat**

Genetically-resistant wheat cultivars are often considered as sustainable, most effective and most economical management methods for the control of tan spot (De Wolf et al. 1998; Gamba et al. 1998; Reide et al. 2003). However, in Canada, only a limited number of wheat cultivars have resistance to tan spot (Lamari et al. 2005). Tan spot disease severity can be reduced by tillage of the soil (Schuh 1990; Bockus and Claassen 1992; Stover et al. 1996). Crop rotation is also another effective cultural method for reduction of tan spot involving the cultivation of susceptible wheat crops with annual breaks in the same field (De Wolf et. 1998). A 3-year crop rotation between wheat crops can also decrease tan spot incidence (Rees and Platz 1979). Crops such as soybean, alfalfa, peas, and flax (Bailey et al. 1992; Hosford 1971), being non-hosts, are generally good choices for rotation with wheat crops.

Several fungi and bacteria have been regarded as biocontrol agents of *P. tritici-repentis* which ultimately result in yield growth (Gough and Ghazanfani 1982; Pfender et al. 1991). The antifungal metabolites produced by *Pseudomonas fluorescens* strain Pf-5, have shown the inhibitory function against both mycelial growth of *P. tritici-repentis in vitro* and ascocarp formation on wheat straw (Pfender et al. 1993). Six isolates of *Trichoderma harzianum* could reduce tan spot severity under field conditions either as seed treatments or foliar sprays at different growth stages (Perello et al. 2006).

Foliar application of fungicides is another effective approach for the management of wheat tan spot. So far, several fungicides from different classes have been applied to control tan spot (Colson et al. 2003; Wegulo et al. 2011; Patel et al. 2012; Harvey et al. 2015). Application of propiconazole found to decrease tan spot infection in wheat and increase grain yield (Entz et al. 1990). In Denmark, pyraclostrobin, picoxystrobin, propiconazole and prothioconazole were the most effective fungicides for tan spot controlling with efficacies 55-97% (Jorgensen and Olsen 2007). However, a reduced sensitivity to a range of strobilurin fungicides have been found for field isolates of *P. tritici-repentis* in Germany (Reimann and Deising 2005). In western Canada, numerous foliar fungicides are used to control tan spot disease which are listed in the "Crop Protection" e-book (Alberta Agriculture and Forestry 2017a).

**2.2. Barley**

Barley (*Hordeum vulgare* L.) is an economically important cereal crop along with wheat which belongs to the family Poaceae (Gramineae) (NCBI 2017b). The first barley cultivar adopted for the Canadian climate with high yield was released in 1889 by the Ontario Agriculture College. During the 1950-1960s, desirable traits of Canadian six-row barley were identified (i.e. high malt extract levels, quick processing, and high levels of starch-degrading

enzymes), resulting in introduction of the cultivar "Harrington" as the first Canadian-bred two-row malting barley in the world market. Later, "AC Metcalfe" and "CDC Copeland" were developed as two-row malt cultivars and distinguished for their quality, leading to Canada's reputation for premium malt barley (GoBarley 2017). Currently three different classes of barley are cultivated in Canada for general purposes (i.e. cosmetic industry), malting, and food barley (Schouest et al. 2012; Decloedt et al. 2015; Rodbotten et al. 2015; Rosser et al. 2016; Canadian Grain commission 2017a).

Barley is the fourth largest crop among grains behind maize, wheat, and rice; its total production in the world was 141.8 million tons in 2017 (USDA 2017c). The European Union, Russia, Ukraine, Australia, and Canada are, respectively, the top five producers of barley around the world (USDA 2017c). In 2017, Canada had a seeded area of 2.33 million hectares produced 7.9 million tons barley (Statistics 2017c), of which 7.5 million tons was produced in the Prairie provinces Alberta, Saskatchewan and Manitoba (Statistics 2017c). In the Prairie provinces, 60.8% of the total seeded area were allocated to malting barley and 31.8% assigned for barley used for general purposes. A relatively small percentage (1.3%) of seeded area was also devoted to food (Canadian Grain Commission 2017b). In 2017, Canada was ranked as the world's sixth exporter, with 1.5 million tons, after European union, Australia, Russia, Ukraine, and Argentina (USDA 2017d).

### 2.2.1. Important fungal diseases of barley

Pathogenic fungi pose a threat to barley crops and reduce their productivity. *Puccinia hordei* (causal agent of leaf rust), *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn. (causal agent of stem rust), *Puccinia striiformis* Westend. f. sp. *hordei* (causal agent of stripe rust), *Pyrenophora teres* Drechs. (causal agent of net blotch), *Blumeria graminis* (DC.) Golovin: Speer

f. sp. *hordei* Em. (causal agent of powdery mildew), and *Rhynchosporium secalis* (Oudem.) J.J. Davis (causal agent of scald) are the major fungi causing foliar diseases in barley with severe yield losses (Czembor 2001; Sun et al. 2006; Xi et al. 2008; Fetch et al. 2011; Safar Ali Safavi et al. 2012; Akhavan et al. 2017). In 1963, severe losses associated by FHB in barley threatened some of the population with starvation in South Korea (Vestal 1964). Loose smut (caused by *Ustilago nuda* (Jensen) Kellerman & Swingle.), covered smut (caused by *Ustilago hordei* (Pers.) Lagerh.) are other severe fungal diseases of barley affecting its seed, head and root with yield reductions (Menzies et al. 2014; Tekauz 2000). In the subsequent section, the pathosystem of necrotrophic fungi *Pyrenophora teres,* which is used in this research, is described.

### 2.2.2. Pyrenophora teres

#### 2.2.2.1. Taxonomy, epidemiology, and symptoms

*Pyrenophora teres* Drechs. (Anamorph: *Drechslera teres* (Sacc) Shoem.) is an ascomycete fungus which belongs to the family Pleosporaceae (Liu et al. 2011). *Hordeum vulgare* and *H. vulgare* ssp. *spontaneum* are the primary hosts of this pathogen. However, other wild *Hordeum* species, as well as gramineous species from the genera such as *Bromus*, *Avena*, *Triticum*, and *Elymus* are other hosts of this fungus (Shipton et al. 1973; Liu et al. 2011).

*P. teres* has two forms: *P. teres* f. *teres* (*Ptt*) (causal agent of net form of net blotch, NFNB) and *P. teres* f. *maculata* (*Ptm*), (causal agent of spot form of net blotch, SFNB), on barley (Rau et al. 2007; Liu et al. 2011). These two forms are microscopically identical, morphologically similar but genetically different; however, they can be differentiated based on morphological characteristics and the polymerase chain reaction (PCR) based molecular markers (Leisova et al. 2005; Keiper et al. 2008; Lu et al. 2010; Bogacki et al. 2010).

NFNB and SFNB symptoms appear on all upper parts of the barley including leaves, stems, leaf sheaths, and kernels (Liu et al 2011). The pathogen causing NFNB directly penetrates the leaves and initially results small circular to elliptical pin-point lesions. As the symptoms develop in both horizontal and vertical directions, they cause distinct dark-brown net-like patterns with horizontal and vertical reticulations (Steffenson 1997; Liu et al. 2011). The pathogen causing SFNB results dark-brown and circular to elliptical lesions encircled by a chlorotic zone of varying width, depending on the isolates virulence and host resistance (McLean et al. 2009).

#### 2.2.2.2. Importance of net blotch on barley

*P. teres* is an economically important foliar disease of barley throughout the world causing a loss of grain yield and the quality (Tekauz 1990; Steffenson 1997; McLean et al. 2009; Liu et al. 2011). NFNB and SFNB can typically reduce the productivity of barley cultivars up to 10-40% and 44%, respectively. However, they have the potential for causing 100% yield loss of susceptible cultivars under conducive environmental conditions for disease development (Mathre 1997; Steffenson et al. 1991; Jayasena et al. 2007; Murray and Brennan 2010). Each form of net blotch has different importance in barley-growing areas worldwide depending on susceptibility of the barley cultivars to local pathotypes of *P. teres*, availability of cultural control measures, and the climatic conditions (Steffenson 1997). In France, SFNB was found to be the predominant form of the disease in many regions (Arabi et al. 1992; while in Norway, both forms are present and no strong evidence of predominance of any of these two could be found (Wonneberger et al. 2017). In western Canada, the more prevalent form of *P. teres* seems to be the *Ptt,* which comprises 82% of the total isolates in the collection coming from the Prairie provinces (Alberta, Saskatchewan and Manitoba) (Tekauz 1990). However, several studies showed an increase in the

incidence of *Ptm* over time (Tekauz 1990; van den Berg and Rossnagel 1991; Liu and Friesen 2010), indicating changes in resistance in barley cultivars to *Ptt* and *Ptm* in this region, and/or even in climatic conditions (Tekauz 1990; Louw et al. 1996).

#### 2.2.2.3. Management of net blotch in barley

Crop rotation is one of the management strategies available for the control of net blotch of barley in western Canada (Tekauz 2003). Monoculture of barley resulted in increased net blotch severity compared with a non-host crop rotation (Krupinsky et al. 2004; Turkington et al. 2005; Turkington et al. 2012). However, the high demand of barley for livestock feed and market factors make crop rotation as short-term strategy for controlling net blotch severity and sustaining barley productivity (Turkington 2005). Burning stubble and conventional tillage are considered as other cultural control methods in UK (Jordan and Allen 1984). However, several studies showed that there is no significant difference in the adoption of tillage system either conventional tillage or conservation tillage, in the control of net blotch severity (Mathre 1997; Bailey et al. 2000; Martin et al 2001; Turkington et al. 2006).

The biocontrol activity of two *Pseudomonas fluorescens* strains (MKB100 and MKB156) was observed in foliar application against net blotch symptom development in detached leaf experiments in glasshouse conditions and small-scale field trials (Khan et al. 2010). *Pseudomonas chlororaphis* MA 342 was also found to be an effective and consistent biocontrol agent against the fungus *P. teres (*Hökeberg 1998; Tombolini et al. 1999).

Deployment of resistant cultivars is the most effective and environmentally-friendly means of disease management which is also cost-efficient for growers by reduction or elimination of the need for fungicide applications and cultural control practices. In Canada, the incidence of SFNB has been reduced by 61% with cultivation of resistant 6-rowed cultivars

relative to the susceptible 2-rowed barley cultivars (Tekauz 1976; Tekauz 1990). In another study performed by Turkington et al. (2006), susceptible barley cultivars showed 5-12 times greater potential of severe infection by NFNB compared to resistant cultivars. Currently, most of the commercial barley cultivars, especially the malting types, are susceptible to net blotch (Alberta Agriculture and Forestry 2017c), and very few NFNB-resistant cultivars are available (Alberta Agriculture and Forestry 2017a).

Foliar treatment with fungicides is another method of control by growers where the ecofriendly methods are inadequate (Mathre 1997; Tekauz 2003; Turkington et al. 2011; Turkington et al. 2015). Several classes of fungicides have been identified as effective chemicals in controlling SFNB and NFNB; this includes pyraclostrobin, propiconazole, azoxystrobin, epoxiconazole, and trifloxystrobin (Jayasena et al. 2002). The effectiveness of these fungicides for disease control is dependent on many factors such as their concentration, mode of action, active ingredient, and timing and numbering of applications (Khan et al. 1989; van den Berg and Rossnagel 1990). A list of foliar fungicides for the control of barley net blotch in western Canada is provided in the "Crop Protection" e-book (Alberta Agriculture and Forestry 2017a). However, the frequent application of fungicides on genetically diverse populations of NFNB and SFNB poses a high risk for resistance development, so finding an economically viable alternative to current fungicides in crop protection is a necessity.

### 2.3. Canola

Canola (*Brassica napus* L) is an oilseed crop developed from rapeseed and belongs to the Brassicaceae (mustard) family (Casséus 2009). The elimination of undesirable characteristics of rapeseed including high content of erucic acid (C22:1) from seed oil and high level of sulphur compounds glucosinolates from seed meal led to the development of Canada's new version of

rapeseed, called “Canola”, in the early 1970s (Canola Council of Canada 2017a; Busch et al. 1994). Canola is an abbreviated name from "Canadian Oil Low Acid" referring to the cultivars of rapeseed which produce seed oils with less than 2% erucic acid and seed solid components with less than 30 μmol of glucosinolates per gram in the meal (Casséus 2009; Rempel et al. 2014; Canola Council of Canada 2017a). By the 1980's, the production of rapeseed almost replaced by canola in Canada (Casséus 2009).

*Brassica* species including *B. napus, B. rapa,* and *B. juncea* are cultivated for canola grade oil and meal. Canola oil, after extraction and refinement, can be utilized as a cooking oil, deep-frying oil, salad seasoning, and in margarine preparation (Rempel et al. 2014). Canola meal is a nutritive source of vitamins B and E as well as proteins which can be used in aquaculture and livestock feed industries (Rempel et al. 2014). Other usage of canola oil is involved in preparation of many non-edible products, such as biodiesel, cosmetics, detergents, inks, lacquers, lubricants, pharmaceuticals, and plastics (Canola Council of Canada 2017c).

The total worldwide production of brassica oilseeds, as one of the world’s most important crops, was 72.06 million tons in 2017 (USDA 2017e). This crop is widely grown in many countries throughout the world where Canada and China were the two leading countries producing by 19.90 and 13.10 million tons in 2017, respectively (USDA 2017e). Ninty-nine percent of the Canada’s canola is produced in the prairie provinces Saskatchewan, Alberta, and Manitoba while British Columbia, Ontario, Quebec and New Brunswick producing the rest 1% (Statistics Canada 2017c). Canola has been considered as one of the major cash crops in Canada, which contributes more than $26 billion annually. Canada exports 90% of its production to other countries, such as the United States, Japan, Mexico, and China (Rempel et al. 2014; Canola Council of Canada 2017a).

### 2.3.1. Important diseases of canola

There are various sources of fungal pathogens of canola which are transferred from the soil matrix to canola seed and residues, as well as from other susceptible host plants (Canola Council of Canada 2017d). Clubroot is an important soil-borne disease of canola, caused by *Plasmodiophora brassicae* Woronin, resulting in yield loss of 30-100% in severely infected canola crops (Strelkov et al. 2007; Hwang et al. 2011). *Rhizoctonia solani* Kühn*, Pythium* spp.*,* and *Fusarium* spp. are the other soil-borne pathogenic fungi of canola, infecting roots and young seedlings and causing seedling blight, damping off, foot rot*,* and brown girdling root rot diseases. White rust or staghead (*Albugo candida* Pers.), alternaria black spot (*Alternaria brassicae* Berk. *and Alternaria raphani* Groves and Skolko), white leaf spot and gray stem (*Pseudocerosporella capsellae* Ellis and Everh.), and blackleg (*Leptosphaeria maculans* (Desm.) Ces. & De Not.) are known as stubble- or residue-borne diseases of canola (Martens et al. 1988; Guo et al. 2005). The two destructive fungal pathogens, which are studied in this thesis research, *L. maculans* and *S. sclerotiorum,* (West et al. 2001; Canola Council of Canada 2017d) are described in the following sections.

### 2.3.2. Leptosphaeria maculans

#### 2.3.2.1. Taxonomy, epidemiology, and symptoms

*Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph *Phoma lingam* Tode ex Fr.) is an ascomycete fungus which belongs to Leptosphaeriaceae family (Rouxel and Balesdent 2005). Members of this order are mostly saprophytes with an ability to live on dead plant materials, while some can live as parasites on living plants (Kaczmarek and Jedryczka 2011). *L. maculans* affects host species mainly of the genus *Brassica* (Rouxel and Balesdent 2005), including cabbage, cauliflower, broccoli, mustard, rapeseed, and canola.

The fungus gradually grows from lesions of leaves via the veins, colonizes in the petiole, and moves systemically through vascular tissue to the stem base without causing any macroscopic symptoms (West et al. 2001; Hayward et al. 2012). Inside the plant, blockage of the xylem vessels by the fungus restricts water and nutrient transmission which contributes to premature ripening, lodging and ultimately yield reduction (Kaczmarek and Jedryczka 2011). At the end of the growing season, the fungus becomes necrotrophic when it completely expands to the plant's crown and roots (Hayward et al. 2012) and causes crown cankers and stem lesions (West et al. 2001). On stems, lesions are usually found at the base with a distinct dark brown or purple margin (Hammond et al. 1985) and have the potential to girdle and even separate the stem in severe infections (West et al. 2001). Seeds also may become dry and pods fracture easily at harvest time, resulting in seed loss (Davies 1986) and denoting that canola is susceptible to blackleg infection from the seedling to the pod-set stages.

#### 2.3.2.2. Importance of blackleg in canola

Blackleg is one of the most economically important diseases of canola worldwide which cause extensive yield losses in Europe, Australia, and Canada (Gugel and Petrie 1992; Chen and Fernando 2006). It was not considered as a great economic concern until the 1950s, when severe epidemics occurred in France (1950), Australia (1972), and England (1977), with reported yield losses from 50% to 96% (Gugel and Petrie 1992; Toscano- Underwood et al. 2001). In 1998 and 1999, Australia also suffered from significant losses of $18.6 and $ 49.4 million in the oilseed industry as a result of this disease, respectively (Khangura and Barbetti 2001). In western Canada, *L. maculans* was firstly identified on canola stubble in central Saskatchewan in 1975. The disease incidence had increased by ten-fold between 1978 and 1981 (Juska et al. 1997) until it was found in 65% of the Saskatchewan's fields in 1986 (Jesperson 1989); then it dramatically

spread to Ontario (1986), Manitoba (1987) and Alberta (1988) (Gugel and Petrie 1992). In 1997, the Canola Council of Canada estimated the annual loss due to blackleg disease about 50 million CAD annually (Juska et al. 1997).

**2.3.2.3. Management of blackleg in canola**

Crop rotation and the cropping of blackleg resistant cultivars are of utmost importance in cultural control methods of blackleg disease management (Kharbanda and Tewari 1996; Gout et al. 2006). Short rotations or continuous cropping of canola cultivars prevents the complete decomposition of crop residues and readily breaks down the crop qualitative resistance, leading to development of *L. maculans* inoculum on infected canola stubble and debris (Huang et al. 2009; Marcroft et al. 2012; Kutcher et al. 2013). Breakdown of resistance has been reported in France (Rouxel et al. 2003) and Australia (Li et al, 2003). In addition to crop rotation, weed control is also considered as an effective method for blackleg management since *L. maculans* can survive on volunteer canola and other weed species (Kutcher et al. 2011). Other methods of cultural control of blackleg disease in canola are time adjustment of seeding in the season prior to maturation of pseudothecia and peak periods of ascospore discharge (Aubertot et al. 2004), tilling and burning the crop residue (Kharbanda and Tewari 1996; Guo et al. 2005), as well as flooding canola basal stems infected with *L. maculans* (Peluola et al. 2013). In Canada, all registered cultivars of *B. napus* have moderate to high resistance against blackleg disease (with unclear resistance type or specific resistance genes) (Kutcher et al. 2011). The heterothallic nature of *L. maculans* may increase genetic diversity, result in more virulent genotypes of the fungus population, and ultimately defeat the resistance (Aubertot et al. 2006).

Biological control procedures have been found to be useful for the management of blackleg in canola. For example, the Bird's nest fungi, *Cyathus striatus* and *Cyathus olla*, reduce

the stubble food for *L. maculans* survival and available inoculum for further spread (Shinners and Tewari, 1997). Two bacterial strains, *Bacillus endophyticus* and *Bacillus amyloliquefaciens* have been recognized as potential biocontrol agents against *L. maculans* (Danielsson et al. 2007). The bacterial isolate of *Serratia plymuthica* HRO-C48 and fungal isolate of *Gliocladium catenulatum* J1446 found to decrease the disease severity of *L. maculans* on infected cotyledons of *B. napus* by 44% and 52%, respectively (Hammoudi et al. 2012). The antifungal activity of peptides produced by the bacterium *Paenibacillus polymyxa* Prazmowski against *L. maculans* was also reported (Kharbanda et al. 2003).

In western Canada, synthetic fungicides are generally applied on canola both as seed and foliar treatments for the reduction of the incidence of blackleg disease. Seed treatment destroys seed borne inoculum, prevents the spread of blackleg into un-infected areas, and protects the young seedling from airborne ascospores with their systemic activity (Gugel and Petrie 1992). Foliar treatment reduces blackleg disease symptoms on leaves and results in increased yield (Kutcher et al. 2011); however, mixed successes have been reported regarding the efficacy of foliar fungicides in blackleg control (Gugel and Petrie 1992; Khangura and Barbetti 2004). In Alberta, there are currently six registered products for seed treatments and eight registered foliar fungicides for management of blackleg in canola (Alberta Agriculture and Forestry 2017a).

### 2.3.3. Sclerotinia sclerotiorum

#### 2.3.3.1. Taxonomy, epidemiology, and symptoms

*Sclerotinia sclerotiorum* (Lib.) de Bary is an ascomycete fungus which belongs to the Sclerotiniaceae family (Willets and Wong 1980; Bolton et al. 2006). *S. sclerotiorum* is a non-specific and necrotrophic pathogen which is known as the causal agent of disease in over 400 plant species from 75 different families (Boland and Hall 1994; Bolton et al. 2006; Attanayake et

al. 2013). The initial symptoms of infected tissues usually show as water-soaked lesions then they rapidly enlarge and develop into necrotic tissues with fluffy white mycelium (Boland and Hall 1994; Bolton et al. 2006). Later, the old lesions become bleached, shredded, and shattered when dry (Bolton et al. 2006).

#### 2.3.3.2. Importance of Sclerotinia stem rot on canola

Sclerotinia stem rot is one the most destructive diseases of canola in many countries around the world, with the potential to cause severe yield losses and oil quality reduction (Bardin & Huang 2001; Bolton et al. 2006; Gao et al. 2014). In China, this fungal disease could reduce canola yield ranging from 10% to 80% as well as the oil quality (Wang et al. 2014). In 2008, 36% of the canola crops cultivated in UK was damaged by sclerotinia stem rot leading to economic loss of £20 million (Young and Werner 2012). Annual incidence of *S. sclerotiorum* in the United States averaged around 13.6% (Bradley and Lamey 2005; Lamey 2003), which could contribute to an economic loss of $94 million between 1991 and 2002 (Lamey 2003). In western Canada, yield losses ranging from 5 to 10% due to Sclerotinia stem rot are typical in canola fields (Fang and Platford 1995; Platford 1996), although they may reach up to 94% in drastically infected fields (McLaren et al. 2005).

#### 2.3.3.3. Management of Sclerotinia stem rot in canola

Tilling the soil is a potential method for management of Sclerotinia stem rot, causing the sclerotia to be buried and preventing their successful germination (Williams and Stelfox 1980). It also may be effective in increased parasitism of the buried sclerotia resulting in reduced viability of the sclerotia (Kurle et al. 2001). Altering the crop canopy phenology through avoiding dense-planting is another cultural control strategy against *S. sclerotiorum* reducing the disease dispersal via plant to plant contact (Tu and Zeng 1997). Crop rotation is focused on decreasing the amount

of inoculum present in the field (Twengström et al. 1998). However, the ineffectiveness of a 3- or 4-year crop rotation away from a susceptible host in reduction of viable sclerotia populations in the field was also reported (Williams and Stelfox 1980; Morrall and Dueck 1982). This ineffectiveness could be attributed to the scattering of *S. sclerotiorum* ascospores by wind (Suzio and Koayashi 1972; Williams and Stelfox 1979), causing the infection of host crops by externally produced inoculum (Morrall and Dueck 1982). Moreover, the vast host range of *S. sclerotiorum* and presence of susceptible weeds and volunteers may lead to inoculum preservation, even in the absence of a canola crop (Boland and Hall 1994).

More than 30 species of fungi and bacteria exist with antagonistic or mycoparasitic activity against *Sclerotinia* spp (Adams and Ayres 1979). Fungi belonging to the genera *Fusarium, Gliocladium, Hormodendrum, Mucor, Pencillium, Trichoderma, Verticillium, Conithyrium,* and *Ulocladium* were identified as biocontrol agents of *S. sclerotiorum* (Adams and Ayres 1979; McLaren et al. 1996; Li et al. 2003). A bacterial suspension of *Bacillus subtilis* could strongly inhibit the mycelial growth of *S. sclerotiorum* and sclerotia germination at concentrations of $10^9$ and $10^{11}$ CFU/ml. It also significantly reduced the disease severity and incidence in field trials by 50-70% was as effective as the fungicide treatments including tebuconazole and carbendazim used for canola stem rot control (Gao et al. 2014). In Canada, three registered bio-control products are Serenade Max (PCP# 28549, Bayer Crop Science), Serenade CPB (PCP# 30143, Bayer Crop Science), and Contans WG (PCP#29066, Bayer Crop Science) which are used on canola against Sclerotinia stem rot (Government of Saskatchewan 2016; Alberta Agriculture and Forestry 2017a).

Deployment of resistant cultivars is another control measure for the management of Sclerotinia stem rot. However, there are no commercial cultivars with complete resistance

against Sclerotinia stem rot in the Canadian market (Canola Council of Canada 2017e). Some tolerant cultivars such as '45S52' from DuPont Pioneer were introduced to the market which can be cultivated by growers as another control measure, although they still can get damaged when the disease pressure is high (Pratt 2012).

In Canada and United States, application of fungicides at the flowering stage of canola is one of the conventional methods for controlling Sclerotinia stem rot, which often must be made prior to symptoms appearance (del Río et al. 2007; Koch et al. 2007; Turkington et al. 2011). In Alberta, several registered fungicides such as Acapela, Lance AG, Priaxor, Proline 480 SC, and Quadris are used for the management of this disease (Alberta Agriculture and Forestry 2017a). The application of fungicides in disease control may lead be successful, but it may not be guaranteed every year since over time the infection level by *S. sclerotiorum* changes. The extensive application of fungicides also causes fungicide insensitivity via increased selection pressure on pathogen populations. Such an example was confirmed in Canada when *S. sclerotiorum* populations developed resistance to the fungicide benomyl (Benlate), formerly used as chemical agent for control of canola and alfalfa diseases caused by *S. sclerotiorum* (Gossen et al. 2001).

### 2.4. Antimicrobial activity of UFAs against phytopathogens

Difficulties and challenges associated with pathogenic fungi such as severe yield losses, wide host range, lack of highly resistant cultivars, and increased virulence or resistance toward synthetic fungicides have motivated researchers to develop alternative or complementary fungicides.

UFAs and hydroxy unsaturated fatty acids (HUFAs) as organic compounds provide one such alternative. They include compounds with antifungal activity due to their specific structural

characteristics (Prost et al. 2005; Pohl et al. 2011; Cantrell et al. 2008). Coriolic acid (13-hydroxy-9(Z),11(E)-octadecadienoic acid), which was first extracted from the seed oil of *Coriaria nepalensis* (Tallent et al. 1966), could inhibit the spore germination and germ tube elongation of the rice blast fungus, *Pyricularia oryzae* (Namai et al. 1993). The accumulation of coriolic acid in Sasanishiki rice cultivar after infection with the rice blast fungus as anti-rice blast fungus compounds was also reported (Kato et al. 1993). The inhibitory activity of coriolic acid also was demonstrated for the *in vitro* growth of fungal pathogens such as *Cladosporium herbarum*, *Botrytis cinerea*, *Phytophthora infestans*, and *Phytophthora parasitica* var. *nicotianae* (Prost et al. 2005). Didehydrocoriolic acid has been shown to act as a self-defense compound against the rice blast disease (Ding et al. 2012). Application of coriolic acid by 0.15% in bread making could extend the mold-free shelf life of wheat bread from 2 to more than 6 days (Black et al. 2013). The growth-inhibiting activity of coriolic acid against food spoilage as well as fungal pathogens *Aspergillus niger* and *Penicillium roqueforti* was also observed (Liang et al. 2017).

Ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), constituting more than 80% of seed oil of castor plant (*Ricinus communis* L.) (Bafor et al. 1991), showed antifungal activity against pathogenic fungi *Aspergillus niger* and *Penicillium roqueforti in vitro* (Black et al. 2013; Chen et al. 2016; Liang et al. 2017). The methylated derivatives of ricinoleic acid such as methyl-12-(4-hydroxy-3-methoxyphenyl propenoate) octadec-9-en-1-oate and methyl -12-(4-hydroxy-3-methoxybenzoate) octadec-9-en-1-oate also possessed antifungal activity against fungal pathogens *Candida albicans*, *Candida rugosa*, *Saccharomyces cerevisiae*, *Rhizopus oryzae*, and *Aspergillus niger in vitro* experiments via agar well diffusion method. The antifungal activity of coriolic acid and ricinoleic acid accumulated in supernatant of *Pseudomonas aeruginosa* 42A2 against pathogenic fungi *Verticillium dhaliae*, *Macrophomina phaesolina*,

*Arthroderma uncinatum*, *Trycophyton mentagrophytes* and *Penicillium funiculosum* have been also reported (Martin-Arjol et al. 2010).

Other UFAs and their derivatives exert antimicrobial activity against diverse range of species. For example, 13(S)-hydroperoxy-9(Z),11(E),15(Z)-octadecatrienoic acid, 13(S)-hydorxy-9(Z),11(E),15(Z)-octadecatrienoic acid, and 5(Z)-etherolenic acid inhibited the mycelial growth of *Leptosphaeria maculans* and *Alternaria brassicae* at 1 mM concentration but their relative potency of inhibition was different (Graner et al. 2003). However, none of these UFAs were effective in inhibition of mycelial growth of *Sclerotinia sclerotiorum* at the same concentration, indicating species-specific mode of action (Graner et al. 2003). The growth-inhibiting activity of *threo*-12,13-dihydroxy-9(Z)-octadecenoic acid and *cis*-12,13-epoxy-9(Z)-octadecenoic acid was also reported against *Sclerotinia sclerotiorum* (Graner et al. 2003). The antiviral activity of oleic acid against *tobacco mosaic virus* (TMV) was demonstrated *in vivo* (Zhao et al. 2017). Methyl- and ethyl- oleate (3 g/L) showed antifungal activity against the powdery mildew of barley caused by *Blumeria graminis* f. sp. *hordei* (Choi et al. 2010). However, many of these UFAs are not stable chemically and tend to be degraded in the presence of biomolecules (Prost et al. 2011) or to be metabolized by several enzymes (Schaller et al. 2001; Chechetkin et al. 2004), explaining the transient nature of growth inhibitory effect and making intricate the antifungal functionality of the UFAs against the target fungi (Graner et al. 2003).

Apart from the direct antifungal activity of UFAs, they may also act simultaneously as signaling molecules to regulate plant self-defense pathways through their phytohormone mediators such as salicylic acid (SA) and jasmonic acid (JA) (as reviewed by Kunkel and Brooks 2002; and Kachroo and Kachroo 2009). Accumulation of oxylipins such as coriolic acid in rice plants led to enhanced resistance to rice blast fungal pathogen (Yara et al. 2008). Rice plants

treated with 2,6-dichloroisonicotinic acid (a synthetic resistance-inducer) and JA were efficiently protected from infection by the rice blast fungus being related to the induction of a set of pathogenesis-related (*PR*) genes (Schweizer et al. 1997). Increased expression of PR genes resulted in increased resistance to TMV also was illustrated in oleic acid-treated tobacco leaves (Zhao et al. 2017). Exogenous application of arachidonic acid (10 µM) on wild-type leaves of Arabidopsis demonstrated an increase in basal levels of JA which was related to upregulation of JA biosynthesis pathways gene (Allene oxide synthase, *AOS*) and JA-responsive gene (Vegetative storage protein 2, *VSP2*) in response to arachidonic acid treatment (Savchenko et al. 2010). However, this treatment reduced the SA levels and downregulated the expression of the genes protein arginine deiminase 4 (*PAD4*), isochorismate synthase 1 (*ICS1*), WRKY transcription factor 70 (*WRKY70*), and pathogenesis-related protein 1 (*PR1*), indicating the antagonistic interactions of JA and SA pathways to facilitate the fine-tuning of defense responses to different plant pathogens (Savchenko et al. 2010).

Signaling in plant defense pathways leads to the formation of UFAs, as signaling molecules or antimicrobial agents, which ultimately contributes to pathogen defense (Kachroo and Kachroo 2009). To date, several studies have been conducted regarding the application of UFAs and HUFAs against phytopathogens illustrating their antifungal activity (Graner et al. 2003; Prost et al. 2005; Pohl et al. 2011) and their potential as environmentally-friendly fungicides because of their molecular configuration. However, the exogenous application of UFAs on major Canadian crops as protective agents against pathogenic fungi has not been reported yet. Therefore, in the present research work, the efficacy of two HUFAs, coriolic acid and ricinoleic acid will be assessed.

## 2.5. Research objectives

The first objective of this project was to determine the antifungal activity and efficacy of coriolic acid (13-hydroxy-9(Z),11(E)-octadecadienoic acid) and ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid) against important fungal pathogens of canola (*Leptosphaeria maculans* and *Sclerotinia sclerotiorum*), wheat (*Fusarium graminearum* and *Pyrenophora tritici-repentis*), barley (*Pyrenophora teres* f. *teres*), and fruits (*Aspergillus niger*) *in vitro*. The second objective was to figure out the potential contribution of coriolic acid and ricinoleic acid in crop protection against the selected fungal pathogens of canola, wheat, and barley when they are applied exogenously on plants and on seeds (*in vivo*).

## 2.6. Research Hypotheses

The specific hypotheses of this study are as follows:

- Coriolic acid and ricinoleic acid could have significant inhibitory effects on mycelial growth of pathogenic fungi including *L. maculans, S. sclerotiorum, F. graminearum, P. tritici-repentis. P. teres* f. *teres,* and *A. niger in vitro*.
- Exogenous application of coriolic acid and ricinoleic acid on plants and/or seeds (*in vivo*) may inhibit the disease development and control plant infection.

# 3. MATERIALS AND METHODS

## 3.1. Preparation of UFAs

Coriolic acid (13-hydroxy-9(*Z*),11(*E*)-octadecadienoic acid) was generously provided by the Food Microbiology lab (Nuanyi Liang, PhD Candidate) of the Department of Agricultural, Food and Nutritional Science, University of Alberta. It was produced either through enzymatic oxidation of linoleic acid or extracted from seed oil of *Coriaria nepalensis* Wall (XinTai Seed Production and Wholesale Company, Jiangsu, China), then purified by high speed counter current chromatography (Nanda and Yadav 2003; Liang et al. 2017). Ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic) and oleic acid (9-cis-octadecenoic acid), each with purity > 99%, were also purchased from Nu-Check Prep, Inc. (Elysian, MN).

## 3.2. Preparation of fungal isolates

The fungal isolates *Pyrenophora tritici-repentis* AB 7-2 (Aboukhaddour et al. 2013), *Pyrenophora teres* f. *teres* AB 06 and AB 34 (Akhavan et al. 2016; 2017), and *Sclerotinia sclerotiorum* SS-01 (Navabi et al. 2010) were used in this study. *Aspergillus niger* FUA5001 was kindly provided by Dr. M. Gänzle, Department of Agricultural, Food and Nutritional Science, University of Alberta. *Fusarium graminearum* G-1, *Leptosphaeria maculans* RL-60 were generously provided by Dr. S.E. Strelkov, Department of Agricultural, Food and Nutritional Science, University of Alberta. The identities of both *F. graminearum* and *L. maculans* were confirmed via DNA sequence analysis (Demeke et al. 2005; Liu et al. 2006). Briefly, DNA was extracted from actively growing mycelia on PDA plates using Promega DNA isolation kit (Promega Corporation, Madison, WI, USA) and quality and quantity of the isolated DNA were determined using NanoDrop 1000 spectrophotometer (Thermo scientific, Waltham, MA, USA).

Two specific primer pairs were also selected from the literature (Table 3-1) for these two fungi and used for PCR amplification reactions (Demeke et al. 2005; Liu et al. 2006). PCR amplification was performed in a 25 µl of reaction including 2.5 µl of 10X PCR buffer (150 mM Tris-HCl, 500 mM KC), 2 mM $Mgcl_2$, 0.2 mM of dNTPs, 1 units of *Taq* DNA Polymerase, 0.2 µM each forward and reverse primer (Table 3-1) and 1 µl of mycelial DNA. The entire reaction was then incubated at 95 °C for 3 min for initial denaturation, followed by 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 62 °C for *F. graminearum* and 68°C for *L. maculans*, and 1 min extension at 72 °C, plus a final extension of 5 min at 72 °C in GeneAmp PCR System 9700 (Applied Biosystems, USA). PCR products were separated by electrophoresis in 2.0% (wt/vol) agarose gels stained with 0.6 µl of syber safe DNA gel stain (Invitrogen, USA). Then, the amplified products of expected size were isolated from agarose gels using a sterile scalpel blade on UV exposure box. DNA sequences were determined at the Molecular Biology Service Unit (MBSU), Department of Biological Sciences, University of Alberta. DNA sequences were confirmed using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 3-1. Specific primers used for identification of *Fusarium graminearum* and *Leptosphaeria maculans*

| **Fungal pathogens** | **Primer sequences** | **References** |
|---|---|---|
| ***Leptosphaeria maculans*** | Lm-F: CTTGCCCACCAATTGGATCCCCTA<br>Lm-R: GCAAAATGTGCTGCGCTCCAGG | Demeke et al. 2005 |
| ***Fusarium graminearum*** | Fg16-F: CTCCGGATATGTTGCGTCAA<br>Fg16-R: GGTAGGTATCCGACATGGCAA | Liu et al. 2006 |

### 3.3. Preparation of fungal inoculum

The required growth conditions for sporulation of all fungal isolates is described in Table 3-2. When fungal isolates sporulated on their specific media, approximately 5 ml of sterile distilled water was added to sporulating cultures and gently scraped using a sterile inoculation loop. Under sterile conditions, the spore suspensions were collected and passed through four layers of cheesecloth to eliminate mycelial cells. Spores were counted using a Neubauer counting chamber (Fein-Optik, Jena, Germany) and spore concentrations were adjusted for subsequent experiments.

Table 3-2. Fungal pathogens, pathogenicity and their specific sporulation conditions

| Fungal isolates | Pathogenicity | Culture conditions for sporulation |
|---|---|---|
| *Pyrenophora tritici-repentis* AB 7-2 | Tan spot in wheat | The fungus was grown on V8-PDA medium (150 ml V8 juice, 3 g $CaCO_3$ and 10 g agar, 10 g PDA for 1 L) at room temperature (RT: 21 ± 2 °C) under continuous darkness until colonies reached a diameter up to 5 cm. Mycelia were covered by sterile distilled water (SDW) and gently streaked with the bottom of a sterilized glass tube. Subsequently, plates were incubated for 16-19 h under fluorescent light at RT. Then they were incubated at 14ºC for 24 h (Aboukhaddour et al. 2013). |
| *Pyrenophora teres* f. *teres* AB 06 & AB 34 | Net boltch of barley (NFNB) | The fungus was grown on V8-agar (100 ml V8 juice, 3 g $CaCO_3$ and 20 g agar, for 1 L) at RT under continuous darkness until colonies reach the diameter up to 5 cm. Mycelia were covered by sterile distilled water and gently streaked with the bottom of a sterilized glass tube. Subsequently, plates were incubated for 16-19 h under fluorescent light at RT. Then they were incubated at 15 ºC for 24 h (Akhavan et al. 2017). |
| *Sclerotinia sclerotiorum* SS 01 | Sclerotinia stem rot on canola | The fungus was grown on Difco potato dextrose agar (PDA; Dickinson and Company, Sparks, MD, USA) medium at RT for 3 days. Then sub-cultured on fresh PDA media for additional 3 days at RT to use for inoculation of canola seedlings (Joshi et al. 2016). |
| *Leptosphaeria maculans* RL-60 | Blackleg on canola | The fungus was grown on V8-agar medium (150 ml V8 juice, 1.5 g $CaCO_3$ and 15 g agar for 1 L) at RT under continuous cool-white fluorescent light and incubated for 14 days (Feng et al. 2014). |
| *Fusarium graminearum* G-1 | Fusarium head blight on wheat and barley | The fungus was grown on PDA plates sealed with parafilm at 25°C for 12 h light/darkness for 5 days. (Geddes et al. 2008). |
| *Aspergillus niger* FUA5001 | Mold spoilage | The fungus was grown on PDA plates at 25 ºC in darkness for 7 days (Magnusson snd Schnurer 2001). |

### 3.4. Minimum Inhibitory Concentration (MIC) Assay

Antifungal activity of fatty acids was determined using the broth micro-dilution method as described by Magnusson and Schnurer (2001) with some modifications. Pure fatty acids were dissolved in 100% ethanol at a concentration of 53.33 g/L. The fatty-acid stock solution (100 µl) was mixed with 100 µl of the specific liquid medium used to grow each fungus (described in Table 3-3) in a 96-well microtiter plate to make a series of 2-fold diluted of HUFAs at 20, 10, 5, 2.5, 1.25. 0.625, 0.312, 0.156, 0.078 and 0.039 g/L concentrations. Ethanol was evaporated by placing the microtiter plate without a lid in a laminar flow hood until 100 µl of 50% ethanol placed in an empty well of the same plate was totally evaporated. Subsequently, the wells were inoculated with 33.33 µl of spore suspensions of the aforementioned pathogenic fungi at concentration of $10^4$ spores/ml. The well consisting of only the medium was considered as the negative control and the well including the medium plus spore suspension served as the positive control. The MIC of samples was defined as the lowest concentration to completely inhibit the visible growth of the fungal strains and determined visually one day after that the growth was visible in the positive-control wells. The MIC values were determined from the average of three independent experiments using replicate preparations of the conidiospores. The entire experiment was also repeated twice.

Table 3-3. Specific liquid media used in MIC for each fungal pathogen

| Fungal pathogens | Specific liquid media used for MIC |
|---|---|
| *Pyrenophora tritici-repentis* | Potato Dextrose Broth (PDB) |
| *Pyrenophora teres* f. *teres* | PDB |
| *Leptosphaeria maculans* | V8-juice broth |
| *Sclerotinia Sclerotiorum* | PDB |
| *Aspergilus niger* | mMRS broth |

### 3.5. Plant materials

*Brassica napus* cv. 'Westar' and DH12075 line used as susceptible genotypes for experiments with *L. maculans* and *S. sclerotiorum*, respectively (Sharma et al. 2010; Joshi et al. 2016). Their seeds were sown in plastic inserts (5 cm × 5 cm; one seed per insert) filled with Sunshine potting mix (W.R. Grace and Co., Fogelsville, PA, USA) and grown in controlled growth cabinets under 22 °C day/18 °C night and 16 hours photoperiod for three weeks. Seedlings were watered regularly and were fertilized by Peters ® NPK (20-20-20) solution on 2-week old seedlings at concentration of 200 ppm (Sharma et al. 2010). *Triticum aestivum* cv. 'Katepwa' and *Hordeum vulgare* cv. 'Xena' were treated as susceptible genotypes for *P. tritici-repentis* and *P. teres* f. *teres* experimentation, respectively. Their seeds were sown in plastic square pots (5 inches; 6 seeds per pot) filled with Sunshine potting mix (W.R. Grace and Co., Fogelsville, PA, USA) and grown in growth cabinets under 20 °C day/18 °C night and 16 hours photoperiod and watered regularly for 10-13 days to reach the 2-3 leaf stage (Aboukhaddour et al 2013; and Akhavan et al. 2016). No fertilizer was used for wheat and barley cultivars.

### 3.6. Foliar treatment and plant inoculation procedures

To evaluate the reaction of canola cv. 'Westar' to *L. maculans* after fatty acids treatment, 3-week old seedlings were placed in a misting chamber 24 hour prior to inoculation with a relative humidity of > 95%. Then seedlings were air-dried in greenhouse for about 2 hours and treated manually using a plastic-bottle sprayer with approximately 10 ml of coriolic acid and ricinoleic acid per seedling at concentrations of 0.12, 0.25, 0.5, 1, and 2 g/L. Four hours later, two true leaves of each seedlings were wounded at two sites of the mid-rib using a sterile needle gauge and inoculated with 10 µl of spore suspension with concentration of $10^7$ spores/ml. To prevent the falling of inoculum droplets, the seedlings were left for about two hours at the same

place and then returned to the humidity chamber for 48 hours. After transferring plants to the previous growth cabinets, disease severity was measured 14 days post inoculation (Sharma et al. 2010). Briefly, all treated leaves and control leaves were excised from seedlings, gently wiped clean with a wet sponge to remove any debris that might interfere with the analysis, and then scanned using EPSON Perfection V19 Scanner (Epson America Inc, USA). Subsequently, all pictures were analyzed by APS Assess 2.0 software (The American Phytopathological Society 2008) and the mean disease severity was calculated as the percentage of lesion area divided by leaf area for each leaf.

Inoculation of the canola line DH12075 by *S. sclerotiorum* was done based on the methodology described by Joshi et al. (2016) with some modifications. Briefly, 21-day old seedlings were placed in the humidity chamber 24 hours prior to inoculation. The seedlings were air-dried in greenhouse for about 2 hours and then were treated manually using a plastic-bottle sprayer with approximately 10 ml of coriolic acid and ricinoleic acid per each one at five different concentrations including 0.12, 0.25, 0.5, 1, and 2 g/L. Four hours later, two true leaves of each seedlings were wounded at two sites of the mid-rib using a sterile needle gauge and inoculated by 3-day old mycelial plug (5 mm) of actively growing *S. sclerotiorum* excised from PDA plates. Similarly, leaves of negative control plants were inoculated with sterile PDA plugs without fungal cultures. After inoculation, the plants were left in the humidity chamber for 24 h and then transferred to the growth cabinets. Disease severity also was measured 48 hours post inoculation using APS Assess 2.0 software as described for 'Westar' cultivar (The American Phytopathological Society 2008).

Seedlings of the wheat cv. 'Katepwa' were treated manually using a plastic-bottle sprayer with approximately 8 ml of coriolic acid, ricinoleic acid, and oleic acid at five different

concentrations with two-fold dilutions including 0.12, 0.25, 0.5, 1, and 2 g/L four hours before inoculation. Inoculation with *P. tritici-repentis* was performed as described by Aboukhaddour et al. (2013) with some modifications. Briefly, seedlings in two- to three-leaf stage were sprayed by conidial suspension with concentration of $4 \times 10^3$ spores/ml using a pressurized sprayer connected to an air-line. Immediately, the inoculated seedlings were covered with moist plastic bags for 24 hours to maintain the relative humidity at almost 100%. Subsequently, the bags were removed, plants were returned to the previous growth conditions, and disease severity was measured six days after inoculation using APS Assess 2.0 software as described for 'Westar' cultivar (The American Phytopathological Society 2008).

Seedlings of the barley cv. 'Xena' were also treated manually using a plastic-bottle sprayer with approximately 8 ml of coriolic acid, ricinoleic acid, and oleic acid at 0.12, 0.25, 0.5, 1, and 2 g/L concentrations four hours before inoculation. Infection by *P. teres* f. *teres* was performed as described by Akhavan et al. (2016) with some modifications. The seedlings at 3-leaf stage were inoculated by conidial suspension with concentration of $1 \times 10^4$ spores/ml using an automatic sprayer connected to an air-line, then they were covered with moist plastic bags for 24 hours to preserve the relative humidity around 100%. After bag removal and plant transition to the growth cabinets (mentioned as above), the disease severity was measured six days post inoculation using APS Assess 2.0 software as described for 'Westar' cultivar (The American Phytopathological Society 2008).

In all plant experiments, sterile distilled water containing 0.05% Tween-20 (PH= 7.5) was used as the solvent of fatty acids at such low concentrations with two-fold ranging from 0.12 to 2 g/L (Bajpai et al. 2009). Treatment of seedlings with sterile distilled water containing 0.05% Tween-20 served as positive control when inoculated with specific fungal spore suspension and

served as negative control when un-inoculated. A fungicide, Bumper 418 EC (Propiconazole 418 g/L), also was used as a chemical control at a concentration of 1.5 ml/L which was applied on seedlings 36 hours before inoculation. Oleic acid also was applied on wheat and barley crops as another control of fatty acids without hydroxy group in its structure. Experiments were arranged in a randomized complete block design (RCBD) with three biological replicates for each treatment and were repeated twice for each crop. Six plastic inserts each with one seedling represented one biological replicate in case of 'Westar' cultivar and DH12075 line. A single pot with six seedlings represented one biological replicate in case of the 'Katepwa' and 'Xena' cultivars. In total, there were 18 replicates (6 technical replications × 3 biological replicates) for each treatment.

### 3.7. Seed germination assay

The effects of coriolic acid, ricinoleic acid, and oleic acid was assessed on seed germination of 'Katepwa', 'Xena', 'Westar', and DH12075 cultivars/lines as described by Nwachukwu and Umechuruba (2001) with some modifications. Six seeds of each cultivar, as one biological replicate, were soaked in aqueous form of coriolic acid, ricinoleic acid and oleic acid at five different concentrations with two-fold dilution ranging from 0.12 g/L to 2 g/L for two hours. Seeds then were air-dried for two hours and placed on Petri-dishes covered with Whatman filter paper saturated with SDW. Petri-dishes were kept in an incubator set at of 20 °C and 16/8 hours of light/darkness to prepare suitable condition for seed germination. The Petri-dishes were watered regularly and monitored for 7 days for seed germination and seedling growth. A seed was considered to have germinated if both roots and shoots were present. Soaked-seeds in the fatty acid solvent (water-tween 0.05%) and in SDW served as the controls. The experiment was

designed as randomized complete block design (RCBD) and performed with three biological replicates over time for each treatment and repeated twice for each crop.

### 3.8. Seed treatment assay

The efficacy of coriolic acid, ricinoleic acid, and oleic acid in crop protection as seed treatment compounds was assessed on 'Katepwa', 'Westar', and 'DH12075' cultivars/lines. Six seeds of each crop cultivar, as one biological replicate, were soaked in aqueous form of coriolic acid, ricinoleic acid, and oleic acid at five different concentrations ranging from 0.12 g/L to 2 g/L for two hours. Following that, seeds were sown in plastic inserts (5 cm x 5 cm; one seed per insert) filled with sterile Sunshine potting mix (W.R. Grace and Co., Fogelsville, PA, USA) and watered regularly. Seeds soaked in the solvent (water-tween 0.05%), without fatty acids, which were later inoculated with specific phytopathogens, served as the positive control. Seeds soaked in water-tween 0.05% served as a negative control and were not inoculated with the pathogen. Plant infection procedures by specific phytopathogens and disease severity measurements were performed as described previously in plant inoculations section. The experiment was designed as randomized complete block design (RCBD) and performed in three biological replicates for each treatment and repeated twice for each crop.

### 3.9. Histological and morphological studies

The effect of coriolic acid, ricinoleic acid, and oleic acid treatment on clogging of stomatal pores was investigated using light microscopy. Two hours after spraying the 3-week old 'Westar' seedlings by two concentrations of these fatty acids (2 and 1 g/L), a small piece of leaf from each treatment including fatty acids-treated as well as controls was prepared (8 × 10 mm). The tissues were fixed in a solution consisting of formaldehyde solution (37%), acetic acid, and

ethanol (50%) overnight at RT (Yeung and Saxena 2005). After fixation, the tissues were sectioned paradermally (8 μm thickness), affixed to glass slides, and stained as described by Sharma et al. (2010) with some modifications. Briefly, the affixed tissues were de-waxed in two series of toluene solutions each for 5 min, rehydrated to 50% ethanol and then stained with Lacto Phenol Cotton Blue (LPCB) for 20 minutes. Subsequently, the stained sections were washed 3 times with water (3 min each), counterstained with Safranin for 20 seconds, dehydrated in ethanol and toluene, and then mounted with DPX (Electron Microscopy Sciences, Hatfield, PA, USA) mounting medium. The sections were viewed using an Optronics digital camera interfaced to an Axio Scope.A1 - Zeiss Microscope (Carl Zeiss MicroImaging, Oberkochen, Germnay).

The stomatal pores were also observed directly by a layer of leaf surface using an Axio Scope.A1 - Zeiss Microscope (Carl Zeiss MicroImaging, Oberkochen, Germnay) for all treated and untreated leaves of the 'Westar' cultivar, then photographed with an Optronics digital camera. In addition, a relief map of the surface of all treated and untreated Westar leaves was prepared through painting each side of the midrib with a clear nail polish. When the nail polish thoroughly dried, the edge of the nail polish was carefully peeled off using a small piece of scotch tape. Then the nail polish was placed on a microscope slide and visualized under Axio Scope.A1 - Zeiss Microscope by an Optronics digital camera.

### 3.10. Determination of reactive oxygen species (ROS) levels

Accumulation of two major types of ROS, $O_2^-$ (superoxide) and $H_2O_2$ (hydrogen peroxide) (O'Brien et al. 2012) was determined through histochemical staining of 'Westar' canola cultivars treated with coriolic acid, ricinoleic acid, and oleic acid at the two highest concentrations used in this study, 2 g/L and 1 g/L. Two different methods of fatty acids treatment were used for determination of ROS level including foliar and wounding treatments. Briefly, the

second leaf of 3-week old of ‘Westar’ seedlings were wounded at two sides of the mid-rib using a sterile needle gauge. Then 10 µl of coriolic acid, ricinoleic acid, and oleic acid at the mentioned concentrations was applied on each wounded site. While, in foliar treatment method, the 3-week ‘Westar’ seedlings were sprayed with 10 ml of coriolic acid, ricinoleic acid, and oleic acid at the aforementioned concentrations. Six hours later, the second leaf was excised from each wounded- and foliar-sprayed seedling (three seedlings for each treatment) and placed in staining falcon tubes separately. Untreated seedlings and treated ones by the solvent of the fatty acids which was water-tween 0.05%, served as the controls. Staining of the leaves with Nitroblue Tetrazolium (NBT) and 3,3’-Diaminobenzidine (DAB) was conducted as described by Kumar et al. (2014) Daudi and O’ Brien (2012) with some modifications. Briefly, 16 ml of 1 M sodium phosphate monobasic solution ($NaH_2PO_4$) and 84 ml of 1 M sodium phosphate dibasic solution ($Na_2HPO_4$) were mixed together and then the volume increased to 2 L with SDW to prepare 50 mM sodium phosphate buffer (pH=7.5). Subsequently, 50 mg of DAB was dissolved in 45 ml of SDW in a bottle covered with aluminum foil. The pH was also adjusted to 3.8 while the solution was mixing properly on a magnetic stirrer. The total volume of the solution was also increased to 50 ml to get 1 mg/ml of DAB solution. For NBT staining, 100 mg of NBT was dissolved in 50 mM sodium phosphate buffer (pH=7.5) with volume of 50 ml to make a 0.2% NBT solution. The solution was blended on magnetic stirrer thoroughly in an amber bottle and prepared freshly before use. After preparation of DAB and NBT solutions freshly and incubation of treated and control leaves over-night in the staining falcon tubes covered with aluminum foil, the staining solutions were poured and replaced by bleaching solution (ethanol: acetic acid: glycerol= 3:1:1). After, the tubes were placed in boiling water bath at 90-95 °C for 15 ± 5 min to bleach out the

chlorophyll of leaves, then the leaves were photographed on a white background under uniform lighting.

### 3.11. Statistical analysis

Disease scores and seed germination data were analyzed using the MIXED procedure of SAS version 9.4 (Statistical Analysis system; SAS Institute Inc., Cary, NC, USA). The model for the experiment included different treatments (Negative control, Positive control, coriolic acid 0.12, coriolic acid 0.25, coriolic acid 0.5, coriolic acid 1, coriolic acid 2, oleic acid 0.12, oleic acid 0.25, oleic acid 0.5, oleic acid 1, oleic acid 2, ricinoleic acid 0.12, ricinoleic acid 0.25, ricinoleic acid 0.5, ricinoleic acid 1, ricinoleic acid 2 g/L, and Fungicide (Bumper 418 EC at concentration of 1.5 ml/L)) as the fixed independent variables and disease severity as the dependent variable. If the data for certain treatments were not normally distributed, the Box-Cox transformation within PROC TRANSREG was used to find the most appropriate data transformation. Differences between means were analyzed using a Tukey's Multiple Comparison Test with a 95% confidence level and were reported as a mean ± the standard error of the mean (SEM). The cut of p-value of a significance was $P < 0.05$.

# 4. RESULTS

## 4.1. MICs of coriolic acid and ricinoleic acid against pathogenic fungi

The antifungal activity of coriolic acid and ricinoleic acid was determined using the broth micro-dilution method (Table 4-1). Coriolic acid and ricinoleic acid possessed the strongest inhibitory activity against *L. maculans* with MIC of 0.73 and 0.83 g/L, respectively, followed by *A. niger* with MIC values of 0.78 g/L for coriolic acid and 0.88 g/L for ricinoleic acid. A weaker inhibitory activity of coriolic acid was observed against the phytopathogens *S. sclerotiorum*, *P. teres* f. *teres* and *P. tritici-repentis* with MIC values of 2.92, 1.66, and 1.64 g/L, respectively. Ricinoleic acid also could inhibit the mycelial growth of *P. teres* f. *teres* and *P. tritici-repentis* at concentrations of 2.08 and 2.18 g/L, respectively. Amongst all pathogenic fungi used in this study, the weakest inhibitory activity of coriolic acid and ricinoleic acid were observed for *F. graminearum* with MIC of 7.50 and 8.33 g/L, respectively (Table 4-1).

Table 4-1. Minimum Inhibitory Concentrations (MICs) of coriolic acid and ricinoleic acid against phytopathogenic fungi

| Fungal species | Mean MIC (g/L) ± SE[a] | |
|---|---|---|
| | **Coriolic acid (13-OH C 18:2)** | **Ricinoleic acid (12-OH C 18:1)** |
| *Leptosphaeia maculans* | 0.73 ± 0.10 | 0.83 ± 0.10 |
| *Aspergilus niger* | 0.78 ± 0.10 | 0.88 ± 0.34 |
| *Pyrenophora teres* f. *teres* | 1.66 ± 0.20 | 2.08 ± 0.41 |
| *Pyrenophora tritici-repentis* | 1.64 ± 0.46 | 2.18 ± 0.32 |
| *Sclerotinia sclerotiorum* | 2.92 ± 1.09 | ND[b] |
| *Fusarium graminearum* | 7.50 ± 1.44 | 8.33 ± 1.66 |

[a] SE: standard error of the mean
[b] ND: not determined

### 4.2. Effects of foliar treatment of UFAs on plant-pathogen interaction

#### 4.2.1. Dicotyledons

The effects of coriolic acid and ricinoleic acid were investigated through foliar spraying on whole plant systems. Two genotypes of canola including Westar and DH12075 infected by *L. maculans* and *S. sclerotiorum*, respectively, were tested for the inhibitory activity of coriolic acid and ricinoleic acid. There was no significant reduction on disease severity of blackleg caused by *L. maculans* in 'Westar' cultivar treated with five different concentrations of coriolic acid and ricinoleic acid including 0.12, 0.25, 0.5, 1, and 2 g/L compared with the disease severity of a positive control (3.99%) (Figure 4-1). Similar observations were also made for coriolic acid- and ricinoleic acid-treated DH12075 canola line inoculated with *S. sclerotiorum* in comparison with the disease severity of positive control 18.33% (Figure 4-2). The highest concentration of coriolic acid and ricinoleic acid (2 g/L) used in this study caused extensive necrotic lesions on the leaves of both genotypes of canola including 'Westar' and DH12075, which ultimately contributed to death of these cultivars after couple of days (Figure 4-3).

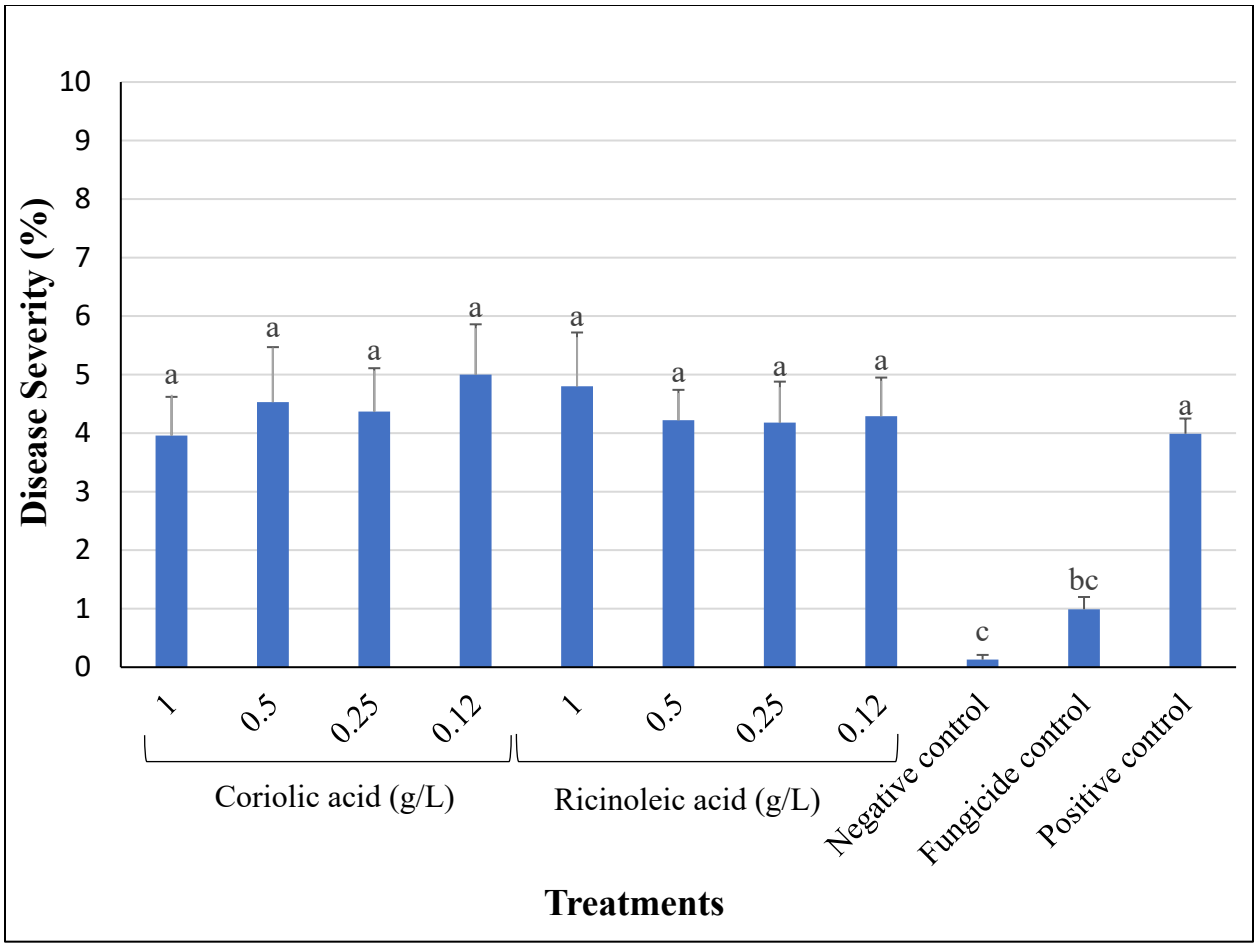



Figure 4-1. Effects of foliar treatment of coriolic acid and ricinoleic acid on disease severity induced by *Leptosphaeria maculans* in *Brassica napus* cv. 'Westar' under controlled-environment growth chambers conditions. Bars with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

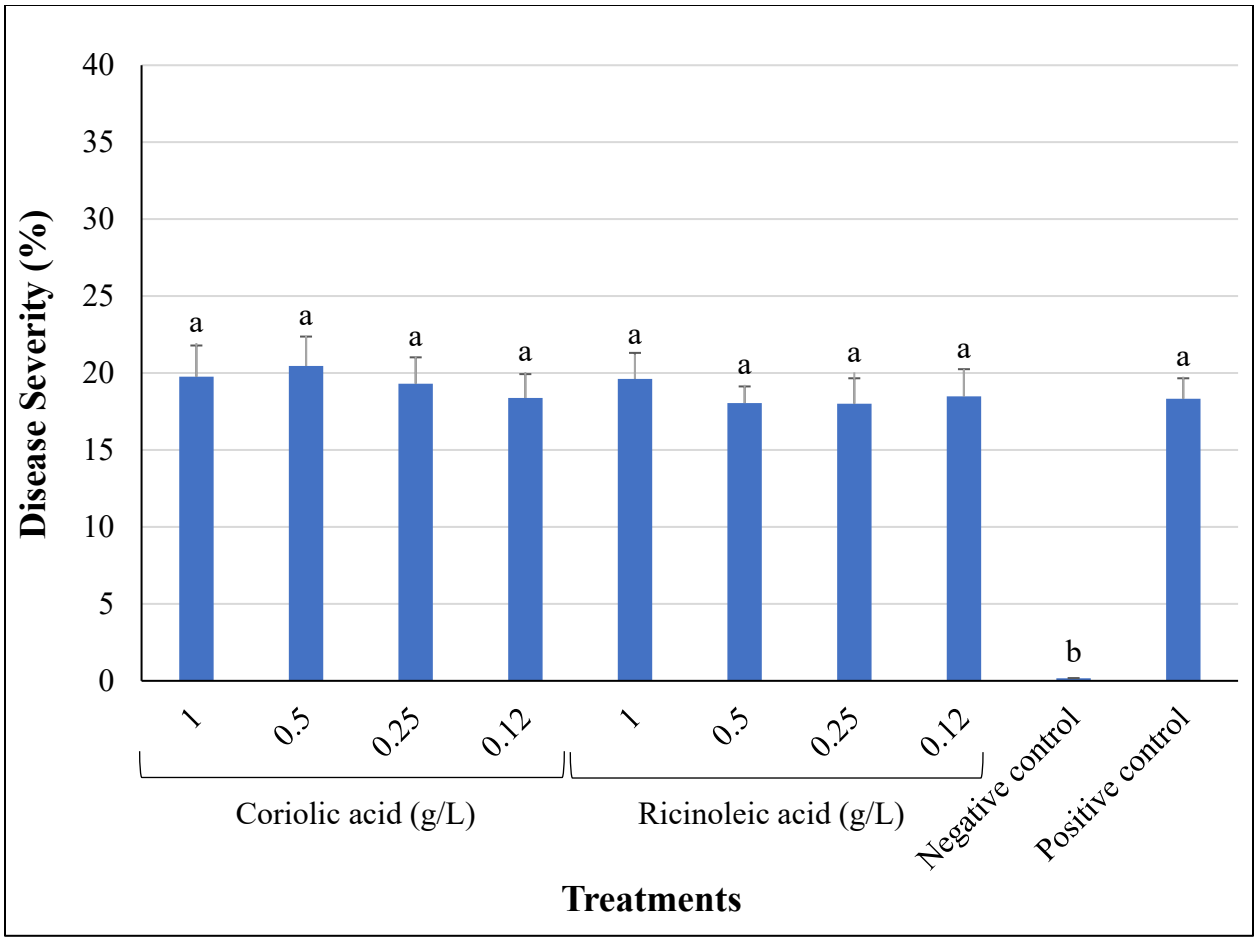



Figure 4-2. Effects of foliar treatment of coriolic acid and ricinoleic acid on disease severity induced by *Sclerotinia sclerotiorum* in *Brassica napus* line DH12075 under controlled-environment growth chambers conditions. Bars with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

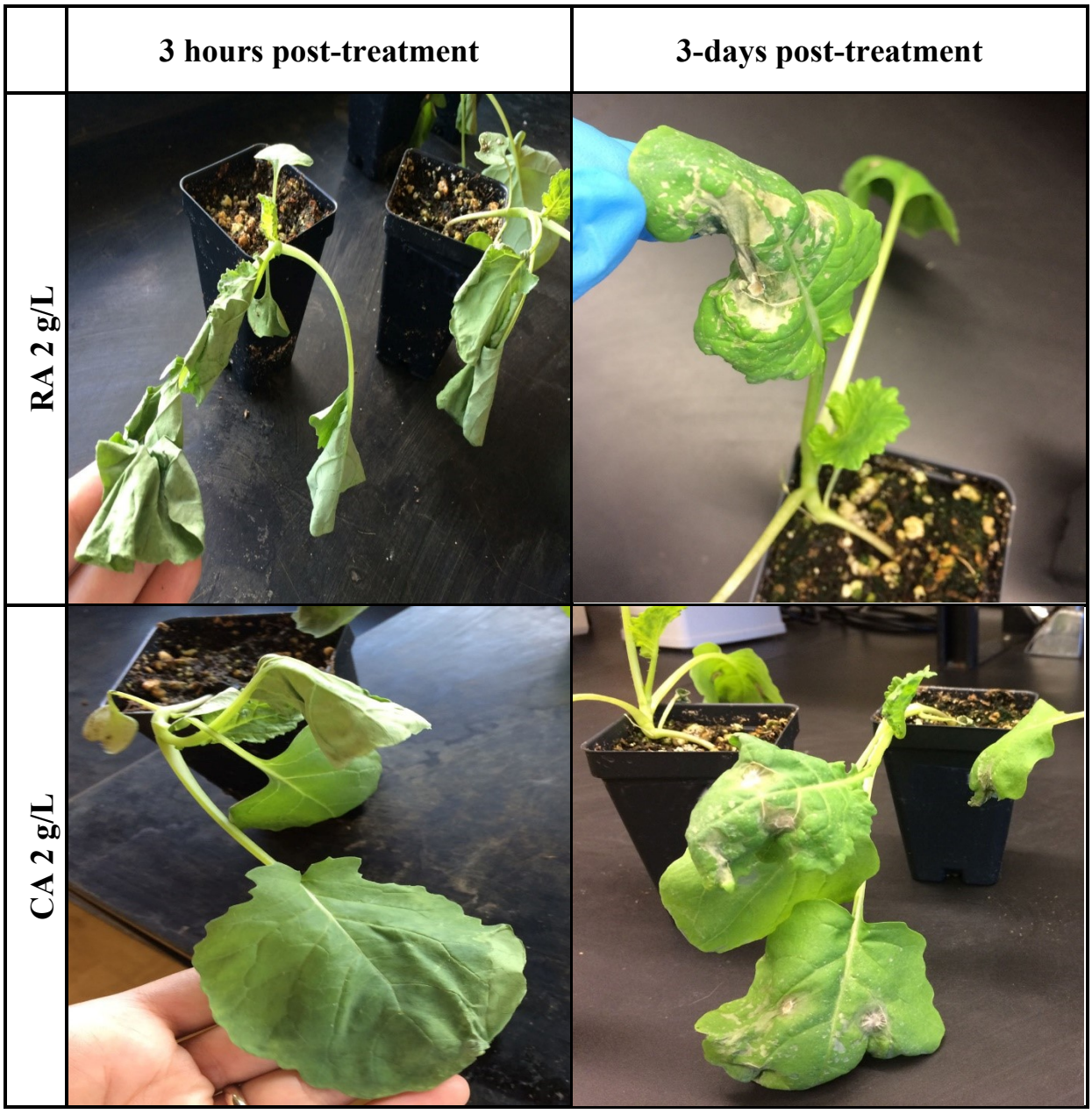


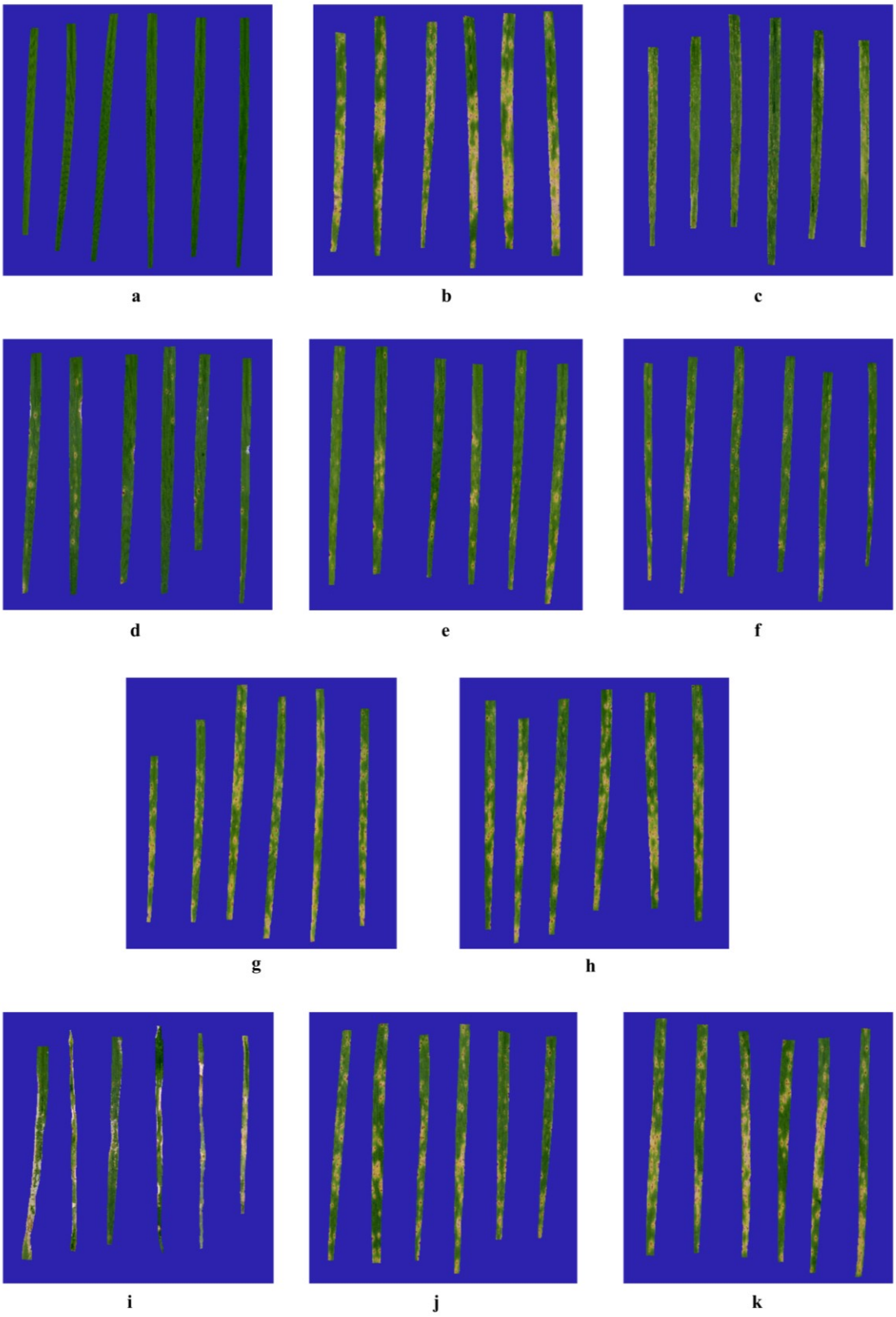

Figure 4-3. Effects of foliar treatment of coriolic acid and ricinoleic acid at 2 g/L concentration in three-week old *Brassica napus* cv. 'Westar' 3 hours and 3 days post treatment, which caused wilting and formation of necrotic lesions on the seedlings.

### 4.2.2. Monocotyledons

The effects of coriolic acid, ricinoleic acid, and oleic acid were investigated through foliar spraying on whole plant systems. Coriolic acid-treated wheat inoculated with *P. tritici-repentis* (isolate AB 7-2) resulted in a reduction of visual symptoms (Figure 4-4) and a significant ($P < 0.05$) reduction in disease severity at concentrations of 2 (1.26%), 1 (3.16%), and 0.5 (12.30%) g/L when compared with the positive control with disease severity of 21.23% (Figure 4-5). Additionally, higher concentrations of coriolic acid (i.e. 2 and 1 g/L) showed significant ($P < 0.05$) disease reduction in comparison with a concentration of 0.5 g/L. A statistically significant effect on disease severity was not observed in wheat seedlings treated with ricinoleic acid and oleic acid. These results suggest that, under the experimental conditions tested, coriolic acid afforded a higher degree of protection in wheat against this pathogen.

In barley, coriolic acid-treated seedlings at 0.5 g/L concentration showed a significant ($P < 0.05$) reduction in disease symptoms caused by *P. teres* f. *teres* (isolate AB 34) (30.64%) compared with the positive control (59.00%) (Figure 4-6). Furthermore, coriolic acid at 2 and 1 g/L concentrations decreased the disease severity on barley seedlings infected by *P. teres* f. *teres* by 40.93% and 36.25%, respectively. No significant difference was observed amongst the five different concentrations of ricinoleic acid and oleic acid including 2, 1, 0.5, 0.25, and 0.12 g/L on the disease severity caused by *P. teres* f. *teres* in barley seedlings compared with the positive control (Figure 4-6). Similar to wheat, ricinoleic acid and oleic acid were not effective to control the disease severity caused by *P. teres* f. *teres* in barley seedlings. Although coriolic acid was effective to control the disease severity on barley but it was not as effective as on wheat. Formation of necrotic lesions on wheat and barley seedlings were in a similar manner with the

canola cultivars but their numbers were much fewer which did not kill these monocotyledonous crops.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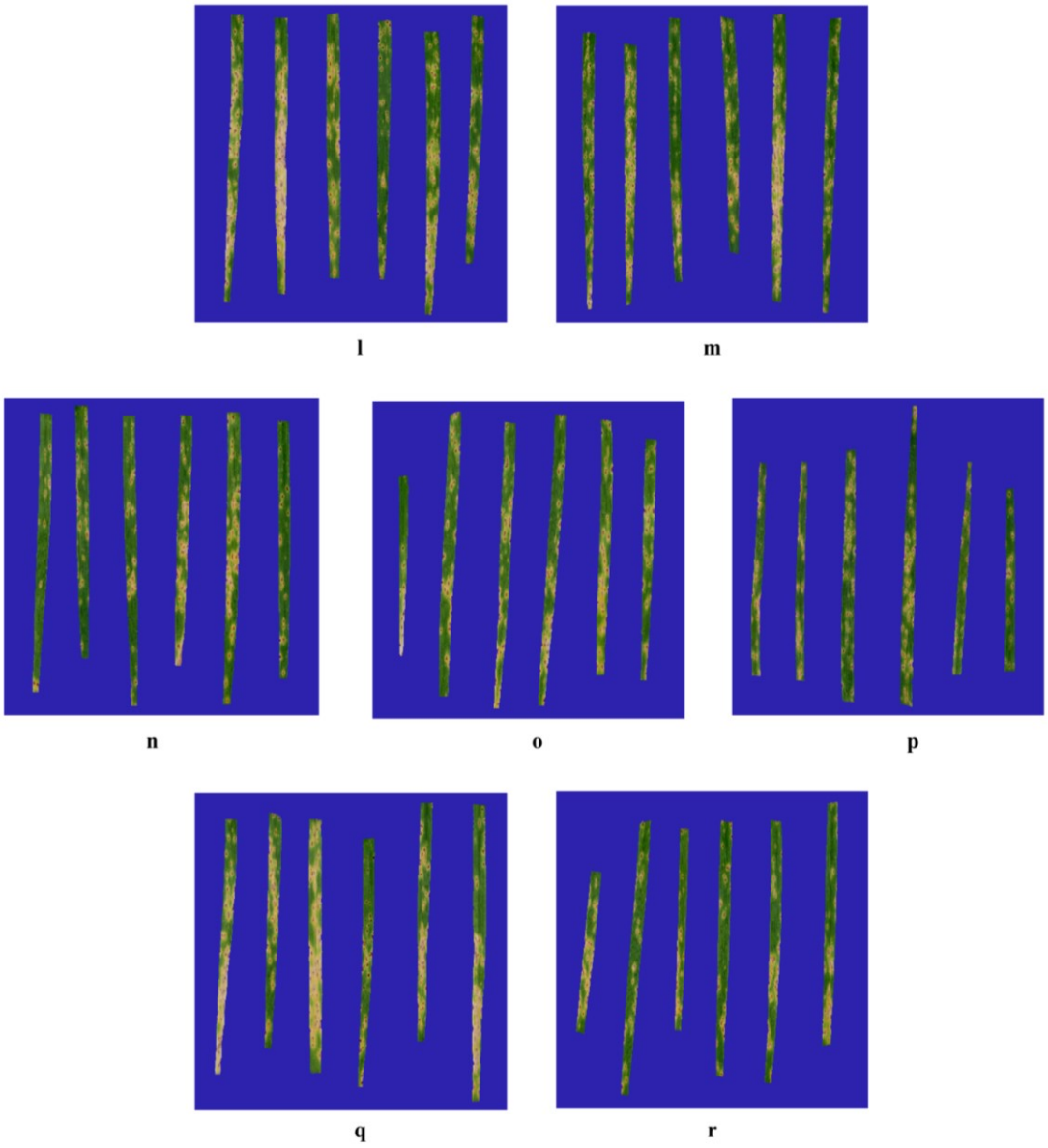



Figure 4-4. Effects of foliar treatment of unsaturated fatty acids on the disease severity induced by *Pyrenophora tritici-repentis* in *Triticum aestivum* cv. 'Katepwa'. [a] negative control, [b] positive control, [c] fungicide control, [d] coriolic acid 2 g/L, [e] coriolic acid 1 g/L, [f] coriolic acid 0.5 g/L, [g] coriolic acid 0.25 g/L, [h] coriolic acid 0.12 g/L, [i] ricinoleic acid 2 g/L, [j] ricinoleic acid 1 g/L, [k] ricinoleic acid 0.5 g/L, [l] ricinoleic acid 0.25 g/L, [m] ricinoleic acid 0.12 g/L, [n] oleic acid 2 g/L, [o] oleic acid 1 g/L, [p] oleic acid 0.5 g/L, [q] oleic acid 0.25 g/L, [r] oleic acid 0.12 g/L. Photos were taken 6 days after inoculation.

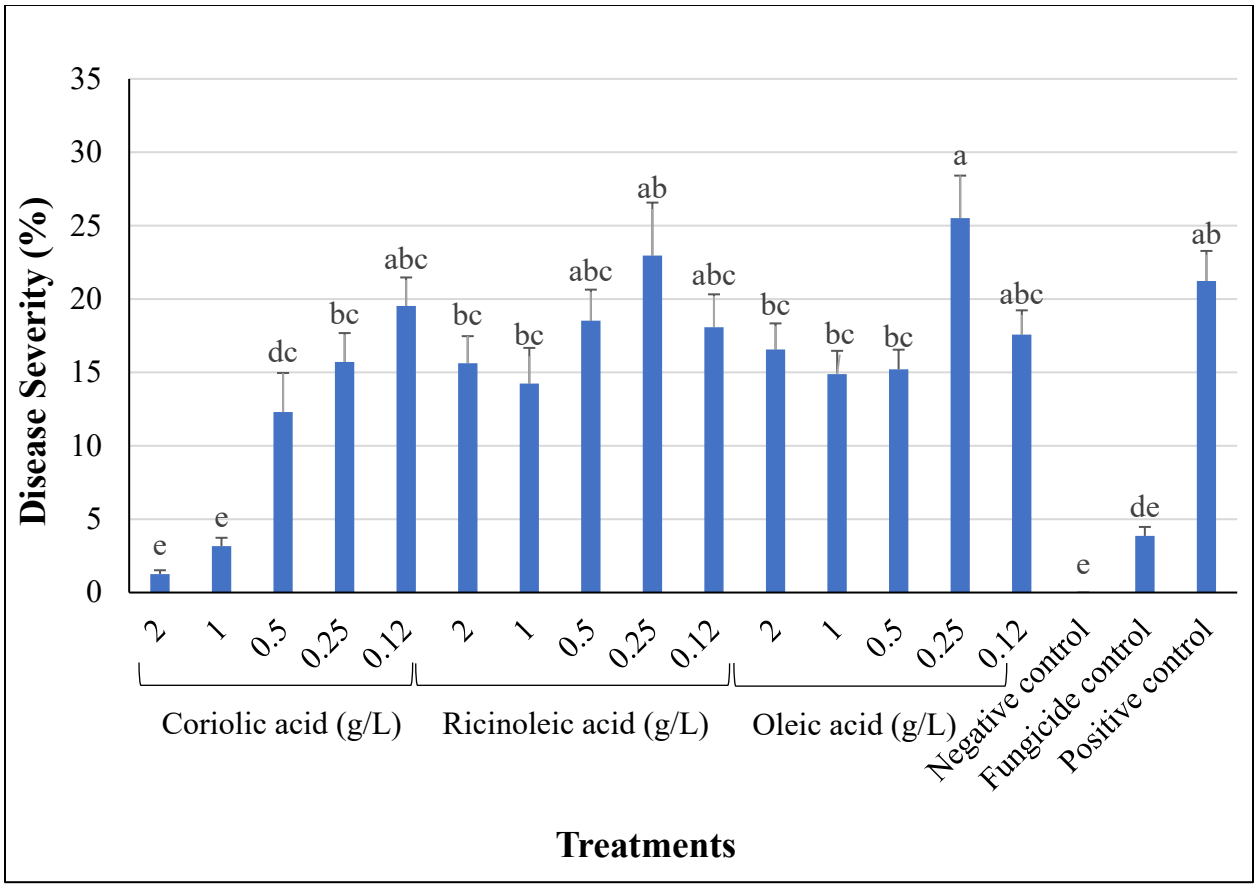



Figure 4-5. Effects of foliar treatment of coriolic acid, ricinoleic acid, and oleic acid on the disease severity induced by *Pyrenophora tritici-repentis* in *Triticum aestivum* cv. 'Katepwa' under controlled-environment growth chambers conditions. Bars with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

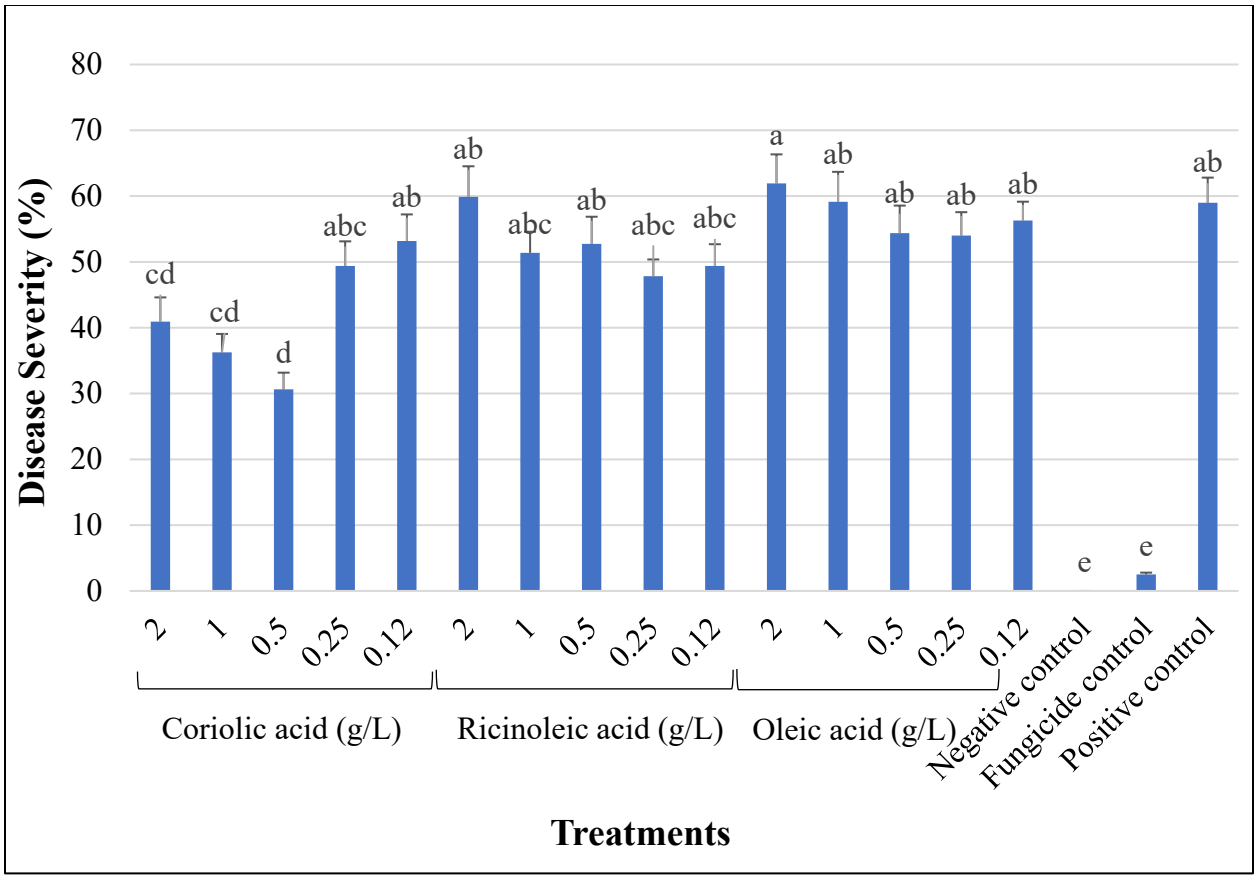



Figure 4-6. Effects of foliar treatment of coriolic acid, ricinoleic acid, and oleic acid on the disease severity induced by *Pyrenophora teres* f. *teres* in *Hordeum vulgare* cv. 'Xena' under controlled-environment growth chambers conditions. Bars with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

### 4.3. Effects of coriolic acid, ricinoleic acid and oleic acid on seed germination

The effects of coriolic acid, ricinoleic acid and oleic acid were assessed on seed germination of wheat, barley and canola genotypes (Westar and DH12075). The results indicated that coriolic acid, ricinoleic acid, and oleic acid did not have any inhibitory effect on seed germination of wheat and 'Westar' compared with the controls when their seeds were tested on moist Whatman paper (Table 4-2). However, in terms of canola line DH12075, coriolic acid at 2 g/L concentration showed lower seed germination compared to controls and other treatments. On barley, ricinoleic acid with 0.25 and 0.12 g/L concentrations had a better seed germination compared to other treatments, with the lowest germination rate for coriolic acid at 2 g/L concentration (Table 4-2).

Table 4-2. Effects of treatment of coriolic acid, ricinoleic acid, and oleic acid at concentrations with two-fold dilutions ranging from 0.12 to 2 g/L on seed germination of wheat, Barley, and Canola

| Treatment | Canola-Westar (%) | Canola-DH12075 (%) | Wheat-Katepwa (%) | Barley-Xena (%) |
|---|---|---|---|---|
| Coriolic acid 2 g/L | 94.44 $^{a}$ ± 5.55 | 88.88 $^{b}$ ± 5.55 | 88.88 $^{b}$ ± 5.55 | 27.77 $^{c}$ ± 5.55 |
| Coriolic acid 1 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 88.88 $^{b}$ ± 5.55 | 50.18 $^{abc}$ ± 16.85 |
| Coriolic acid 0.5 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 94.44 $^{a}$ ± 5.55 | 66.85 $^{abc}$ ± 9.78 |
| Coriolic acid 0.25 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 88.88 $^{b}$ ± 5.55 | 61.11 $^{abc}$ ± 5.55 |
| Coriolic acid 0.12 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 88.88 $^{b}$ ± 5.55 | 61.11 $^{abc}$ ± 5.55 |
| Ricinoleic acid 2 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 83.33 $^{a}$ ± 9.62 | 38.88 $^{bc}$ ± 5.55 |
| Ricinoleic acid 1 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 66.85 $^{abc}$ ± 9.78 |
| Ricinoleic acid 0.5 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 55.55 $^{abc}$ ± 5.55 |
| Ricinoleic acid 0.25 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 94.44 $^{a}$ ± 5.55 | 83.88 $^{a}$ ± 0.00 |
| Ricinoleic acid 0.12 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 88.88 $^{b}$ ± 5.55 | 83.51 $^{a}$ ± 9.62 |
| Oleic acid 2 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 83.33 $^{a}$ ± 9.62 | 55.55 $^{abc}$ ± 11.11 |
| Oleic acid 1 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 83.33 $^{a}$ ± 9.62 | 78.14 $^{ab}$ ± 5.74 |
| Oleic acid 0.5 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 88.88 $^{b}$ ± 5.55 | 55.55 $^{abc}$ ± 5.55 |
| Oleic acid 0.25 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 94.44 $^{a}$ ± 5.55 | 72.40 $^{ab}$ ± 5.74 |
| Oleic acid 0.12 g/L | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 94.44 $^{a}$ ± 5.55 | 66.66 $^{abc}$ ± 0.00 |
| Solvent control | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 94.44 $^{a}$ ± 5.55 | 61.11 $^{abc}$ ± 5.55 |
| SDW control | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 100 $^{a}$ ± 0.00 | 61.29 $^{abc}$ ± 14.83 |

Values show the mean percentage of germination for each treatment ± standard error of the mean which is followed by different letters, showing significant ($P < 0.05$) difference between treatments based on Tukey's Multiple Comparison Test.

### 4.4. Effects of seed treatment of UFAs on plant-pathogen interaction

The effect of seed treatment with coriolic acid, ricinoleic acid, and oleic acid was also investigated on the disease severity induced by *P. tritici-repentis*, *L. maculans*, and *S. sclerotiorum* in wheat cv. 'Katepwa', canola cv. 'Westar', and canola line DH12075, respectively. No significant reduction was observed on disease severity caused by *P. tritici-repentis* in wheat cv. 'Katepwa' (Figure 4-7), *L. maculans* in canola cv. 'Westar' (Figure 4-8), and *S. sclerotiorum* in canola line DH12075 (Figure 4-9) compared with the positive control, when their seeds were treated with five different concentrations of coriolic acid, ricinoleic acid, and oleic acid at two-fold dilutions ranging from 0.12 to 2 g/L.

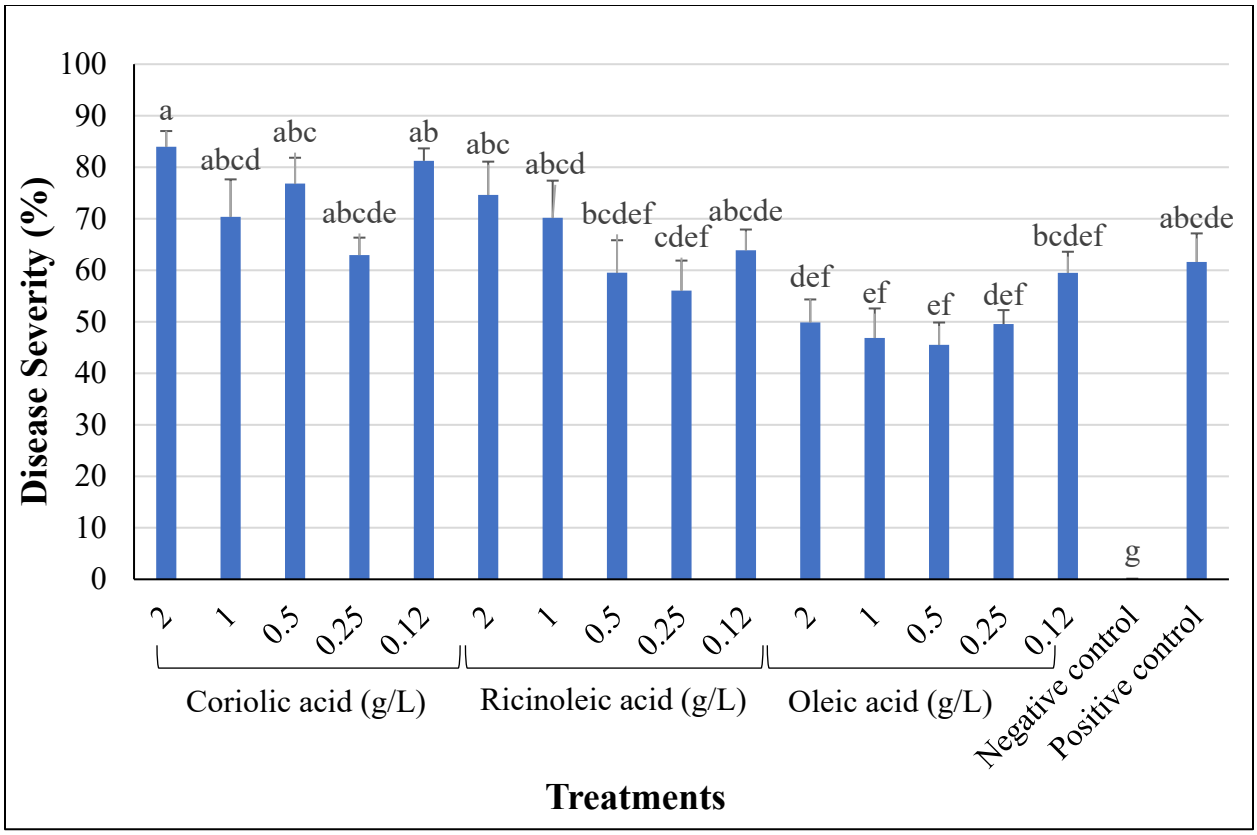



Figure 4-7. Effects of seed treatment of coriolic acid, ricinoleic acid, and oleic acid on the disease severity induced by *Pyrenophora tritici-repentis* in *Triticum aestivum* cv. 'Katepwa' under controlled-environment growth chambers conditions. Columns with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

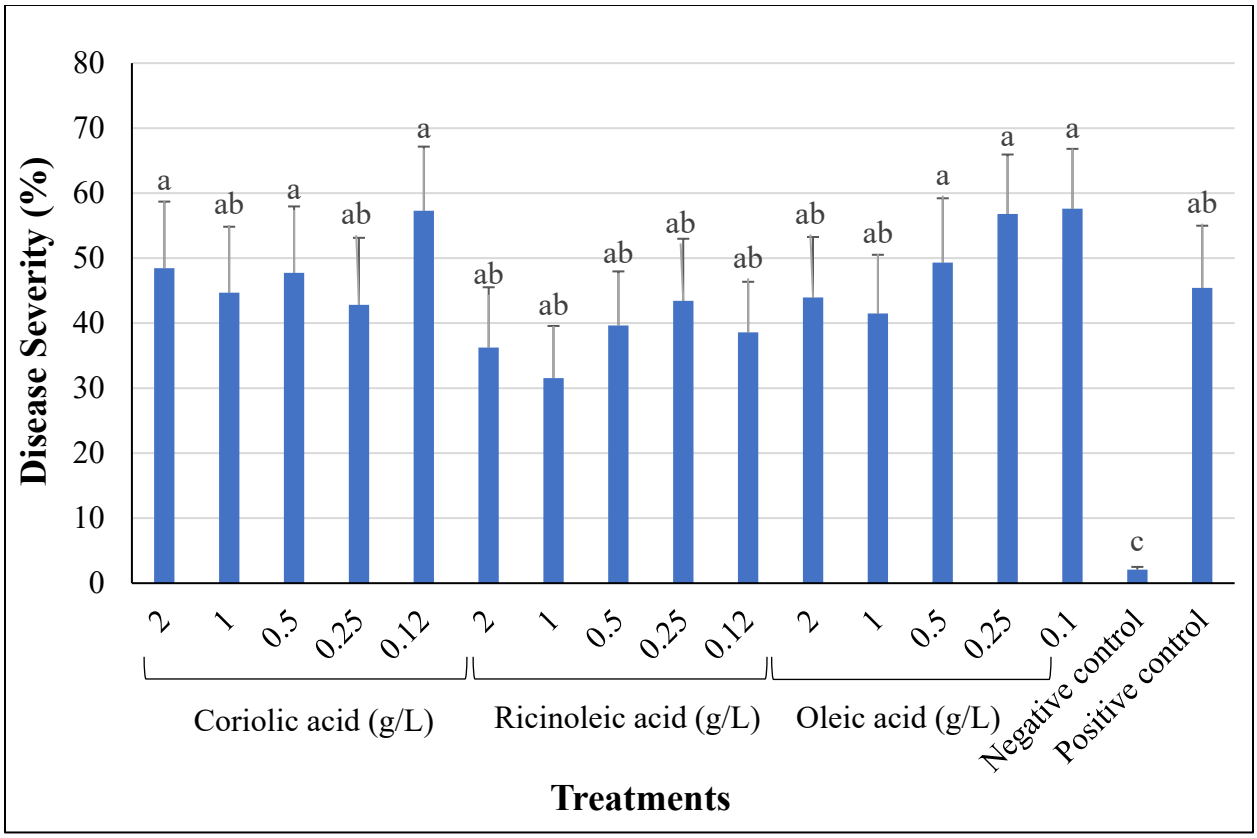



Figure 4-8. Effects of seed treatment of coriolic acid, ricinoleic acid, and oleic acid on the disease severity induced by *Leptosphaeria maculans* in *Brassica napus* cv. 'Westar' under controlled-environment growth chambers conditions. Columns with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented ± standard error of the means.

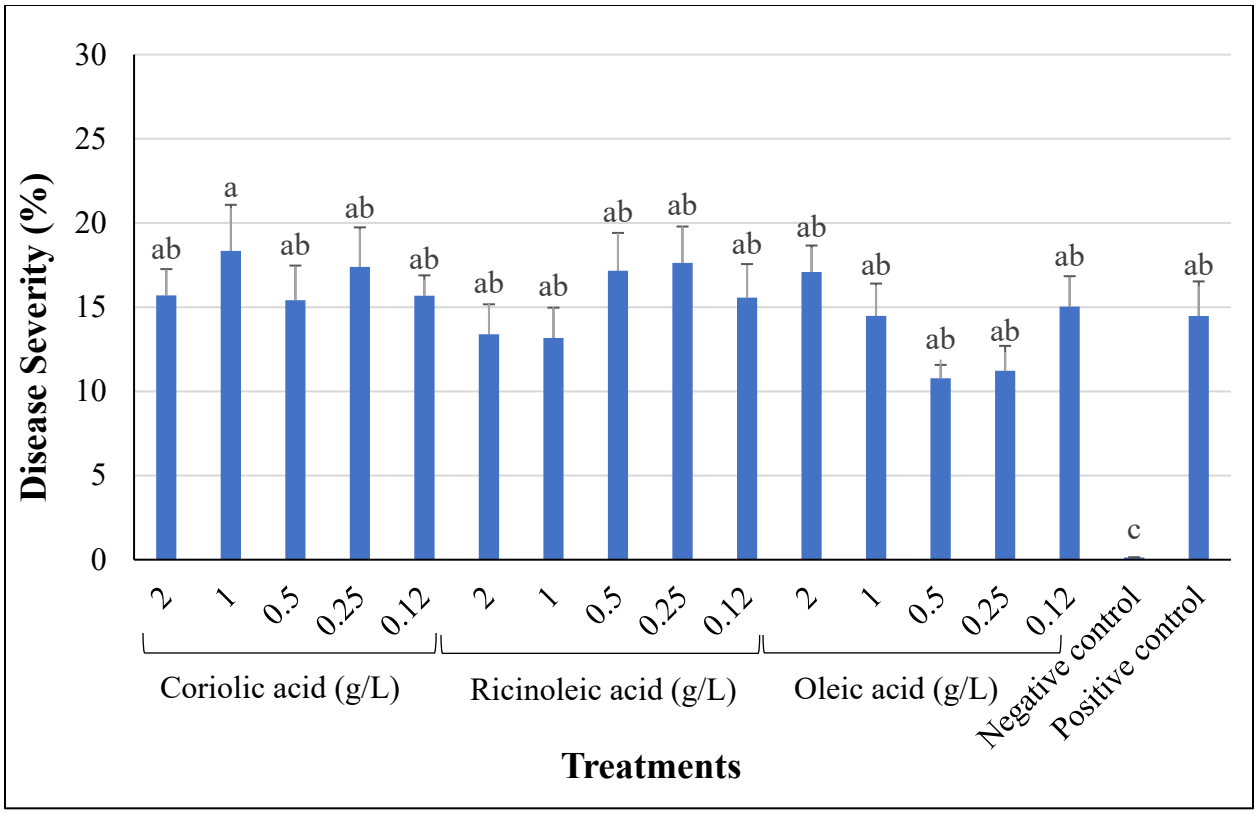
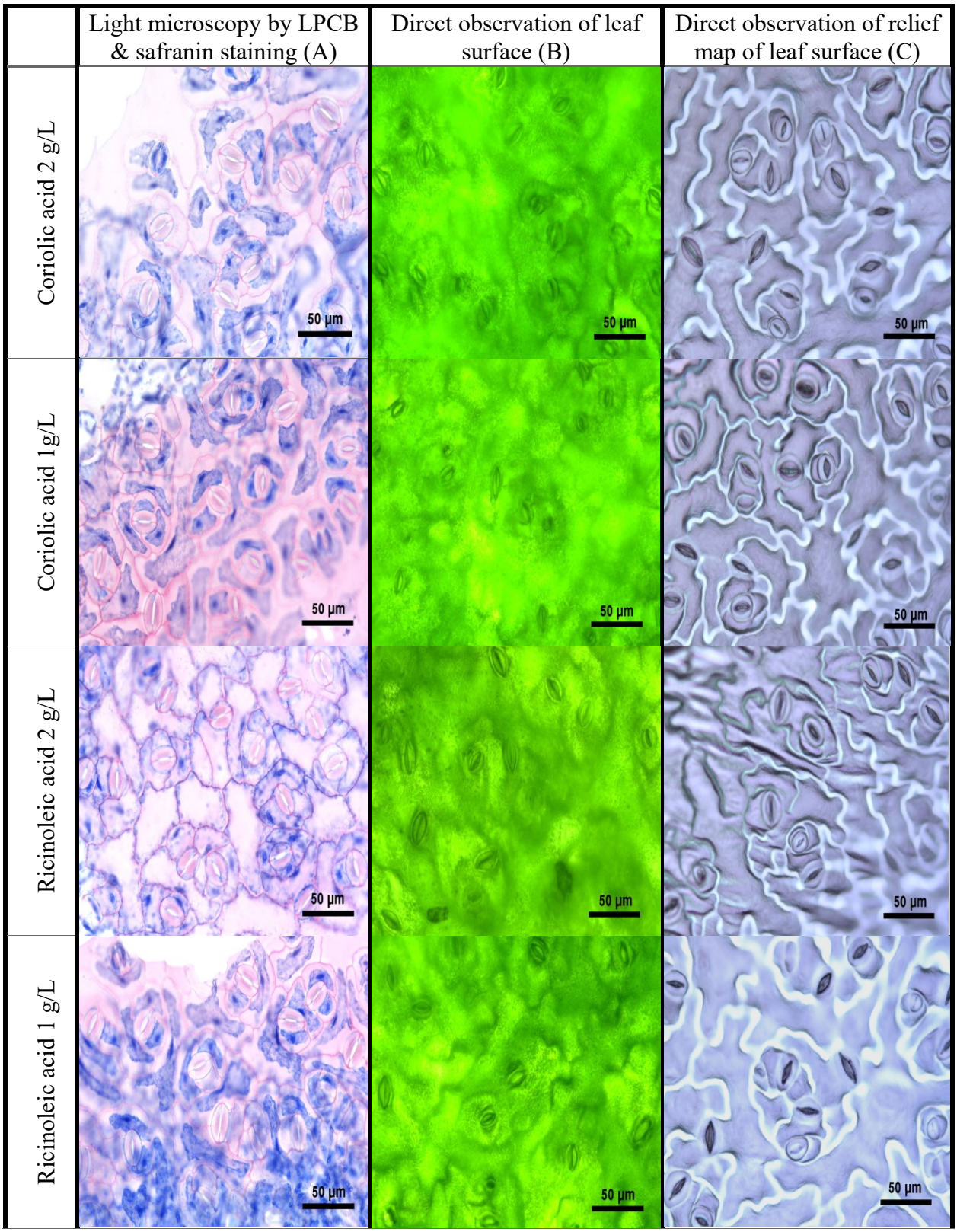



Figure 4-9. Effects of seed treatment of coriolic acid, ricinoleic acid, and oleic acid on the disease severity induced by *S. sclerotiorum* in *Brassica napus* line DH12075 under controlled-environment growth chambers conditions. Columns with the same lowercase letter indicate means that do not differ significantly ($P < 0.05$) based on Tukey's Multiple Comparison Test. Untransformed means are presented Untransformed means are presented ± standard error of the means.

### 4.5. Histological and morphological studies

Spraying canola seedlings with coriolic acid and ricinoleic acid at 2 and 1 g/L concentrations led to formation of necrotic lesions on the leaves, followed by wilting of the plants. It, was hypothesized that such high concentrations may clogged the stomatal pores and inhibit transpiration, which ultimately contributes to the senescence and death of the canola seedlings. Therefore, the effects of coriolic acid, ricinoleic acid, and oleic acid treatments at 2 and 1 g/L concentrations were investigated via light microscopy method as well as direct observation of leaves under the microscope to understand whether there are any lipid layer levels on stomatal pores and evidence of clogging. The results revealed no evidence of oily layers on stomatal pores between all the UFAs-treated leaves as well as control leaves (Figure 4-10).


Light microscopy by LPCB & safranin staining (A)
Direct observation of leaf surface (B)
Direct observation of relief map of leaf surface (C)
Coriolic acid 2 g/L
Coriolic acid 1g/L
Ricinoleic acid 2 g/L
Ricinoleic acid 1 g/L
50 µm

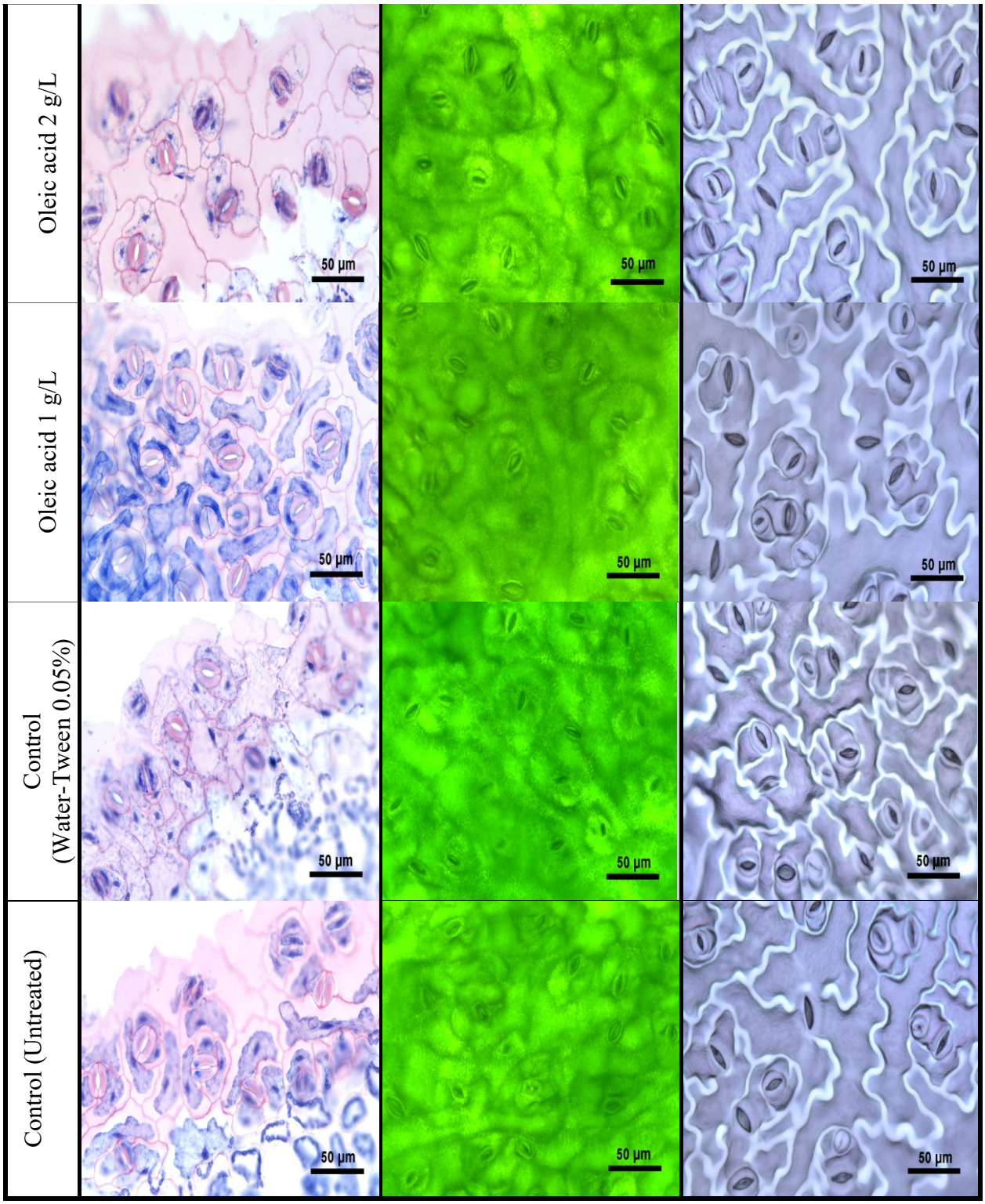
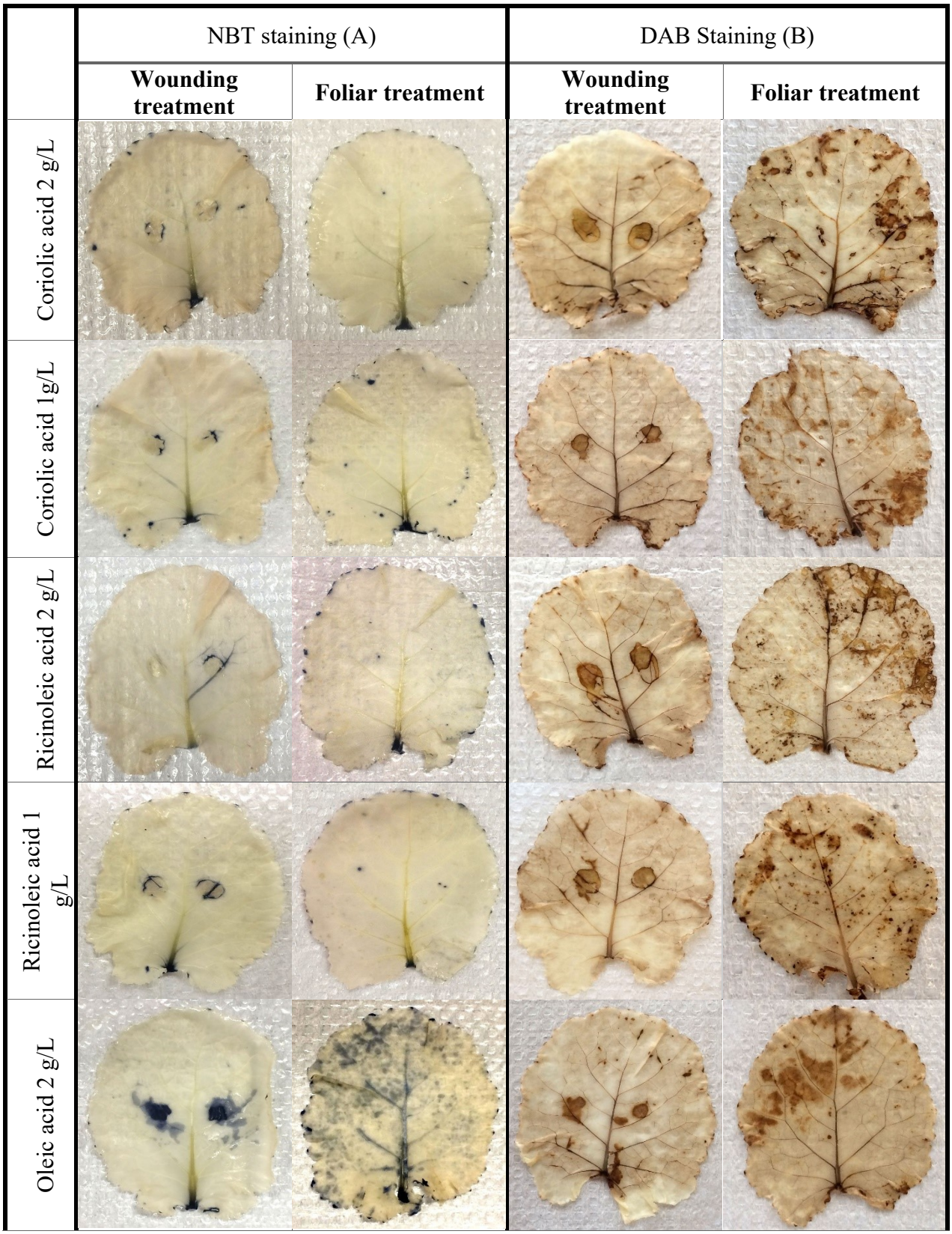



Figure 4-10. Effects of high concentrations (2 and 1 g/L) of coriolic acid, ricinoleic acid, and oleic acid on stomatal pores of *Brassica napus* cv. 'Westar' using light microscopy via Lacto Phenol Cotton Blue and safranin staining (A), direct observation of leaf surface (B), and relief map (C), via optronics digital camera interfaced to Axio Scope.A1 - Zeiss microscope with magnification of 40× (Scale bar = 50 µm).

### 4.6. Effects of coriolic acid, ricinoleic acid, and oleic acid on the ROS levels

To understand whether coriolic acid, ricinoleic acid and oleic acid triggered the oxidative stress through disturbance of a steady-state level of ROS in canola plants, accumulation of $H_2O_2$ and $O^-_2$ was detected in the canola cv. 'Westar' after treatment with concentrations of 1 and 2 g/L of coriolic acid, ricinoleic acid and oleic acid using histochemical staining. The histochemical staining of $O^-_2$ with NBT indicated that the oleic acid-treated leaves stained heavily as a result of $O^-_2$ accumulation (Figure 4-11A). However, compared with controls, no staining of $O^-_2$ was observed for coriolic acid- and ricinoleic acid-treated leaves using both foliar- and wounding-treatment procedures (Figure 4-11A). $H_2O_2$ accumulation was also detected using histochemical staining with DAB. DAB reacts with $H_2O_2$ in the presence of peroxidases to produce a brown polymerization product. Treatment of leaves with coriolic acid, ricinoleic acid and oleic acid at 2 g/L concentration induced intense staining as compared with the controls, indicating the accumulation of $H_2O_2$ in 2 g/L UFA-treated leaves (Figure 4-11B). In contrast, coriolic acid-, ricinoleic acid-, and oleic acid-treated leaves at 1 g/L concentration had similar staining patterns as controls, suggesting that all these treatments probably had similar accumulation of $H_2O_2$ in their leaves (Figure 4-11B).


NBT staining (A)
DAB Staining (B)
Wounding treatment
Foliar treatment
Wounding treatment
Foliar treatment
Coriolic acid 2 g/L
Coriolic acid 1g/L
Ricinoleic acid 2 g/L
Ricinoleic acid 1 g/L
Oleic acid 2 g/L

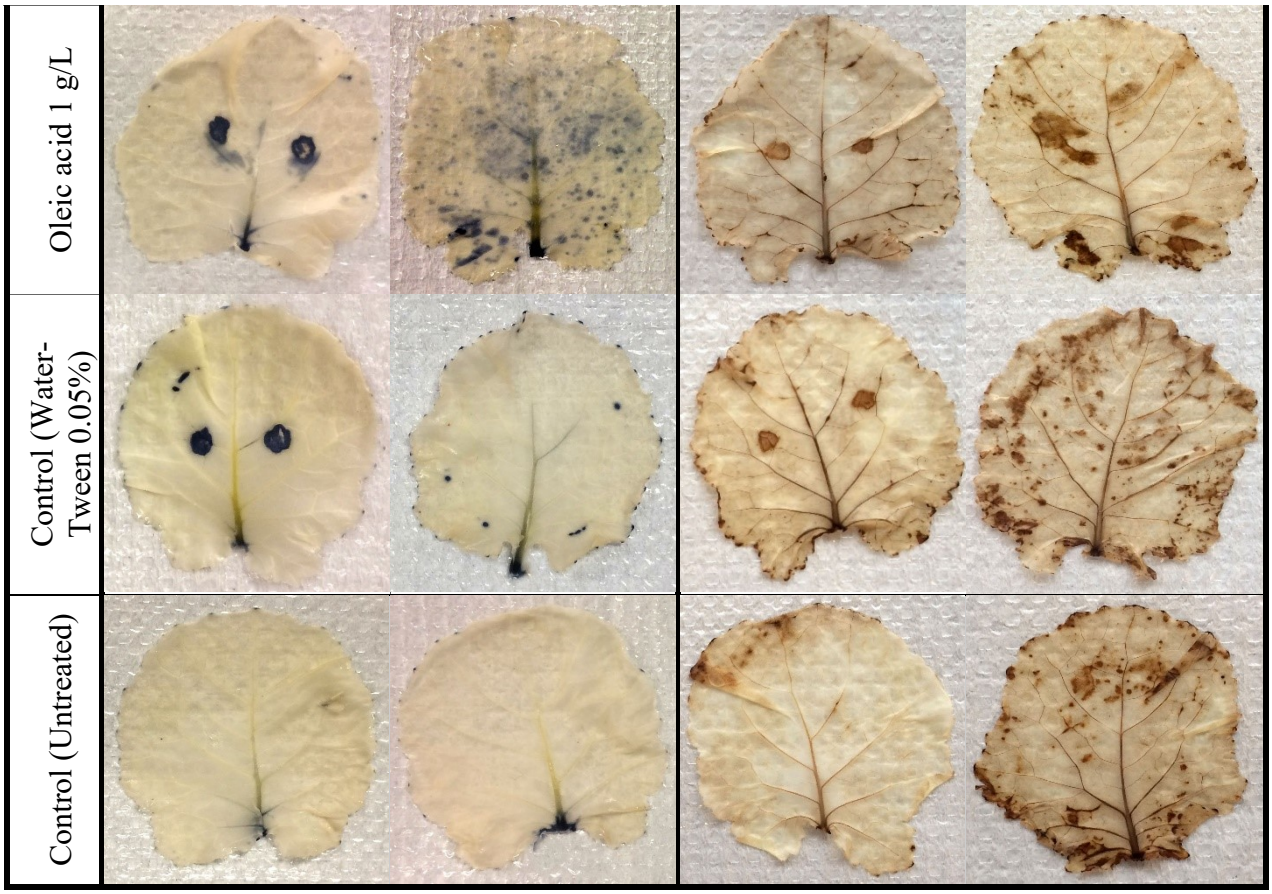



Figure 4-11. Effects of coriolic acid, ricinoleic acid and oleic acid at 2 and 1 g/L concentrations on the accumulation of $O^{-}_{2}$ and $H_2O_2$ in the leaves of *Brassica napus* cv. 'Westar' using Nitroblue Tetrazolium (A) and 3,3'-Diaminobenzidine (B) staining procedures.

# 5. DISCUSSION

Results described in this thesis provide evidence that HUFAs such as coriolic acid are potent inhibitors of plant pathogenic fungi *in vitro* and *in vivo*. It appeared that coriolic acid and ricinoleic acid exhibited large antifungal activity in active concentrations *in vitro*, affecting different pathogenic fungi. The lowest MICs of coriolic acid and ricinoleic acid were observed for *L. maculans* respectively by 0.73 and 0.83 g/L, indicating that coriolic acid and ricinoleic acid at lower concentrations than that had no effect on *L. maculans* mycelial growth. Our observation is similar with the results obtained by Graner et al. (2003), where the mycelial growth of *L. maculans* could not be inhibited by 0.29 g/L concentration of coriolic acid. *A. niger* was the second most sensitive fungi to coriolic acid and ricinoleic acid with MICs of 0.78 and 0.88 g/L, respectively, being in general agreement with the results obtained in previous studies (Chen et al. 2016; Liang et al. 2017). Two closely-related pathogens, *P. tritici-repentis* and *P. teres* f. *teres,* showed a similar range of MICs for coriolic acid and ricinoleic acid. Although there is no published research in the literature indicating the antifungal activity of coriolic acid and ricinoleic acid against these two species, there are studies showing the antifungal activity of other related UFAs such as linoleic acid and linolenic acid against *P. avenae* (Walters et al. 2004). Coriolic acid at concentrations lower than 2.92 g/L had no inhibitory activity against *S. sclerotiorum.* Similarly, a Graner et al. (2003) study on inhibitory effect of different concentrations of coriolic acid against *S. sclerotiorum* indicated that coriolic acid at 0.29 g/L or lower did not affect the growth rate of *S. sclerotiorum* mycelium, considering 0.29 g/L was the highest concentration they used in their experiment while the highest concentration that we used was 20 g/L to determine MIC. The differences in the MICs of coriolic acid and ricinoleic acid against *F. graminearum* were as high as ten-fold as compared with MICs against *L. maculans*,

indicating antifungal activity to be specific for different fungi (Graner et al. 2003; Prost et al. 2005; Pohl et al. 2011).

An explanation for differences in antifungal activities may be related to the diversity in the chemical composition of the membranes of different fungi such as sterol content (Avis and Belanger 2001). The general mechanism of antifungal UFAs with cell membranes has been described as detergent-like properties, in which antifungal UFAs directly insert into the phospholipid bilayer of the fungal membranes, disturb the membrane physically, and release intracellular electrolytes and proteins due to increased fungal membrane fluidity, ultimately leading to cytoplasmic disintegration of fungal cells (Avis and Belanger 2001; Pohl et al. 2011). Fungal sterols such as ergosterol, can moderate such stress-induced elevations in the membrane fluidity (Avis and Belanger 2001; Pohl et al. 2011); therefore, differences in sterol content of fungal membranes may lead to variations in fungal sensitivity. However, the antifungal activity of UFAs may also be linked to other features of fungal physiology. For example, laetisaric acid ((R)8-hydroxy-*cis*-9*cis*-12-octadecadienoic acid), an isomer of coriolic acid, acts as a premature-sexual-inducer (psi) Bα factor, regulating the sexual development of the ascomycetous fungus *Aspergillus nidulans* (Mazur et al. 1991), while showing antifungal properties against phycomycetous fungi (Bowers et al. 1986). The association of laetisaric acid in regulation of conidia formation and production of fumonisins by *Fusarium verticillioides* was also reported (Scala et al. 2014). Consequently, these diverse roles of HUFAs in fungal physiology and ecology may illustrate their species-specific modes of action.

Our observation from the *in vivo* studies illustrated that coriolic acid exhibited the most control activity against tan spot of wheat and subsequently on net blotch of barley. Both ricinoleic acid and oleic acid did not control the disease severity of tan spot and net blotch on

wheat and barley cultivars, respectively. The protective and curative applications of oleic acid at 0.33 and 1 g/L concentrations also could not control the powdery mildew caused by *Podosphaera xanthii* on cucumber plants, while its methyl and ethyl esters at the same concentrations could effectively control the disease severity (Choi et al. 2010). Methyl- and ethyl-oleate also showed control activity against the powdery mildew of barley at 3 g/L concentration (Choi et al. 2010). However, another *in vivo* study illustrated that oleic acid at 0.5 or 1 g/L concentrations exerted antiviral activity against tobacco mosaic virus in tobacco seedlings, attributed to the activated expression levels of defense-related genes such as PR-1 and PR-5 (Zhao et al. 2017).

The difference in antifungal activity of coriolic acid, ricinoleic acid, and oleic acid against the tested pathogenic fungi of wheat and barley may also be explained by their structural differences, such as the presence and location of hydroxy groups, providing the specific structure-function relationships of UFAs in inhibition of pathogenic fungi (Prost et al. 2005; Pohl et al. 2011; Black et al. 2013; Liang et al. 2017). The 12,13,17-trihydroxy-9-octadecenoic acid did not inhibit the growth of rice blast fungus (*Pyricularia oryzae*) in rice crops (Hou and Forman 2000), while 9,12,13-trihydroxy-10-octadecenoic acid shown to be active against the fungus (Kato et al. 1985), indicating that the position of hydroxy groups plays a key role in growth inhibition of rice blast fungus. Furthermore, the presence and number of carbon-carbon double bonds in long-chain UFAs contributes to the higher levels of antifungal activity of UFAs (Prost et al. 2005; Black et al. 2013). Thus, our results can support the conclusion that HUFAs with more C=C bonds also show higher levels of antifungal activity against pathogenic fungi *in vivo*, although their antifungal activity were observed to be similar in *in vitro* experiments.

Control of tan spot of wheat and net blotch of barley by coriolic acid at 0.5 g/L concentration or higher, may relate to coriolic acid effects on germination of their conidiospores, germ-tubes growth, or development of the infection structure, appressorium. To the best of knowledge, there is no published article showing the effects of coriolic acid on fungal infection development, but it has been previously indicated that coriolic acid can reduce spore germination in *L. maculams* (Garner et al. 2003) and inhibit mycelial growth in *Aspergillus spp*, *Penicillium roqueforti*, *Mucor plumbeus*, *Cladosporium herbarium*, *Botrytis cinerea*, *Phytophtora infestans* and *Fusarium oxysporum* (Prost et al. 2005; Pohl et al. 2011; Black et al. 2013; Liang et al. 2017).

The effects of coriolic acid and ricinoleic acid on disease severity caused by *L. maculams* and *S. sclerotiorum* on canola cultivars were not as promising as the results obtained for monocotyledons crops. None of these fatty acids at 1 g/L or lower concentrations were effective against the pathogenic fungi affecting canola cultivars. Canola cultivars treated with coriolic acid and ricinoleic acid at the highest concentration (2 g/L) showed soaking and wilting symptoms which ultimately caused necrotic leaf tissues and dead seedlings. Although little information about the phytotoxicity of long chain fatty acids has been documented, the phytotoxic activity of stearic acid (C18:0), myristic acid (C14:0), and decanoic acid (C 12:0), extracted from root exudates of barnyard grass (*Echinochloa crusgalli* (L.) Beauv.), at 100 ppm concentrations has been reported on germination and growth rate of alfalfa, lettuce, monochoria, Indian jointvetch, and sesame (Xuan et al. 2006). Foliar treatment of middle-chain fatty acids including caproic acid (C6), caprylic acid (C8), peralgonic acid (C9), capric acid (C10), undecanoic acid (C11), lauric acid (C12), and myristic acid (C14) at 0.1 M concentration also resulted in severe to low levels of damage on crabgrass (*Digitaria ciliaris* Koel.) by degradation of cell membranes and

thylakoid membranes of the treated leaves (Fukuda et al. 2004; Lederer et al. 2004). It was also indicated that the observed variations in phytotoxicity is related to differences in hydrophobicity of the applied fatty acids which increases by increasing in the number of carbon-chain (Fukuda et al. 2004). According to these observations and the fact that amphiphilic monocarboxylic acids can disturb membrane-mediated processes by their partition into the bilayer of cell membranes (Gruber and low 1988), it can be suggested that coriolic acid and ricinoleic acid, as amphiphilic molecules with 18 carbons in their alkyl chains and high levels of hydrophobicity, possibly have the potential to damage the cell and thylakoid membranes of canola cultivars especially when uses at such high concentration (2 g/L). Although this phytotoxicity effect can be specific to some species, as indicated by this study, the monocotyledons crops were affected much lesser compared to dicotyledons ones.

The difference in functional doses of HUFAs to inhibit the disease severity caused by various pathogenic fungi illustrates that the inhibitory effects are not only dose-dependent but also fungus and/or host specific. However, the role of UFAs as signaling molecules to regulate plant defense pathways in the induction of resistance responses to the diseases cannot be ignored. Oleic acid treatment in tobacco leaves induced resistance responses to TMV, resulting in increased activity of defense related enzymes (phenylalanine ammonia lyase) and increased expression levels of defense related genes (PR-1a and PR-5) (Zhao et al. 2017). The exogenous application of UFAs such as arachidonic acid, docosahexaenoic acid, linolenic acid, eicosapentaenoic acid, oleic acid and linoleic acid on potato plants also induced systemic resistance against infection of potato by *Phytophthora infestans* (Cohen et al. 1991). Consequently, it can be speculated that coriolic acid treatments on monocotyledons crops probably triggered the defense mechanisms such as SA-dependent and/or JA-dependent

signaling pathways, resulting in induced resistance against subsequent challenge by the pathogenic fungi. Although further research is needed to understand the biology behind this defense mechanism.

Under various abiotic and biotic stresses, generation of ROS superoxide, hydrogen peroxide, hydroxyl radicals, and peroxy radicals are increased, thereby imposing a secondary oxidative burst in plant cells which can damage cellular structures and macromolecules and ultimately lead to cell death (Bhattacharjee 2005; Sewelam et al. 2016). In the present study to establish a link between formation of necrotic tissues on the canola cv. Westar treated by coriolic acid and ricinoleic acid at 2 and 1 g/L concentrations and enhanced ROS levels, we detected the accumulation of $H_2O_2$ and $O^-_2$ in the treated leaves. The histochemical staining of coriolic acid- and ricinoleic acid-treated leaves showed no apparent accumulation of $O^-_2$ compared with control leaves at both concentrations; however, increased staining suggested that the accumulation of $H_2O_2$ was probably higher in coriolic acid- and ricinoleic acid-treated leaves at 2 g/L concentration six hours post-treatment. It can be speculated that coriolic acid and ricinoleic acid at a 2 g/L concentration are able to trigger the enhanced production of $H_2O_2$ in foliar tissues of 'Westar' as a result of the oxidative burst through degradation of membrane lipids which eventually lead to oxidative damage and plant cell death (Bhattacharjee 2005; Gill 2010; Sharma 2012). This enhancement in accumulation of $H_2O_2$ may relate to changes in the activity of enzymatic antioxidant defense systems developed in plants against oxidative stress damage or non-enzymatic systems (Mittler 2004; Gill 2010), which will require further investigations to reveal. In contrast, increased staining for $O^-_2$ observed in oleic acid-treated leaves at 2 and 1 g/L concentrations compared with controls suggests that structural differences of fatty acids may play a role in production of various ROS indicators such as free radicals ($O^-_2$) or non-radical

molecules ($H_2O_2$). Although there is no literature regarding the exogenous application of coriolic acid, ricinoleic acid, and oleic acid on ROS accumulation in plant cells, several studies on mammalian cells illustrated the activity of long chain fatty acids including oleic acid in stimulation of the oxidative burst and enhancement of ROS levels (Inoguchi et al. 2000; Cury-Boaventura and Curi 2005; John Aitken et al. 2006; Hatanaka et al. 2013).

Long-chain fatty acids are known to be germination-inhibiting compounds and their inhibitory activity increases by increasing the number of carbon atoms present in the alkyl chains (Marambe et al. 1993; Edney and Rizvi 1996). In this study, however, ricinoleic and oleic acid had no effect on seed germination of the tested cultivars compared with controls, supporting the results obtained by Ferrarese et al. (1998) in which different concentrations of oleic acid had no effect on seed germination of *B. napus* L. cv Iciola 41. Likewise, among the long-chain fatty acids in animal-waste compost, saturated fatty acids are more potent germination-inhibitors of sorghum seeds than the UFAs (Marambe et al. 1993). In the case of coriolic acid, we observed a significant ($p<0.05$) germination-inhibiting effect at 2 g/L concentration for DH12075 but not at other concentrations. Currently, to our knowledge, there are no reports of coriolic acid effects on seed germination rate of monocotyledons and dicotyledons. Therefore, this inhibitory effect may be attributed to the threshold effect of fatty acids on seed germination (Buller et al. 1976) or changes in the rate of physiological processes of treated seeds such as water uptake and α-amylase activity (Marambe et al. 1992; Marambe et al. 1993). The diversity in germination rate of treated seeds by coriolic acid 2 g/L between crops may also be explained by the species-specific differences in the responses of crop species to the germination-inhibiting compounds (Marambe et al. 1991). The results of seed treatment assays shown the ineffectiveness of coriolic acid, ricinoleic acid, and oleic acid as seed treatment compounds against the tested

pathogenic fungi, which suggests the chemical instability and biodegradability of UFAs because of the high activity of double bonds (Pohl et al. 2011; Liu et al. 2014), as well as intricacy of antifungal functionality of the UFAs against the target fungi.

## 6. CONCLUSION AND FUTURE PROSPECTS

In conclusion, a comparison amongst the antifungal activity of coriolic acid, ricinoleic acid, and oleic acid provided insights into the structure-function relationships of UFAs as well as their host-pathogen interactions, meaning that the antifungal activity of UFAs is dependent on their chemical structure as well as the type of fungus and its host. Until now, a limited number of fatty acids were developed as commercial crop protective compounds against fungal diseases. As shown by the present study, UFAs such as coriolic acid possess superior activity against pathogenic fungi of moncotyledons both *in vitro* and *in vivo*. This suggests the potential of UFAs as environmentally-friendly crop protective agents for commercial application to control fungal diseases of plants, reduce environmental pollution in agricultural ecosystems and keep human and animal health safe. As the future line of works, evaluating the antifungal activity of coriolic acid against a wide range of fungal pathogens is valuable. Conducting seed treatment experiments to assess the efficacy of coriolic acid against seed-borne and/or soil-borne fungal pathogens affecting the seed or seedling viability is also worthwhile in management of diseases caused by them. Furthermore, it has been demonstrated that the antifungal activity, both *in vitro* and *in vivo*, does not explain what is happening in the plant; thus, the molecular mechanism behind this antifungal activity should be investigated further to understand how these fatty acids can stimulate plant defense signaling pathways. Investigating the metabolic profiling between coriolic acid treated plants and untreated ones is also important for identification of accumulated and/or diminished metabolites inside the plants which are involved in resistance induction. These

results will hopefully provide some useful insights into future control strategies of fungal diseases.

## LITERATURE CITED


Aboukhaddour R, Turkington TK, and Strelkov SE. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. Can J Plant Pathol. 35:256-268.

Aboukhaddour R, Cloutier S, Lamari L, Strelkov SE. 2011. Simple sequence repeats and diversity of globally distributed populations of Pyrenophora tritici-repentis. Can J Plant Pathol. 33:389-399.

Adams PB, and Ayers WA. 1979. Ecology of *Sclerotinia* spp. Phytopathol. 69:896-899.

Agarwal PK, Agarwal P, Reddy MK, and Sopory SK. 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep. 25:1263-1274.

Agriculture and Agri-food Canada. 2017. From a single seed-Tracing the Marquis wheat success story in Canada to its roots in Ukraine. [Online] Available from: http://www.agr.gc.ca/eng/news/scientific-achievements-in-agriculture/from-a-single-seed-tracing-the-marquis-wheat-success-story-in-canada-to-its-roots-in-ukraine-4of11/?id=1181305178350 [accessed 20 December 2017].

Akhavan A, Strelkov SE, Kher SV, Askarian H, Tucker JR, Legge WG, Tekauz A, and Turkington TK. 2017. Resistance to *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* in Canadian Barley Genotypes. Crop Sci. 57:151-160.

Akhavan A, Turkington TK, Askarian H, Tekauz A, Xi K, Tucker JR, Kutcher HR, and SE Strelkov. 2016. Virulence of *Pyrenophora teres* populations in western Canada. Can. J. Plant Pathol. 1:1-14.

Alavanja MCR, Hofmann JN, Lynch CF, Hines CJ, Barry KH, Barker J, Buckman DW, Thomas K, Sandler DP, Hoppin JA, Koutros S, Andreotti G, Lubin JH, Blair A, and Beane Freeman LE. 2014. Non-Hodgkin Lymphoma risk and insecticide, fungicide and fumigant use in the agricultural health Study. PLoS ONE. 9(10): e109332.

Alberta Agriculture and Forestry. 2017a. Crop protection. (H. Brook and M. Cutts, eds.). Alberta Agriculture and Forestry, Edmonton, AB.

Alberta Agriculture and Forestry. 2017b. Wheat Utilization. Government of Alberta, Agriculture and Forestry. [Online] Available form: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop1274 [accessed 25 December 2017].

Alberta Agriculture and Forestry. 2017c. Varieties of Cereal and Oilseed Crops for Alberta-2017. Government of Alberta, Agriculture and Forestry. [Online] Available from: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex4069 [accessed 01 January 2018].

Arabi MIE, Barrault G, Sarra fi A, Albertini L (1992) Variation in the resistance of barley cultivars and in the pathogenicity of *Drechslera teres* f. sp. *maculata and D. teres* f. sp. *teres* isolates from France. Plant Pathol. 41:180-186.

Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, and Willett WC. 1996. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ. 313:84-90.

Attanayake RN, Carter PA, Jiang D, del Río-Mendoza L, and Chen W. 2013. *Sclerotinia sclerotiorum* populations infecting canola from China and the United states are genetically and phenotypically different. Phytopathology 103:750-761.

Atkinson NJ, and Urwin PE. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot. 63:3523-3544.

Aubertot JN, West JS, Bousset-Vaslin L, Salam MU, Barbetti MJ, and Diggle AJ. 2006. Improved resistance management for durable disease control: a case study of phoma stem canker of oilseed rape (*Brassica napus*). Eur J Plant Pathol.

Aubertot JN, Pinochet X, and Dore T. 2004. The effects of sowing date and nitrogen availability during vegetative stages on *Leptosphaeria maculans* development on winter oilseed rape. Crop Prot. 23:635-645.

Aune D, Chan DS, Lau R, Vieira R, Greenwood DC, Kampman E, and Norat T. 2011. Dietary fibre, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. BMJ. doi: 10.1136/bmj.d6617.

Avis TJ, and Belanger RR. 2001.Specificity and mode of action of the antifungal fatty acid *cis*-9-heptadecenoic acid produced by *Pseudozyma flocculosa*. Appl. Environ. Microbiol. 67:956-960.

Bafor M, Smith MA, Jonsson L,Stobart K, and Stymne S. 1991. Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (Ricinus communis) endosperm. Biochem. J. 280:507-514.

Bailey KL, Johnston AM, Kutcher HR, Gossen BD, and Morrall RAA. 2000. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can J Plant Sci. 80:169-175.

Bailey KL, Mortensen K, and Lafond GP. 1992. Effects of tillage systems and crop rotations on root and foliar diseases of wheat, flax, and peas in Saskatchewan. Cann J Plant Sci. 72:583-591.

Bajpai VK, Kim HR, Hou CT, and Kang SC. 2009. Microbial conversion and in vitro and in vivo antifungal assessment of bioconverted docosahexaenoic acid (bDHA) used against agricultural plant pathogenic fungi. J. Ind. Microbiol. Biotecnol. 36:695-704.

Bardin SD, and Huang HC. 2001. Research on biology and control of *Sclerotinia* diseases in Canada. CanJ Plant Pathol. 23:88-98.

Barley Council of Canada. 2017. [Online] Available from: http://cafta.org/export/barley [accessed 15 November 2017].

Bhattacharjee S. 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. Current Sci. 89:1113-1121.

Black BA, Zannini E, Curtis JM, and Gänzle MG. 2013. Antifungal hydroxy fatty acids produced during sourdough fermentation: microbial and enzymatic pathways, and antifungal activity in bread. Appl Environ Microbiol. 79:1866-1873.

Bockus WW, and Claasen MM. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Dis. 76:633-636.

Bogacki P, Keiper FJ, and Oldach KH. 2010. Genetic structure of South Australian *Pyrenophora teres* populations as revealed by microsatellite analyses. Fungal Biol. 114:834-841.

Boland GJ, and Hall R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum.* Can J Plant Pathol. 16:93-108.

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

Boosalis MG, Doupnik BL, Wysong DS, and Watkins JE. 1983. The wheat scab epidemic of 1982. Univ. Nebr. Farm, Ranch Home Quart. 29:7-9.

Bottalico A, and Perrone G. 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol. 108:611-624.

Bowers WS, Hoch HC, Evans PH, and Katayama M. Thallophytic Allelopathy - isolation and identification of laetisaric acid. Science. 232:105-106.

Bradley CA, and Lamey HA. 2005. Canola disease situation in North Dakota, USA, 1993–2004. Pages 33-34 in: Proc. 14th Aust. Res. Assembly on Brassicas, Port Lincoln, Australia.

Broekaert WF, Courtin CM, Verbeke K, Van de Wiele T, Verstraete W, and Delcour JA. 2011. Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. Crit Rev Food Sci Nutr. 51:178-194.

Brown S, Koike ST, Ochoa OE, Laemmlen F, and Michelmore RW. 2004. Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, Bremia lactucae. Plant Dis. 88:502-508.

Buller DC, Parker W, and Reid JSG. 1976. Short-chain fatty acids as inhibitors of gibberellin-induced amylolysis in barley endosperm. Nature. 260:169-170.

Busch L, Gunter V, Mentele T, Tachikawa M, and Tanaka K. 1994. Socializing Nature: Technoscience and the Transformation of Rapeseed into Canola. Crop Sci. 34:607-614.

Canadian Grain Commission. Barley – Chapter 6 | Official Grain Grading Guide. 2017a. [Online] Available from: https://www.grainscanada.gc.ca/oggg-gocg/06/oggg-gocg-6-eng.htm [accessed 20 December 2017].

Canadian Grain Commission. Quality of western Canadian malting barley 2017-Barley production. 2017b. [Online] Available from: http://www.grainscanada.gc.ca/barley-orge/harvest-recolte/2017/qbsm17-qosm17-2-en.html [accessed 31 December 2017].

Canola Council of Canada. 2017a. Good as Gold. Celebrating 50 years of canola. [Online] Available from: https://canolahistory.ca [accessed 20 November 2017].

Canola Council of Canada. 2017a. Industry overview. [Online] Available from: https://www.canolacouncil.org/markets-stats/industry-overview [accessed 15 November 2017].

Canola Council of Canada. 2017b. Canola Oil. [Online] Available from: https://www.canolacouncil.org/media/501782/canola_oil.pdf [accessed 15 November 2017].

Canola Council of Canada. 2017c. Canola FAQ's. [Online] Available from: https://www.canolacouncil.org/oil-and-meal/what-is-canola/canola-faq's [accessed 21 November 2017].

Canola Council of Canada. 2017d. Canola Encyclopedia. [Online] Available from: https://www.canolacouncil.org/canola-encyclopedia [accessed 28 November 2017].

Canola Council of Canada. 2017e. Canola Encyclopedia. Sclerotinia Stem Rot. [Online] Available from: https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/# [accessed 22 December 2017].

Cantrell CL, Case BP, Edward Mena E, Kniffin TM, Duke SO, and Wedge DE. 2008. Isolation and identification of antifungal fatty acids from the Basidiomycete *Gomphus floccosus*. J. Agric. Food Chem. 56: 5062-5068.

Casséus L. 2009. Canola: a Canadian success story. [Online] Available from: http://www.statcan.gc.ca/pub/96-325-x/2007000/article/10778-eng.htm [accessed 16 November 2017].

Chechetkin IR, Medvedeva NV, and Grechkin AN. 2004. The novel pathway for ketodiene oxylipin biosynthesis in Jerusalem artichoke (Helianthus tuberosus) tubers. Biochim. Biophys. Acta. 1686:7-14.

Chen YY, Liang N, Curtis JM, and Gänzle MG. 2016. Characterization of Linoleate 10-hydratase of *Lactobacillus plantarum* and Novel Antifungal Metabolites. Frontiers in Microbiology. 7:1-11.

Chen XM. 2005. Epidemiology and control of stripe rust [Puccinia striiformis f. sp. tritici] on wheat. Can J Plant Pathol. 27:314-337.

Chen Y, and Fernando WGD. 2006. Prevalence of pathogenicity groups of *Leptosphaeria maculans* in western Canada and North Dakota, USA. Can J Plant Pathol. 28:533-539.

Choi GJ, Jang KS, Choi YH, Yu JH, and Kim JC. 2010. Antifungal activity of lower alkyl fatty acid esters against powdery mildews. Plant Pathol. J. 26:360-366.Cury-Boaventura MF, and Rui Curi. 2005. Regulation of reactive oxygen species (ROS) production by C 18 fatty acids in Jurkat and Raji cells. Clinical Sci. 108:245-253.

Colson ES, Platz GJ, and Usher TR. 2003. Fungicidal control of *Pyrenophora tritici-repentis* in wheat. Aust Plant Pathol. 32: 241-246.

Cook RJ, Yarham DJ, 1989. Occurrence of tan spot of wheat caused by *Pyrenophora tritici-repentis* on wheat in England and Wales in 1987. Plant Pathol. 38:101-102.

Cooper R. 2015. Re-discovering ancient wheat varieties as functional foods. J Tradit Complement Med. 5:138-143.

Czembor JH. 2001. Sources of resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*) in Moroccan barley land racesCan J Plant Pathol. 23:260-269.

Danielsson J, Reva O, and Meijer J. 2007. Protection of oilseed rape (*Brassica napus*) toward fungal pathogens by strains of plant-associated *Bacillus amyloliquefaciens*. Microbial Ecol. 54:134-140.

Daudi A, and O'Brien JA. 2012. Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. Bioprotocol 2: e263.

Davies JML. 1986. Diseases of oilseed rape London: Collins. 195-236.

Day L, Augustin MA, Batey IL, and Wrigley CW. 2006. Wheat-gluten uses and industry needs. Trends Food Sci Tech. 17:82-90.

Decloedt A, Watson H, Van Landschoot A, Vanderputten D. 2015. Gluten-free barley malt beers. Fermentatio studiedag, Samenvattingen. Koninklijke Oud-Studentenbond Fermentatio Hogeschool Gent.

Demeke T, Clear RM, Patrick SK, and Gaba D. 2005. Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. food Microbiol. 103: 271-284.

del Río LE, Bradley CA, Hensen RA, Endres GJ, Hanson BK, McKay K, Halvorson M, Porter PM, Le Gare DG, and Lamey HA. 2007. Impact of sclerotinia stem rot on yield of canola. Plant Dis. 91:191-194.

De Wolf ED, Effertz RJ, Ali S, and Francl LJ. 1998. Vistas of tan spot research. Can J Plant Pathol. 20:349-370.

Djoussé L, Pankow JS, Eckfeldt JH, Folsom AR, Hopkins PN, Province MA, Hong Y, and

Ellison RC. 2001. Relation between dietary linolenic acid and coronary artery disease in the National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr. 74:612-619.

Ding L, Peschel G, and Hertweck C. 2012. Biosynthesis of archetypal plant self-defensive oxylipins by an endophytic fungus residing in mangrove embryos. Chembiochem. 13: 2661-2664.

Edney NA, and Rizvi M. 1996. Phytotoxicity of fatty acids present in dairy and hog manure. J. Environ. Sci. Health. 31:269-281.

Entz MH, Berg CVD, Stobbe EH, Rossnagel BG, Lafond GP, and Austenson HM. 1990. Effect of late-season fungicide application on grain yield and seed size distribution in wheat and barley. Can J Plant Sci. 70: 699-706.

FAOSTAT. 2017. Food and Agriculture Organization of the United Nations. [Online] Available from: http://www.fao.org/docrep/006/y4011e/y4011e04.htm [accessed 20 December 2017].

Fajardo JE, Dexter JE, Roscoe MM, and Nowicki TW. 1995. Retention of ergot alkaloids in wheat during processing. Cereal Chem. 72:291-298.

Fang J, and Platford RG. 1995. Distribution, Prevalence, and incidence of canola diseases in Manitoba 1994. Can Plant Dis Surv. 75:145-147.

Feng J, Zhang H, Strelkov SE, Hwang SF. 2014. The *LmSNF1* gene is required for pathogenicity in the canola blackleg pathogen *Leptosphaeria maculans*. PlosOne. 9:e92503.

Fernandez MR, Depauw RM, and Clarke JM. 2001. Reaction of common and durum wheat cultivar to infection of kernels by *Pyrenophora tritici-repentis*. Can J Plant path. 23:158-162.

Fernandez MR, Clarke JM, De Pauw RM, and Lefkovitch LP. 1997. Emergence and growth of durum wheat derived from red smudge-infected seed. Crop Sci. 37:510-514.

Ferrarese MLL, Baleroni CRS, Ferrarese-Filho O. 1998. Effects of fatty acids on carbohydrates and lipids of canola seeds during germination. Braz. Arch. Biol. Technol. 41:3.

Fetch TG, Mitchell Fetch J, and Xue A. 2011. Races of Puccinia graminis on barley, oat and wheat in Canada in 2006. Can J Plant Pathol: 33:54-60.

Francl LJ. 1998. Genesis and liberation of conidia of *Pyrenophora tritici-repentis*. Can J Plant Pathol. 20:387-393.

Francl LJ and Jordhal JG. 1992. Seed symptomology of durum wheat. In Proceedings of the Second International Tan Spot Workshop.25-26 June 1992, Fargo, N.D. Edited by L.J. Francl, J.M. Krupinsky, and M.P. McMullen. North Dakota State University, Fargo, N.D. P: 61-64.

François IE, Lescroart O, Veraverbeke WS, Marzorati M, Possemiers S, Evenepoel P, Hamer H, Houben E, Windey K, Welling GW, Delcour JA, Courtin CM, Verbeke K, and Broekaert WF.

2012. Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: a double-blind, randomised, placebo-controlled, cross-over trial. Br J Nutr. 108:2229-2242.

Fukuda M, Tsujino Y, Fujimori T, Wakabayashi K, and Boger P. 2004. Phytotoxic activity of middle-chain fatty acids I: effects on cell constituents. Pesticide Biochem. Physiol. 80:143-150.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, and Shinozaki K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol. 9:436-442.

Gao X, Han Q, Chen Y, Qin H, Huang L, and Kang Z. 2014. Biological control of oilseed rape Sclerotinia stem rot by *Bacillus subtilis* strain Em. Biocontrol Sci and Tech. 24:39-52.

Gamba FM, and Lamari L. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). Can J Plant Path. 20:408-414.

Gamba FM, Lamari L, and Brule-Babel AL. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Can J Plant Pathol. 20:401-407.

Geddes J, Eudes F, Tucker JR, et al. 2008. Evaluation of inoculation methods on infection and deoxynivalenol production by *Fusarium graminearum* on barley. Can. J. Plant Pathol. 30: 66-73.

Gill SS, and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stresstolerance in crop plants. Plant Physiol. Biochem. 48:909-930.

Gill BS, Appels R, Botha-Oberholster AM, Robin Buell C, Bennetzen JL, Chalhoub B, Chumley F, Dvorˇa´k J, Iwanaga M, Keller B, Li W, McCombie WR, Ogihara Y, Quetier F, Sasaki T. 2004. A Workshop Report on Wheat Genome sequencing: International Genome Research on Wheat Consortium. Genetics. 168:1087-1096.

GoBarley. 2017. Barley in Canada: A Heritage of Cultivating Quality. [Online] Available from: http://gobarley.com/wp-content/uploads/2016/06/Canadas-Barley-Heritage-FINAL.pdf [accessed 31 December 2017].

Government of Saskatchewan. 2016. Guide to Crop Protection. Government of Saskatchewan, Regina, Saskatchewan, Canada. [Online]. Available from: http://publications.gov.sk.ca/documents/20/870922017%20Guide%20to%20Crop%20Protection%20-%20disease%20control.pdf [accessed 20 December 2017].

Gossen BD, Rimmer SR, and Holly JD. 2001. First report of resistance to benomyl fungicides in *Sclerotinia sclerotiorum*. Plant Dis. 85:1206.

Gossen BD, Carisse O, Kawchuk LM, Van Der Heyden H, and McDonald MR. 2014. Recent changes in fungicide use and the fungicide insensitivity of plant pathogens in Canada. Can J Plant Pathol. 36:327-340.

Gough FJ, and Ghazanfani J. 1982. Biological antagonists of *Pyrenophora tiritici-repentis*. Pages 36-39 in R.M. Hosford Jr., ed. Tan Spot of Wheat and Related Diseases Workshop. North Dakota Agric. Exp. Station, Fargo.

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

Graner G1, Hamberg M, and Meijer J. 2003. Screening of oxylipins for control of oilseed rape (Brassica napus) fungal pathogens. Phytochemistry. 63:89-95.

Gruber HJ, and Low PS. 1988. Interaction of amphiphiles with integral membrane proteins. I. Structural destabitlization of the anion transport protein of the erythrocyte membrane by fatty acids, fatty alcohols, and fatty amines. Biochimica Biophysica Acta. 944: 414-424.

Gugel RK, and Petrie GA. 1992. History, occurrence, impact, and control of blackleg of rapeseed. Can J Plant Pathol. 14:36-45.

Gunstone F. 2011. Vegetable Oils in Food Technology: Composition, Properties and Uses. Oxford: Blackwell.

Guo XW, Fernando WGD and Entz M. 2005. Effects of crop rotation and tillage on blackleg disease of canola. Can J Plant Pathol. 27:53-57.

Hammond KE, Lewis BG, and Musa TM. 1985. A systemic pathway for the infection of oilseed rape plants by *Leptosphaeria maculans*. Plant Pathol. 34:557-565.

Hammoudi O, Salman M, Abuamsha R, and Ehlers RU. 2012. Effectiveness of bacterial and fungal isolates to control Phoma lingam on oilseed rape *Brassica napus*. Am J Plant Sci. 3:773-779.

Harvey IC, Craigie RA, and McCloy B. 2015. The control of tan spot of wheat (caused by *Pyrenophora tritici-repentis*): a possible emerging disease in New Zealand. New Zealand Plant Protec. 68: 428-433.

Hatanaka E, Dermargos A, Hirata AE, Vinolo MAR, Carpinelli AR, Newsholme P, Armelin HA, Curi R. 2013. Oleic, Linoleic and Linolenic Acids increase ROS production by fibroblasts via NADPH oxidase activation. PLosOne. 8:e58626.

Hawkesford MJ, Araus JL, Park R, Calderini D, Miralles D, Shen T, Zhang J, Parry MAJ. 2013. Prospects of doubling global wheat yields. Food En Sec. 2: 34-48.

Hayward A, McLanders J, Campbell E, Edwards D, and Batley J. 2012. Genomic advances will herald new insights into the Brassica: *Leptosphaeria maculans* pathosystem. Plant Biol. 14:1-10.

Hökeberg M. 1998. Seed bacterization for control of fungal seed-borne diseases in cereals. Ph.D. thesis.Uppsala, Sweden: Swedish University of Agricultural Sciences.

Hosford RM Jr. 1982. Tan spot: developing knowledge 1902–1981. In: Tan spot of wheat and related disease workshop. July. 1981. Fargo, ND. Edited by Hosford RM Jr. North Dakota State University, Fargo, ND, USA. P:1-24.

Hosford RM Jr. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopatol. 62:28-32.

Hou CT, and Forman RJ. 2000. Growth inhibition of plant pathogenic fungi by hydroxy fatty acids. J. Industrial Microbiol. Biotech. 24:275-276.

Hu FB, Stampfer MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, Hennekens CH, and Willett WC. 1999. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr. 69:890-897.

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, and Mohler V. 2000. Molecular mapping of the wheat powdery mildew resistance gene Pm24 and marker validation for molecular breeding. Theor App Genet. 101: 407-414.

Huang YJ, Pirie EJ, Evans N, Delourme R, King GJ, and Fitt BDL. 2009. Quantitative resistance to symptomless growth of *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape). Plant Pathol. 58:314-323.

Hwang SF, Ahmed HU, Strelkov SE, Gossen BD, Turnbull GD, Peng G, Howard RJ. 2011. Seedling age and inoculum density affect clubroot severity and seed yield in canola. Can J Plant Sci. 91:183–190.

Hwang SF, Strelkov SE, Peng G, Ahmed H, Zhou Q, and Turnbull G. 2016. Blackleg (*Leptosphaeria maculans*) severity and yield loss in canola in Alberta, Canada. Plants. 5(3):31.

Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, and Nawata H. 2000. High Glucose Level and Free Fatty Acid Stimulate Reactive Oxygen Species Production Through Protein Kinase C–Dependent Activation of NAD(P)H Oxidase in Cultured Vascular Cells. Diabetes. 49:1939-1945.

Jayasena KW, Van Burgel A, Tanaka K, Mejewski J, and Loughman R. 2007. Yield reduction in barley in relation to spot-type net blotch. Austr Plant pathol. 36:429-433.

Jayasena KW, Loughman R, Majewski J. 2002. Evaluation of fungicides in control of spot-type net blotch on barley. Crop Protec. 21:63-69.

Jesperson GD. 1989. Survey of blackleg, sclerotinia and footrot in Saskatchewan canola crops, 1986. Can Plant Dis Surv. 69:60-61.

John Aitken R, Wingate JK, De Iuliis GN, Koppers AJ, and McLaughlin EA. 2006. *Cis*-Unsaturated Fatty Acids Stimulate Reactive Oxygen Species Generation and Lipid Peroxidation in Human Spermatozoa. J. clinical Endocrin. Metabol. 91:4154-4163.

Jonnalagadda SS, Harnack L, Liu RH, McKeown N, Seal C, Liu S, and Fahey GC. 2011. Putting

the whole grain puzzle together: health benefits associated with whole grains-- summary of American Society for Nutrition 2010 Satellite Symposium. J Nutr. 141:1011S-1022S.

Jordan VWL, and Allen EC. 1984. Barley net blotch: influence of straw disposal and cultivation methods on inoculum potential, and on incidence and severity of autumn disease. Plant pathol. 33:547-559.

Jorgensen LN, and Olsen LV. 2007. Control of tan pot (*Drechslera tritici-repentis*) using cultivar resistance, tillage methods and fungicides. Crop Protec. 26:1606-1616.

Joshi RK, Megha S, Rahman MH, Basu U, Kav NVV. 2016. A global study of transcriptome dynamics in canola (*Brassica napus* L.) responsive to *Sclerotinia sclerotiorum* infection using RNA-Seq. Gene. 590: 57-67.

Juska A, Busch L, and Tanaka K. 1997. The blackleg epidemic in Canadian rapeseed as a " Normal Agricultural Accident". Ecol Appl. 7:1350-1356.

Kachroo A, and Kachroo P. 2009. Fatty acids-derived signals in plant defense. Annu. Rev. Phytopathol. 47:153 -176.

Kaczmarek J, and Jedryczka M. 2011. Characterization of two coexisting pathogen populations of Leptosphaeria spp., the cause of stem canker of brassicas. Acta Agrobot. 64:3-13.

Kader KA. 2010. Fitness, virulence and genetic variability in *Pyrenophora tritici-repentis* isolates causing tan spot of wheat in Oklahoma. Unpublished PhD thesis, Oklahoma State University, USA.

Kato TY, Yamaguchi N, Abe T, Uyehara T, Namai M, Kodama M and Shiobara Y. 1985. Structure and synthesis of unsaturated trihydroxy C-18 fatty acids in rice plant suffering from rice blast disease. Tetrahedron Lett.26: 2357-2360.

Kato T, Yamaguchi Y, Namai T and Hirukawa T. 1993. Oxygenated fatty acids with anti-rice blast fungus activity in rice plants. Biosci. Biotechnol.and Biochem. 57: 283 - 287.

Keiper FJ, Grcic M, CapioE, and Wallwork, H. 2008. Diagnostic microsatellite markers for the barley net blotch pathogens, Pyrenophora teres f. maculata and Pyrenophora teres f. teres. Australas Plant Pathol. 37:428-430.

Khan MR, Eleanor OB, Carney BF, and Doohan FM. 2010. A fluorescent pseudomonad shows potential for the control of net blotch disease of barley. Biol control. 54:41-45.

Khan TN. 1989. Effect of spot-type net blotch (*Drechslera teres* (Sacc.) Shoem) infection on barley yield in short season environment of northern cereal belt of Western Australia. Austr J Agric Res. 40:745-752.

Khangura RK, and Barbetti MJ. 2004. Time of sowing and fungicides affect blackleg (*Leptosphaeria maculans*) severity and yield in canola. Aust J Exp Agr. 44:1205-1213.

Khangura RK, and Barbetti M. 2001. Prevalence of blackleg (*Leptosphaeria maculans*) on canola (*Brassica napus*) in Western Australia. Aust J Exp Agri. 41:71-80.

Kharbanda PD, Coleman RN, Beatty PH, Jensen SE, Tewari JP, and Yang J. 2003. *Paenibacillus polymyxa* strain ATCC 202127 for biocontrol of bacteria and fungi. The Governors of the University of Alberta, Edmonton, Canada; Alberta Research Council, Edmonton, Canada.

Kharbanda PD, and Tewari JP. 1996. Integrated management of canola diseases using cultural methods. Can J Plant Pathol. 18:168-175.

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

Kohli M, Mehta Y, and Ackermann M. 1992. Spread of tan spot in the Southern Cone Region of South America. In: L. Francl, J. Krupinsky, & M. Mc Mullen (Eds.) Advances in Tan Spot. Proceedings of the Second International Tan Spot Workshop. Fargo, ND: North Dakota State University. P:86-90.

Kumar D, Yusuf MA, Singh P, Sardar M and Sarin NB. 2014. Histochemical detection of superoxide and $H_2O_2$ accumulation in Brassica juncea seedlings. Bio-Protocol 4:e1108.

Kurle JE, Grau CR, Oplinger ES, and Mengistu A. 2001. Tillage, crop sequence and cultivar effects on Sclerotinia stem rot incidence and yield in soybean. Agronomy J. 93:973-982.

Krupinsky JM, Tanaka DL, Lares MT, and Merrill SD. 2004. Leaf spot diseases of barley and spring wheat as influenced by preceding crops. Agron J. 96:259-266.

Krupinsky JM. 1992. Grass hosts of *Pyrenophora tritici repentis*. Plant Dis. 76:92-95.

Kunkel BN, and Brooks DM. 2002. Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant Biol. 5:325-331.

Kutcher HR, Brandt SA, Smith EG, Ulrich D, Malhi SS, and Johnston AM. 2013. Blackleg disease of canola mitigated by resistant cultivars and four-year crop rotations in western Canada. Can J Plant Pathol. 35:209-221.

Kutcher HR, Fernando WGD, Turkington TK, and McLaren DL. 2011. Best management practices for blackleg disease of canola. Prairie Soils and Crops. 4:122-134.

Lamari L, and Strelkov SE. 2010. The wheat/*Pyrenophora tritici- repentis* interaction: progress towards an understanding of tan spot disease. Can J Plant Pathol. 32:4-10.

Lamari L, Strelkov SE, Yahyaoui A, Orabi J, and Smith RB. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathol. 93:391-396.

Lamey HA. 2003. The status of Sclerotinia sclerotiorum on canola in North America. Proc. 2003 Sclerotinia Initiative Annual Meeting, Bloomington, MN.

Lederer B, Fujimori T, Tsujino Y, Wakabayashi K, and Boger P. 2004. Phytotoxic activity of middle-chain fatty acids II: peroxidation and membrane effects. Pesticide Biochem. Physiol. 80:151-156.

Leisova L, Hanzalová A, Kucera L, 2008. Genetic diversity of *Pyrenophora tritici- repentis* isolates as revealed by AFLP analysis. J Plant Pathol. 90:233-245.

Leisova L, Kucera L, Minarikova V, Ovesna J. 2005. AFLP based PCR markers that differentiate spot and net forms of *Pyrenophora teres*. Plant Pathol. 54:66-73.

Leonard KJ, and Szabo LJ. 2005. Stem rust of small grains and grasses caused by Puccinia graminis. Mol. Plant Pathol. 6:99-111.

Liang N, Cai P, Wu D, Pan Y, Curtis JM, and Gänzle, MG. 2017. High-Speed Counter-Current Chromatography (HSCCC) purification of antifungal hydroxy unsaturated fatty acids from plant-seed oil and *Lactobacillus* cultures. J. Agric. Food Chem. DOI: 10.1021/acs.jafc.7b05658.

Li GQ, Huang HC, and Acharya SN. 2003. Antagonism and biocontrol potential of *Ulocaldium atrum* on *Sclerotinia sclerotiorum*. Biol Control. 28:11-18.

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

Li X, Dodson J, Zhou X, Zhang H, and Masutomoto R. 2007. Early cultivated wheat and broadening of agriculture in Neolithic China. The Holocene. 17:555-560.

Liu X, Han R, Wang Y, Li X, Zhang M, and Yan Y. 2014. Fungicidal activity of a medium-chain fatty acids mixture comprising caprylic, pelargonic, and capric acids. Plant Pathol. J. 13:65-70.

Liu Z, Ellwood SR, Oliver RP, Friesen TL. 2011. *Pyrenophora teres*: profile of an increasingly damaging barley pathogen. Mol Plant Pathol. 121:1-19.

Liu Z, and Friesen TL. 2010. Identification of *Pyrenophora teres* f. *maculata*, causal agent of spot type net blotch in North Dakota. Plant Dis. 94:480.

Liu SY, Liu Z, Fitt BDL, Evans N, Foster SJ, Huang YJ, Latunde-Dada AO, and Lucas JA. 2006. Resistance to *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape) induced by *L. biglobosa* and chemical defence activators in field and controlled environments. Plant pathol. 55: 401- 412.

Louw JP, Crous PW, Holz G. 1996. Relative importance of the barley net blotch pathogens *Pyrenophora teres* f. *teres* (net type) and *P. teres* f. *macu*lata (spot type) in South Africa. Afr Plant Protect. 2:89-95.

Lu S, Platz GJ, Edwards MC, and Friesen TL. 2010. Mating type locus-specific polymerase chain reaction markers for differentiation of Pyrenophora teres f. teres and P. teres f. maculata, the causal agents of barley net blotch. Phytopathology. 100:1298-1306.

Lu ZX, Walker KZ, Muir JG, Mascara T, and O'Dea K. 2000. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. Am J Clin Nutr. 71:1123-1128.

Magnusson J, and Schnurer J. 2001. Lactobacillus coryniformis subsp. coryniformis Strain Si3 Produces a Broad-Spectrum Proteinaceous Antifungal Compound. Appl. and Environ. Microbiol. 67:1-5.

Marambe B, Nagaoka T, and Ando T. 1993. Identification and biological activity of germination-inhibitoin long-chain fatty acids in animal-waste compost. Plant cell Physiol. 34: 605-612.

Marambe B, Ando T, and Kouno K. 1992. Alpha-amylase and protease activities and water relations in germinating sorghum (*Sorghum bicolor* Moench) seeds as affected by animal-waste composts. Soil Sci. Plant Nutr. 38:123-131.

Marambe B, Ando T, and Kouno K. 1991 The interspecific and inter-parametric differences of forage crops in response to seed treatment with aqueous extracts of animal-waste com- posts. J. Fac. Appl. Biol. Sci., *Hiroshima Univ*., Japan 30:19-29.

Marcroft SJ, Van de Wouw AP, Salisbury PA, Potter TD, and Howlett BJ. 2012. Effect of rotation of canola (*Brassica napus*) cultivars with different complements of blackleg resistance genes on disease severity. Plant Pathol. 61:934-944.

Martens JW, Seaman WL, and Atkinson TG. 1988. Diseases of Field Crop Crops in Canada; Revised Edition. The Canadian Phytopathological Society, Harrow, Ontario.

Martin K, Kutcher HR, and Johnston AM. 2001. Impact of tillage system, rotation, and fungicide application on field crop production. Can J Plant Pathol. 23:187.

Martin RA, and Johnston HW. 1982. Effects and control of fusarium diseases of cereal grains in the Atlantic Provinces. Can. J. Plant Pathol. 4:210-216.

Martin-Arjol I, Bassas-Galia M, Bermudoc E, Garcia F, Manresaa A. 2010. Identification of oxylipins with antifungal activity by LC–MS/MS from the supernatant of Pseudomonas 42A2. Chem Phys Lipids. 163:341-346.

Mathre DE. 1997. Compendium of Barley Diseases, 2nd edn. St. Paul, MN: American Phytopathological Society.

Mazur P, Nakanishi K, El-Zayat A, and Champe SP. 1991. Structure and synthesis of sporogenic Psi factors from *Aspergillus nidulans*. J. chem. Soc., Chem. Commun. 1486-1487.

McLaren DL, Graham AD, Kaminski DA, and Lange R. 2005. Canola diseases in Manitoba: distribution, prevalence, and incidence in 2004. Can Plant Dis Surv. 85:76-77.

McLaren DL, Huang HC, and Rimmer SR. 1996. Control of apothecial production of *Sclerotinia sclerotiorum* by *Coniothrium minitans* and *Talaromyces flavus*. Plant Dis. 80:1373-1378.

McLean MS, Howlett BJ, and Hollaway GJ .2009. The epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculate*) of barley: a review. Crop Pasture Sci. 60:303-315.

McMullen M, Jones R, and Gallenberg D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

Menzies JG, Thomas PL and Woods S. 2014. Incidence and severity of loose smut and surface-borne smuts of barley on the Canadian prairies from 1972 to 2009. Can J Plant Pathol.36:300-310.

Mittler R, Vanderauwera S, Gollery M, and Breusegem FV. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9:490-498.

Montesinos E, Bonaterra A, Badosa E, Francés J, Alemany J, Llorente I, and Moragrega C. 2002. Plant-microbe interactions and the new biotechnological methods of plant disease control. Int Microbiol. 5:169-175.

Morrall RAA, and Dueck J. 1982. Epidemiology of sclerotinia stem rot of rapeseed in Saskatchewan. Can J Plant Pathol. 4:161-168.

Murray GM, and Brennan JP. 2010. Estimating disease losses to the Australian barley industry. Austr Plant Pathol. 39:85-96.

Murray G, Brown J. 1987. The Incidence and relative importance of wheat diseases in Australia. AustPlant Pathol. 16:34-37.

Mwale VM, Chilembwe EHC, Uluko HC. 2014. Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*): Damage effects and genetic resistance developed in wheat (*Triticum aestivum*). Int Res J Plant Sci. 5:1-16.

Namai T, Kato T, Yamaguchi Y, and Hirukawa T. 1993. Anti-rice blast activity and resistance induction of C-18 oxygenated fatty acids. Biosci. Biotechnol. Bioche. 57: 611- 613.

National Center for Biotechnology Information. 2017a. Organism Overview, *Triticum aestivum* (bread wheat). [Online]. Available from: https://www.ncbi.nlm.nih.gov/genome/?term=wheat%5Borgn%5D [accessed 20 December 2017].

National Center for Biotechnology Information. 2017b. Organism Overview, *Hordeum vulgare* [Online]. Available from: https://www.ncbi.nlm.nih.gov/genome/?term=barley [accessed 29 December 2017].

Navabi ZK, Strelkov SE, Good AG, Thiagarajah MR, and Rahman MH. 2010. *Brassica* B-genome resistance to stem rot (*Sclerotinia sclerotiorum*) in a doubled haploid population of *Brassica napus* × *Brassica carinata.* Can J. Plant Pathol. 32:237-246.

Nwachukwu EO, and Umechuruba CI. 2001. Antifungal Activities of Some Leaf Extracts on Seed-borne Fungi of African Yam Bean Seeds, Seed Germination and Seedling Emergence. J Appl. Sci. Environ. Mgt. 5: 29-32.

O'Brien JA, Daudi A, Butt VS, and Bolwell GP. 2012. Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta. 236:765-779.

Pageau D, Lajeunesse J, and Lafond J. 2006. Impact of clubroot [Plasmodiophora brassicae] on the yield and quality of canola. Can J Plant Pathol. 28:137-143.

Parry DW, Jenkinson P, and McLeod L. 1995. Fusarium ear blight (scab) in small grain cereals—a review. 44:207-238.

Patel JS, Gudmestad NC, Meinhardt S, Adhikari TB. 2012. Pyraclostrobin sensitivity of baseline and fungicide exposed isolates of Pyrenophora tritici-repentis. Crop Protec. 34:37-41.

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Bergès H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, and Feuillet C. 2008. A physical map of the 1Gb bread wheat chromosome 3B. Science. 322(5898):101-104.

Peluola C, Fernando WGD, Huvenaars C, Kutcher HR, Lahlali R, and Peng G. 2013. Effect of flooding on the survival of *Leptosphaeria spp.* in canola stubble. Plant Pathol. 62:1350-1356.

Perello AE, Monaco CI, Moreno MV, Cordo CA, and Simon MR. 2006. The effect of *Trichoderma harzianum* and *T. koningii* on the control of tan spot (*Pyrenophora tritici-repentis*) and leaf blotch (*Mycosphaerella graminicol*a) of wheat under field conditions in Argentina. Biocontrol Sci and Tech. 16: 803-813.

Pfender WF, King LG, and Rabe JR. 1991. Use of dual- stain fluorescence microscopy to observe antagonism of *Pyrenophora tritici-repe*ntis by *Limonomyces roseipellis* in wheat straw. Phytopathol. 81:109-112.

Pfender WF, Kraus J, and Loper JE. 1993. A genomic region from Pseudomonas Fluorescens Pf-5 required for Pyrrolnitrin production and inhibition of *Pyrenophora tritici-repentis* in wheat straw. Phytopathol. 83:1223-1228.

Platford RG. 1996. Distribution, Prevalence and incidence of canola diseases in Manitoba in 1995. Can Plant Dis Surv. 76:103-105.

Pohl CH, Kock JLF, and Thibane VS. 2011. Antifungal free fatty acids: A review. Science against microbial pathogens: current research and technological advances. 1:61-71.

Ponomarenko A, Goodwin SB, and Kema GHJ. 2011. Septoria tritici blotch (STB) of wheat. Plant Heal. Instr. [Online] Available from: https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/pages/septoria.aspx [accessed 27 December 2017].

Pratt S. 2012. Sclerotinia resistant canola provides new option. West Prod. [Online]. Available from: http://producer.com/2012/07/sclerotinia-resistant-canola-provides-new-option/

Prost I, Dhondt S, Rothe G, Vicente J, Rodriguez MJ, Kift N, Carbonne F, Griffiths G, Esquerré-Tugayé MT, Rosahl S, Castresana C, Hamberg M, and Fournier J. 2005. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. Plant Physiol. 139:1902-1913.

Przybylski R, Mag T, Eskin NAM, McDonald BE. 2005. Bailey's Industrial Oil and Fat Products, Sixth Edition. Hoboken, NJ: JohnWiley & Son. Volume 2, Chapter 2, Canola oil.

Rau D, Attene G, Brown AHD, Nanni L, Maier FJ, Balmas V, Saba E, Schäfer W, and Papa R. 2007. Phylogeny and evolution of mating-type genes from *Pyrenophora teres*, the causal agent of barley 'net blotch' disease. Curr Genet. 51:377-392.

Rees RG, and Platz GJ. 1983. Effect of yellow leaf spot on wheat: Comparison of epidemics at different stages of crop development. Aust J Agric Res. 34:39-46.

Rees RG, and Platz GJ. 1979. The occurrence and control of yellow spot of wheat in north-eastern Australia. Aust J Exp Agric Anim Husb. 19: 369-372.

Reide CR, Francl LJ, Anderson JA, Jordahl JG, and Meinhardt SW. 2003. Additional sources of resistance to tan spot of wheat. Crop Sci. 36:771-777.

Reimann S, and Deising HB. 2005. Inhibition of efflux transporter-mediated fungicide resistance in *Pyrenophora tritici-repentis* by a derivative of 4′-hydroxyflavone and enhancement of fungicide activity. Applied Enviro Microbiol. 71:3269- 3275.

Rempel CB, Hutton SN, and Jurke CJ. 2014. Clubroot and the importance of canola in Canada. Can J Plant Pathol. 36:19-26.

Rodbotten M, Tomic O, Holtekjolen AK, Grini IS, Lea P, Granli BS, Grimsby S, Sahlstrom S. 2015. Barley bread with normal and low content of salt; sensory profile and consumer preference in five European countries. J Cereal Sci. 64:176-182.

Rosser CL, Beattie AD, Block HC, McKinnon JJ, Lardner HA, Górka P, Penner GB. 2016. Effect of maturity at harvest for whole-crop barley and oat on dry matter intake, sorting, and digestibility when fed to beef cattle. J Anim Sci. 94(2):697-708.

Rouxel T, and Balesdent MH. 2005. The stem canker (blackleg) fungus. *Leptosphaeria maculans*, enters the genomic era. Mol Plant Pathol. 6:225-241.

Rouxel T, Penaud A, Pinochet X, Brun H, Gout L, Delourme R, Schmit J, and Balesdent MH. 2003. A ten-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the Rlml resistance gene in oilseed rape. Eur J Plant Pathol. 109:871-881.

Safar Ali Safavi, Assadollah Babai-Ahari, Farzad Afshari and Mahdi Arzanlou. 2012. Effect of yellow rust on yield components of barley cultivars with race-specific and slow rusting resistance to yellow rust. Archs Phytopathol Plant Protec. 45:1488-1498.

Savary S, Ficke A, Aubertot JN, and Hollier C. 2012. Crop losses due to diseases and their implications for global food production losses and food security. Food sec. DOI 10.1007/s12571-012-0200-5.

Savchenko T, Walley JW, Wassim Chehab E, Xiao Y, Kaspi R, Pye MF, Mohamed ME, Lazarus CM, Bostock RM, Dehesh. 2010. Arachidonic Acid: An Evolutionarily Conserved Signaling Molecule Modulates Plant Stress Signaling Networks. Plant Cell. 22:3193-3205.

Scala V, Giorni P, Cirlini M, Ludovici M, Visentin I, Cardinale F, Fabbri AA, Fanelli C, Reverberi M, Battilani P, Galaverna G and Dall′Asta C. 2014. LDS1-produced oxylipins are negative regulators of growth, conidation and fumonisin synthesis in the fungal maize pathogen Fusarium verticillioides. Frontiers in Microbiology. 5:1-14.

Schaller F. 2001. Enzymes of biosynthesis of octadecanoid-derived signaling molecules. J. Exp. Bot. 52: 11-23.

Scheffer RP, and Briggs SP. 1981. Introduction: A perspective of toxin studies in plant pathology. In: Toxins in Plant Disease. R.D. Durbin, ed. Academic Press, New York, USA. P:1-20.

Scheffer RP, and Livingston RS. 1984. Host selective toxins and their role in plant diseases. Science 223:17-21.

Schilder AMC, and Bergstrom GC. 1995. Seed transmission of *Pyrenophora triticirepentis*, causal fungus of tan spot of wheat. Eu J Plant Pathol. 101:81-91.

Schmale III DG, and Bergstrom GC. 2003. Fusarium head blight (FHB) or scab. *The Plant Health Instructor*. DOI:10.1094/PHI-I-2003-0612-01.

Schouest JM, Luu TK, Moy RL. 2012. Improved texture and appearance of human facial skin after daily topical application of barley produced, synthetic, human-like epidermal growth factor (EGF) serum. J Drugs Dermatol. 11:613-620.

Schuh W. 1990. The influence of tillage systems in incidence and spatial pattern of tan spot of wheat. Phytopathol. 80:804-807.

Schweizer P, Buchala A, and Metraux JP. 1997. Gene-expression patterns and levels of jasmonic acid in rice treated with the resistance inducer 2,6-dichloroisonicotinic acid. Plant Physiol. 115: 61-70.

Seaman WL. 1982. Epidemiology and control of mycotoxigenic fusaria on cereal grains. Can. J. Plant Pathol. 4:187-190.

Shabeer A and Bockus WW. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis. 72:599-602.

Sharma RC, Nazari K, Amanov A, Ziyaev Z, and Jalilov AU. 2016. Reduction of winter wheat yield losses caused by stripe rust through fungicide management. J Phytopathol. 164:671-677.

Sharma N, Liang Y, Rahman MH, Kav NNV. 2010. Cytokinin inhibits the growth of *Leptosphaeria maculans* and *Alternaria brassicae*. Can. J. Plant Pathol. 32: 306-314.
Nanda S, and Yadav JS. 2003. Lipoxygenase biocatalysis: a survey of asymmetric oxygenation. J. Mol. Catal. B: Enzym. 26: 3-28.

Shewry PR. 2009. Wheat. J Exp Bot. 60:1537-1553.

Shiferaw B, Smale M, Braun HJ, Duveiller E, Reynolds M, Muricho G. 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Sec. 5:291-317.

Shinners TC, and Tewari JP. 1997. Diversity in crystal production by some bird's nest fungi (Nidulariaceae) in culture. Can J Chem. 75:850-856.

Shipton WA, Khan TN and Boyd WJR. 1973. Net blotch of barley.Re. Plant Pathol. 52: 269-290.

Singh PK, Singh RP, Duveiller E, Mergoum M, Adhikari TB, and Elias EM. 2010. Genetics of wheat-*Pyrenophora tritici-repentis* interactions. Euphytica. 172:1-13.

Sjögren J, Magnusson J, Broberg A, Schnürer J, and Kenne L. 2003. Antifungal 3-hydroxy fatty acids from Lactobacillus plantarum MiLAB 14. Appl Environ Microbiol. 69:7554-7557.

Šramková Z, Gregová E, Šturdík E. 2009. Chemical composition and nutritional quality of wheat grain. Acta Chimica Slovaca. 2:115-138.

Statistics Canada. 2017a. Table 001-0010 - Estimated areas, yield, production and average farm price of principal field crops, in metric units, annual, CANSIM (database). [Online] Available from: http://www5.statcan.gc.ca/cansim/a26?lang=eng&id=0010010&p2=17 [accessed 1 November 2017].

Statistics Canada. 2017b. Table 001-0015 - Exports of grains, by final destination, monthly (tonnes), CANSIM (database). [Online] Available from: http://www5.statcan.gc.ca/cansim/a26?lang=eng&id=0010015&p2=17 [accessed 1 November 2017].

Statistics Canada. 2017c. Table 001-0017 - Estimated areas, yield, production and average farm price of principal field crops, in metric units, annual, CANSIM (database). [Online] Available from:

http://www5.statcan.gc.ca/cansim/a26?lang=eng&retrLang=eng&id=0010017&tabMode=dataTable&p1=-1&p2=9&srchLan=-1 [accessed 21 November 2017].

Steffenson BJ. 1997. Net blotch. In: D.E. Mather, editor, Compendium of barley diseases. 2nd ed. American Phytopathological Society, St. Paul, MN. P:28-31.

Steffenson BJ, Webster RK, and Jackson LF. 1991. Reduction in yield loss using incomplete resistance to *Pyrenophora teres* f. *teres* in barley. Plant Dis. 75:96-100.

Stover RW, Francl LJ, and Jordahl JG. 1996. Tillage and fungicide management of foliar diseases in a spring wheat monoculture. J Prod Agric. 9:261-265.

Strange RN, and Scott PR. 2005. Plant disease: a threat to global food security. Annu Rev Phytopathol. 43:83-116.

Strelkov SE, Manolii VP, Cao T, Xue S, and Hwang SF. 2007. Pathotype classification of Plasmodiophora brassicae and its occurrence in Brassica napus in Alberta, Canada. J Phytopathol. 155:706–712.

Strelkov SE, and Lamari L. 2003. Host–parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. Can J Plant Pathol. 25:339–349.

Sun Y, Zhong S, Steffenson BJ, Friesen TL, Neate SM. 2006. Amplified fragment length polymorphism and virulence polymorphism in *Puccinia hordei*. Can J Plant Pathol. 29:25-34.

Suzio T, and Kobayashi T. 1972. Dispersal of ascospores of *Sclerotinia sclerotiorum* (Lib.) de Bary on kidney bean plants. Part 1. Dispersal of ascospores from a point sourse ofapothecia. Hokkaido Natl Agric Stn Res Bull. 101:151.

Swelam N, Kazan K, and Schenk PM. 2016. Global plant stress signaling: reactive oxygen species at the cross-road. 7:187. doi: 10.3389/fpls.2016.00187.

Tallent WH, Harris J, Wolff IA, and Lundin RE. 1966. Structure and intraglyceride distribution of coriolic acid. Tetrahedron Lett. 7: 4329 - 4334.

Tekauz A. 2003. Diseases of barley. In Diseases of field crops in Canada. 3rd ed. Edited by KL Bailey, BD Gossen, RK Gugel, and RAA Morrall. Canadian Phytopathology Society and University Extention Press, University of Saskatchewan, Saskatoon, Sask. Canada. P:30-53.

Tekauz A, McCallum B, and Gilbert J. 2000. Review: Fusarium head blight of barley in western Canada. Can J Plant Pathol. 22:9-16.

Tekauz A. 1990. Characterization and distribution of pathogenic variation in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculat*a from Western Canada. Can J Plant Pathol. 12:141-148.

Tekauz A, Clear RM, and Cooke LA. 1986. Fusarium head blight outbreak in Manitoba in 1986. Pages 73-78 in 1986 Manitoba Agronomists Annual Conference.

Tekauz A. 1976. Distribution, severity and relative importance of leaf spot diseases wheat in Western Canada in 1974. Can Plant Dis Surv. 56:36-40.

The Canadian Wheat Alliance. 2017. National research council Canada, factsheet. [Online] Available from: https://www.nrc-cnrc.gc.ca/eng/news/releases/2013/wheat_nrc_factsheet.html [accessed 15 November 2017].

Tombolini R, van der Gaag DJ, Gerhardson B, and Jansson JK. 1999. Colonization Pattern of the Biocontrol Strain *Pseudomonas chlororaphis* MA 342 on Barley Seeds Visualized by Using Green Fluorescent Protein. Appl Environ Microbiol. 65:3674-3680.

Toscano-Underwood C, West JS, Fitt BDL, Todd AD, and Jedryczka M. 2001. Development of phoma lesions on oilseed rape leaves inoculated with ascospores of A- group or B-group *Leptosphaeria maculans* (stem canker) at different temperatures and wetness durations. Plant Pathol. 50:28-41.

Tu JC, and Zheng J. 1997. Relationship of row spacing and sowing rate to the development of white mold in azuki and mung bean, 1995–1996. Bean Improv. Coop. 40:136-137.

Turkington TK, O'Donovan JT, Harker KN, Xi K, Blackshaw RE, Johnson EN, Peng G, Kutcher HR, May WE, Lafond GP, Mohr RM, Irvine RB, and Stevenson C. 2015. The impact of fungicide and herbicide timing on foliar disease severity, and barley productivity and quality. Can J Plant Sci. 95:525-537.

Turkington TK, O'Donovan JT, Edney MJ, Juskiw PE, McKenzie RH, Harker KN, Clayton GW, Xi K, Lafond GP, Irvine RB, and Brandt, S. 2012. Effect of crop residue, nitrogen rate and fungicide application on malting barley productivity, quality, and foliar disease severity. Can J Plant Sci. 92:577-588.

Turkington TK, Tekauz A, Xi K, and Kutcher HR. 2011. Foliar diseases of barley: don't rely on a single strategy from the disease management toolbox. Prairie Soils and Crops. 4:142-150.

Turkington TK, Xi K, Clayton GW, Burnett PA, Klein-Gebbinck H, Lupwayi NZ, Harker KN, and O'Donovan, JT. 2006. Impact of crop management on leaf diseases in Alberta barley fields, 1995-1997. Can J Plant Pathol. 28:441-449.

Turkington TK, Xi K, Tewari JP, Lee HK, Clayton GW, and Harker KN. 2005. Cultivar rotation as a strategy to reduce leaf diseases under barley monoculture. Can J Plant Pathol. 27:283-290.

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

USDA (United States Department of Agriculture). Foreign Agriculture Service. 2017a. All Grain Summary Comparison. [Online] Available from: https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads [accessed 21 December 2017].

USDA (United States Department of Agriculture). Foreign Agriculture Service. 2017b. World Wheat Production, Consumption, and Stocks. [Online] Available from: https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads [accessed 21 December 2017].

USDA (United States Department of Agriculture). Foreign Agriculture Service. 2017c. World Barley Production, Consumption, and Stocks. [Online] Available from: https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads [accessed 31 December 2017].

USDA (United States Department of Agriculture). Foreign Agriculture Service. 2017d. World Barley Trade. [Online] Available from: https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads [accessed 31 December 2017].

USDA (United States Department of Agriculture). Foreign Agriculture Service. 2017e. Table 15 Rapeseed Area, Yield, and Production. [Online] Available from: https://apps.fas.usda.gov/psdonline/reporthandler.ashx?reportId=922&templateId=1&format=html&fileName=Table%2015%20Rapeseed%20Area,%20Yield,%20and%20Production [accessed 23 November 2017].

van den Berg CGJ, and Rossnagel BG. 1991. Epidemiology of spot-type net blotch on spring barley in Saskatchewan. Phytopathol. 81:1446-1452.

van den Berg CGJ, and Rossnagel BG.1990. Effect of tilt on severity of spot-type net blotch, grain yield and yield components in barley. Can J Plant Sci. 70:473-480.

Vestal EF. 1964. Barley scab in South Korea in 1963 and 1964. Plant Dis. Rep. 48:754-755.

Walters D, Raynor L, Mitchell A, Walker R, and Walker K. 2004. Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. 157:87-90.

Wang Y, Duan YB, Zhou MG. 2014. Control of *Sclerotinia sclerotiorum* infection in oilseed rape with strobilurin fungicide SYP-7017. Can J Plant Pathol. 36:354-359.

Wegulo SN. 2012a. Rust diseases of Wheat. University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources. G2180.

Wegulo SN, Klein RN, and Harveson RM. 2012b. Tan spot of wheat. University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources. [Online] Available from: http://extensionpublications.unl.edu/assets/pdf/g429.pdf.

Wegulo SN. 2011. Tan spot of cereals. [Online] Available from: https://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/TanSpot.aspx [accessed 25 December 2017].

West JS, Kharbanda PD, Barbetti MJ, and Fitt BDL. 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathol. 50:10-27.

Willets HJ, and Wong JA-L. 1980. The biology of *Sclerotinia sclerotiorum*, S. *trifoliorum* and *S. minor* with emphasis on specific nomenclature. Botanical Rev. 46:101-165.

Williams JR, and Stelfox D. 1980. Influence of farming practices in Alberta on germination and apothecium production of sclerotia of Sclerotinia sclerotiorum. Can J Plant Pathol. 2:169-172.

Williams JR, and Stelfox D. 1979. Disepersal of ascospores of *Sclerotinia sclerotiorumin* relation to sclerotinia stem rot of rapeseed. Plant Dis. Rep. 63:395- 399. Wolf PFJ, and Hoffmann GM. 1993. Biological studies on *Drechslera tritici-repentis* (Died.) Shoem. (teleomorph *Pyrenophora tritici-repentis* (Died.) Drechsler) the causal agent of a leaf spot disease on wheat. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 100:33-48.

Wonneberger R, Ficke A, Lillemo M. 2017. Mapping of quantitative trait loci associated with resistance to net form net blotch (*Pyrenophora teres* f. *teres*) in a doubled haploid Norwegian barley population. PlosOne. Doi.org/10.1371/journal.pone.0175773.

Wong LSL, Tekauz A, Leslie D, Abramson D, and McKenzie RIH. 1992. Prevalence, distribution and importance of Fusarium head blight in winter in Manitoba. Can J Plant Pathol. 14:233-238.

Xi K, Bos C, Turkington TK, Xue AG, Burnett PA and Juskiw PE. 2008. Interaction of net blotch and scald on barley, Can J Plant Pathol. 30:329-334.

Xuan TD, Chung IM, Khanh TD, and Tawata S. 2006. Identification of phytotoxic substances from early growth of barnyard grass (*Echinochloa crusgalli*) root exudates. J. Chem. Ecol. 32:895-906.

Yara A, Yaeno T, Montillet JL, et al. 2008. Biochem Biophys Res Commun. 370: 344-347.

Yoshida M, Nakajima T, Arai M, Suzuki F, and Tomimura K. 2008. Effect of the timing of fungicide application on fusarium head blight and mycotoxin accumulation in closed-flowering barley. Plant Dis. 92:1164-1170.

Young CS, and Werner CP. 2012. Infection routes for *Sclerotinia sclerotiorum* in apetalous and fully petalled winter oilseed rape. Plant Pathol. 61:730-738.

Zhao L, Chen Y, Wu K, Yan H, Hao X, and Wu Y. 2017. Application of fatty acids as antiviral agents against tobacco mosaic virus. Pesticide Biochem. Physiol. 139:87-91.

Zohary D, Hopf M, and Ehud W. 2012. Domestication of Plants in the Old World. Oxford University Press. Fourth edition. P: 4-7.