Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* L. Genotypes and the Relationship with Seed Germination, Vigour and Quality Traits

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in the Partial Fulfilment of the Requirements

for the Degree of Master of Science

in the Department of Plant Sciences

University of Saskatchewan

Saskatoon, SK

By

Caroline H. Brown

Permission to Use

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

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

Head of the Department of Plant Sciences
51 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

Abstract

Volunteer canola (*Brassica napus* L.), defined as canola germinating and emerging after the intended cropping season, is the fourth most occurring weed on the Canadian prairies. The largest contribution of seed into the soil seed bank occurs from pod shatter during maturity and harvest. Gene transfer via pollen flow between different varieties is the major concern with volunteer canola. Gene transfer may result in the stacking of herbicide tolerance types in subsequent volunteer canola populations. Seed dormancy is the physiological mechanism prolonging the presence of viable seed in the soil seed bank. Specifically, canola has a high propensity to be induced into secondary dormancy (SD) under adverse environmental conditions not conducive for germination. Previously screened Canadian commercial material ranged from 0-90% propensity to enter SD. A reduction of SD in modern canola varieties may result in lower seed bank persistence, however, the indirect effect this would have on other seed traits is not known. This study examined a diverse collection of annual *B. napus* genotypes produced in contrasting maternal environments for SD as well as seed germination, vigour and quality traits. Absolute SD values ranged from 0-77% dormant in the diversity collection screened. Genotype was shown to be the main contributor to the variability in SD observed (50%), the interactions between the genotype and environment had a moderate contribution (30%) and very little contribution from maternal environment alone was observed. Genotypes with low SD were more consistent across maternal environments compared to mid and high SD genotypes. No association was observed between SD and germination time or seed vigour traits in the genotypes examined. A significant positive correlation between SD and total protein content was found (r= 0.34; $P < 0.001$). Total oil content was negatively correlated to SD (r= -0.24; $P < 0.05$), likely due to the inverse relationship between oil and protein. From the results in this study, the reduction of SD as a breeding objective in canola breeding programs is feasible as the trait is largely genetically controlled. Likewise, the reduction of SD is unlikely to impact seed germination or vigour traits and SD should not be a large contributor to poor stand establishment of canola.

Acknowledgements

First and foremost I'd like to thank my supervisors, Sally Vail and Steve Shirtliffe, for their mentorship, patience and encouragement throughout this process. Acknowledgement goes out to my advisory committee, Rob Gulden, Kirstin Bett and Rosalind Bueckert for their time and helpful suggestions. A special thank you to Rob for your help with the statistical analysis. Thank you to Allan Feurtado from the National Research Council of Canada for serving as the external on this committee. Thank you to the Agriculture Canada research staff for their help with field work, sample preparation and odd tasks namely, Paul Prodahl, Ryan Vetter, Brad Hope, Christopher Headley and the summer students throughout the years. A big thank you to the GEVES seed phenotyping lab and INRA labs (Marie-Hélène Wagner, Didier Demilly, Carolyn Dürr, Sylvie Ducournau, Nathalie Nesi, Julie Buitink and Oliver Leprince) in France for welcoming me into their lab and spending time teaching me the workings of their high-throughput seed phenotyping platform. Throughout this project, there were many opportunities for collaboration and I'd like to acknowledge Ross Weiss for help with the thermal gradient plate, Gerald Serblowski and Rong Zhou for quality analysis, Carrie Matthiessen at 20/20 seed labs for advice on seed vigour testing and the AAFC greenhouse staff for germination cabinet control.

Thank you to the following funding agencies for making this project possible, SaskCanola, Alberta Canola Growers and Growing Forward 2. I will forever cherish the generous support from the Robert P. Knowles Scholarship fund for providing financial support throughout this project and awarding me the opportunity to travel to France to spend time at GEVES.

Lastly, thank you to my parents, Mark and Lisa, for their unwavering support, interest and words of encouragement throughout all of my schooling. Thank you for always encouraging me to follow my passion and letting me fill the south facing windowsill with overwintering plants. You have taught me that with hard work and perseverance anything is possible and for that, I am truly grateful. Without both of you, none of this would have been possible.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ABA- Abscisic acid

CDT- Controlled Deterioration Test

DH- Doubled Haploid

DOG1- Delay of Germination 1 gene

GA- Gibberellic acid

NAM- Nested Association Mapping population

PCT- Pre-Chill Germination Test

PEG- Polyethylene glycol

QTL- Quantitative Trait Loci

RIL- Recombinant Inbred Line

T50- Time to 50% Germination

List of Equations

1.0 Introduction

Brassica napus L. (*B. napus*, n=19) is an amphidiploid, the combination of two doubled sets of chromosomes, formed from the interspecific hybridization between *Brassica rapa* L. (n=10) and *Brassica oleracea* L. (n=9) (Nagaharu. 1935). Belonging to the Brassicaceae family, *B. napus* is closely related to other cruciferous crops including, broccoli, kale and brown mustard (Downey & Rimmer. 1993). The natural hybridization between the two species that resulted in *B. napus* was believed to have occurred in Mediterranean Europe approximately 0.12 to 1.4 million years ago (Nagaharu, 1935). The hybrid then spread throughout Asia, Europe and North Africa where it was cultivated, and the oil was used as a food source as well as for lamps and soap (Slinkard & Knott. 1995). The oil was later used for industrial purposes due to its lubricating properties. Cultivation in Canada began in the 1900s with the oil mainly used as a lubricant for steam engines (Slinkard & Knott. 1995). Advancement in plant breeding over the last 50 years in Canada has led to the reduction of two anti-nutritional components, glucosinolate content in the meal and erucic acid in the oil (double-low) (Downey et al., 1975). The oil is now primarily used for human consumption in food products and is referred to as canola oil (Canadian oil, low acid) (Downey et al., 1975). Canola oil is low in saturated fat and high in omega acids, making it a healthy alternative to other food oils available on the market. Canola oil possesses superior stability at high heat and has a relatively long shelf-life, two desirable characteristics for food manufacturers (Rakow. 2004).

In western Canada, annual varieties are exclusively produced with the crop planted in the spring and harvested the same autumn. In many other parts of the world, such as Europe, the winter annual is produced. The winter annual is planted and germinates in the autumn, overwinters as a rosette and flowers the following spring. A period of extended cold followed by an increase in day length is required for the winter annual to flower and is called vernalisation (Wang et al., 2011). Winter annuals are not able to survive western Canadian winters but are sometimes crossed into annual varieties to increase genetic diversity. This process, however,

requires intensive backcrossing of the annual to eliminate the vernalisation requirement (Wang et al., 2011). Unless otherwise specified, when reference to canola is made in this thesis it is referring to the annual type.

Genetically-modified, herbicide-tolerant canola was developed in the 1990s to help farmers control weeds within their canola crops (Beckie et al., 2011). Genetically-modified herbicide-tolerant canola, achieved through gene transfer from bacteria, is tolerant to either glyphosate or glufosinate herbicides. Genetically-modified, herbicide-tolerant canola contributes to 99% of all canola planted in Canada (Beckie et al., 2011). A non-genetically-modified herbicide-tolerant system also exists in canola and is marketed as Clearfield®. Mutagenesis was used to inhibit the enzyme acetolactate synthase from functioning and forming branched amino acids, resulting in tolerance to imidazolinone herbicides (Swanson et al., 1989; Tan et al., 2005). Imidazolinone herbicides control both grass and broadleaf weeds while leaving the imidazolinone tolerant crop standing. Several crops have imidazolinone tolerance including wheat, canola and soybeans, making the control of imidazolinone-tolerant volunteer canola in other imidazolinone-tolerant crops difficult (Tan et al., 2005).

While herbicide tolerance has led to many advantages, herbicide-tolerant volunteer canola populations are on the rise. Canola seed in the soil seed bank germinating and emerging after the intended cropping season is referred to as volunteer canola and is the fourth most occurring weed on the Canadian prairies (Beckie, 2015). The persistence of the seed in the soil seed bank is due to the high propensity of the seed to be induced into secondary dormancy under adverse environmental conditions (Baskin and Baskin. 1998). The unintentional movement of genes between cultivars and possibly other Brassica species resulting from pollen-mediated gene transfer, largely by wind and pollinators, can lead to the stacking of herbicide tolerance in volunteer canola (volunteers tolerant to two or more herbicides) (Beckie et al., 2003). Although *B. napus* are largely self-pollinated, outcrossing rates of 30% have been found (Beckie et al., 2003; Rakow and Woods. 1987).

Contamination concerns exist between special oil profile varieties typically produced under contract and commodity/double-low canola. For example, mixing between double-low canola, and high erucic acid rapeseed (HEAR) and/or double-low canola and high oleic/low linolenic (HOLL) varieties. The high erucic acid varieties have greater than 50% erucic acid

(Duncan et al., 2017) and are used for industrial purposes such as in lubricants, plastics and detergents. The high oleic/low linolenic varieties have an enhanced omega-9 oil profile, making the oil more stable under high heat, a desirable quality for frying purposes in the fast food industry (DeBonte et al., 2012). Contamination can happen in one of two ways, first through pollen transfer between closely planted fields with different oil profiles and secondly, by volunteers of different oil profiles germinating in the current canola crop. Of greatest concern is a high erucic acid variety contaminating the oil profile of a double-low variety causing the market class to be downgraded at a loss to the producer.

Another concern with volunteer canola is the perpetuation of the soil-borne pathogen (*Plasmodiophora brassicae*) resulting in clubroot (Hwang et al., 2012). Canola volunteers could serve as a host plant for the pathogen even in rotational crops leading to an increase in inoculum levels (Strelkov & Hwang. 2014). Yield loss from clubroot can be substantial in infected fields and results from the pathogen causing galls on the roots cutting off nutrient and water supply to the rest of the plant (Strelkov & Hwang. 2014). Clubroot resistant varieties are on the market however, the resistance is easily overcome due to a variable pathogen population. Directional selection by resistant volunteers could cause a shift in the clubroot pathogen population.

Planted canola on the Canadian prairies in 2018 totalled 9.2 million hectares, with over half the hectares produced in Saskatchewan (Statistics Canada. 2018). Typical field emergence of canola is around 50% of what is planted, and the other 50% the fate is unknown (Baskin and Baskin 1998; Harker et al., 2003; Harker et al., 2015). Seed existing in the seed bank includes viable seed induced into dormancy, non-viable seed due to attack by disease, seed disappearance due to predation by pests or natural death. It has not been quantifiably described as to why up to 50% of planted canola seed does not emerge in a given year and reasoning is only speculative. First, the environmental conditions may not be favourable for germination causing the seed to be quiescent ('resting' of non-dormant seeds) or induced into secondary dormancy and thereby, remaining in the soil until conditions are suitable for germination (Baskin and Baskin. 1998). Poor vigour resulting in the seedling failing to emerge is another factor contributing to low plant emergence. Certified Number 1 canola seed in Canada must meet 90% germination in order to be sold (CSGA. 2019); however, germination testing is performed under ideal conditions in a laboratory and may not be indicative to spring field conditions in western Canada (Lamichhane et al., 2018). Poor stand establishment in the field is typically classified as plant density below

the recommended 70-100 plants m^{-2} (Harker et al., 2015). Poor canola stands creates a plethora of other management issues including increased weed populations due to lack of competition, harvest management issues and ultimately lower yield.

One way to minimize the persistence of *B. napus* seed in the soil seed bank may be to reduce the genetic potential of the seed to be induced into secondary dormancy under conditions not conductive for germination. Harvest losses from seed with low secondary dormancy would still enter the soil seed bank but cannot be induced into a prolonged dormancy period if poor environmental conditions persist. Low dormancy seeds would germinate in the fall, freeze and die over the winter, whereas early spring germinating seedlings would be killed when the field is sprayed or tilled. Direct selection for low secondary dormancy has not been performed before, therefore, the effect this would have on seed vigour and other seed traits is unknown (Schatzki et al., 2013a; Bus et al., 2011). If selection is effective in reducing secondary dormancy while maintaining seed germination, vigour and quality properties in canola breeding material, volunteer canola populations could be reduced in western Canadian fields.

The last time Canadian canola varieties were screened for secondary dormancy was nearly 20 years ago by Gulden et al. (2004a). Given that such a large percentage of the commercial varieties screened possessed high dormancy, it is possible that genes conferring high dormancy may have been inadvertently maintained because they are prevalent in the breeding programs. Screening against secondary dormancy is not widely practised when selecting for future canola varieties because the process is labour intensive and lengthy. Recently, a Nested Association Mapping (NAM) population comprised of diverse parental lines and Recombinant Inbred Lines (RILs) has been created for annual *B. napus*, capturing the species diversity from across the globe (Parkin et al., 2017). The parental diversity collection contains a large number of genotypes developed in Canada thus, could provide insight into the dormancy dynamics in Canadian germplasm. Given the genetic diversity across the population, low dormancy is likely to be represented. The objectives of this project included examining the diverse parental collection of 51 annual *B. napus* genotypes (NAM) produced in contrasting maternal environments for secondary dormancy, seed germination, vigour and quality traits as well as for precocious germination. The association between secondary dormancy and seed vigour, seed quality traits and precocious germination was examined to help in gaining a stronger understanding of the biological relationships influencing secondary dormancy. The relationship

between secondary dormancy and seed vigour was examined to determine if secondary dormancy contributes to poor stand establishment. It was hypothesized that a wide range in secondary dormancy would exist across different genotypes and maternal environments in which the seed was produced. It was also hypothesized that the high dormancy genotypes would display slower germination speeds, weaker seed vigour properties, lower precocious germination and higher seed protein.

2.0 Literature Review

2.1 Seed Dormancy Overview

Seed dormancy is described as the failure of a viable seed to germinate in favourable environmental conditions (Baskin and Baskin. 1998; Finch-Savage and Leubner-Metzger. 2006). Seed dormancy is observed across all major higher plant species aimed to spread germination out over time and space and is key to the fitness of species. Seed dormancy is largely controlled by genetic factors, synthesis and response to plant hormones including, abscisic acid (ABA) and gibberellins (GA), and the environment (Finch-Savage and Leubner-Metzger. 2006). Seed dormancy is a quantitative trait under the control of numerous genes (Graeber et al., 2012).

The plant hormones, ABA, GA and to a lesser extent ethylene control seed dormancy and germination (Finch-Savage and Leubner-Metzger. 2006; Nonogaki. 2014). Abscisic acid is a plant hormone important in promoting dormancy within seeds (Bewley. 1997). Abscisic acid works by preventing radicle protrusion and thereby, preventing germination from occurring. The dormancy promoting hormone also influences membrane saturation and fluidity causing the membranes to be less sensitive to temperature changes (Hilhorst. 1998). During seed desiccation, ABA is responsible for signalling late embryogenesis abundant proteins (LEAs) to provide protection to the storage materials within the seed (Hilhorst. 2007). The absolute contents of ABA and GA are less important than the relative ratio of the two hormones and as the sensitivity to ABA decreases the sensitivity to GA increases (Finch-Savage and Leubner-Metzger. 2006). Gibberellic acid is present in the seed at all times, but sensitivity to GA only occurs as the ABA:GA ratio decreases (Finch-Savage and Leubner-Metzger. 2006). The addition of exogenous GA to dormant seeds causes an increase in endogenous ABA, supporting the conclusion that GA is not a dormancy-breaking hormone but rather a germination stimulating hormone (Finch-Savage and Leubner-Metzger. 2006). Gibberellic acid stimulates the weakening of seed structures surrounding the radicle in conjunction with an increase in the growth potential of the embryo. The promotion of the growth of radicle cells results in the radicle breaking

through the surrounding seed structures (Finch-Savage and Leubner-Metzger. 2006). The seed coat provides the mechanical resistance to prevent the radicle from emerging therefore, maintaining dormancy until weakened (Finch-Savage and Leubner-Metzger. 2006). Ethylene, a germination stimulating hormone working in association with GA has been found to weaken the endosperm cap allowing for radicle protrusion (Linkies et al., 2009; Graeber et al., 2012). The balance and interplay between these plant hormones have been studied in great depth in terms of germination and dormancy and play a large role in both physiological processes.

Environmental signals such as temperature, oxygen, light and nutrients play a role in dormancy and germination signalling (Seo et al., 2008). Depending on if a plant is a summer or winter annual can determine whether the seed is sensitive to increasing or decreasing temperatures prior to germination (Finch-Savage and Leubner-Metzger. 2006). The primary site for temperature detection occurs in the membranes of the seed. The saturation level of the lipids within the membranes change in response to temperature resulting in changes to membrane fluidity (Hilhorst. 1998). Plants detect light in through the phytochrome proteins and depending on the light perceived it can promote or impede germination (Seo et al., 2008). Red light (R) signals the induction of germination while far-red light (Pfr) reverses that signal (Seo et al., 2008). Phytochromes regulate endogenous levels of ABA and GA. Germination is induced when red light is detected by phytochrome and GA biosynthesis genes are induced (Seo et al., 2008). Whereas, ABA concentration within the seed is regulated opposite to GA concentrations (Seo et al., 2008). Nitrate in conjunction with temperature and light signals have been found to be effective at breaking dormancy (Hilhorst. 2007). In Arabidopsis, the presence of nitrate replaced cold stratification and after-ripening treatments as a dormancy-breaking mechanism (Hilhorst. 2007).

Dormancy and germination studies occur conjointly as dormancy is observed as the lack of germination and germination is observed as the lack of dormancy (Finch-Savage and Leubner-Metzger. 2006). Therefore, it is difficult to determine if environmental signals are dormancy maintaining or germination promoting signals. In summary, the signals that release dormancy may be different than the signals that initiate germination, but both physiological processes are antagonistic and overlapping (Hilhorst. 2007). In current cultivated crops, seeds germinating soon after being planted are selected for thus, seed dormancy has been reduced. Selection for quick germination is performed because it allows the seed to take advantage of environmental

conditions like early soil moisture, escape flowering during the hottest weeks of the year as well as maturing before a killing frost occurs.

2.2 Timing of Dormancy within the Seed

Dormancy induced during development on the mother plant in fresh seed is referred to as primary dormancy and usually as a result of elevated ABA (Baskin and Baskin 1998). Primary dormancy prevents germination while the seed is on the mother plant. A period of after-ripening (dry storage) or cold stratification (storage in cold temperatures) releases primary dormancy (Finch-Savage and Leubner-Metzger. 2006). Abscisic acid production is initiated during seed development with the highest concentration mid-development when storage reserves are deposited and then tapering off towards at the end of development (Bewley. 1997). Only ABA produced within the embryo of a developing seed can cause dormancy with maternal plant ABA having no influence on dormancy of the developing seed (Bewley. 1997).

Secondary dormancy is induced in fully mature seeds due to adverse environmental conditions including, low temperature, light or osmotic stress (Baskin and Baskin 1998; Pekrun. 1997). A typical or often observed characteristic of secondary dormancy is the gradual cycling of the seed from dormant to non-dormant states (Finch-Savage and Leubner-Metzger. 2006). The probability of germination increases as environmental conditions improve at which time the seed transitions from dormant to conditionally dormant, where germination can occur under a narrow range of conditions, and as conditions continue to improve the seed transitions to fully non-dormant (Hilhorst. 1998; Finch-Savage and Leubner-Metzger. 2006).

2.3 Location of Dormancy within the Seed Structures

Endogenous (embryo) dormancy occurs within the embryo and three sub-classifications exist including, physiological, morphological and morphophysiological dormancy (Baskin and Baskin 1998). Physiological dormancy is due to a prohibiting mechanism within the embryo that prevents radicle emergence and subsequently germination (Baskin and Baskin 1998). Physiological dormancy can be divided into three levels depending on the length of time needed to break the dormancy. Non-deep physiological dormancy is broken by a short period, less than 6 months, of cold stratification or after-ripening. Intermediate physiological dormancy requires

greater than 6 months of cold stratification and deep physiological dormancy requires years of cold stratification to break dormancy. Embryos excised from fresh seeds of plant species with non-deep and intermediate physiological dormancy will germinate and produce normal seedlings while deep physiologically dormancy species will not produce normal seedlings when the embryo is excised (Baskin and Baskin 1998). Morphological dormancy results from an underdeveloped or undifferentiated embryo not capable of germinating until the embryo has developed and differentiated (Baskin and Baskin 1998). Lastly, morphophysiological dormancy is a combination of a prohibiting mechanism within the embryo and an immature embryo preventing germination.

Exogenous (coat) dormancy occurs in the surrounding structures of the embryo including the endosperm and testa. The restrictive force of the surrounding structures is stronger than the embryos ability to break through them (Finch-Savage and Leubner-Metzger. 2006). Three sub-classifications also exist including physical, chemical and mechanical dormancy. Physical dormancy is caused by the seed coats impermeability to water due to a thick lignified layer of cells in the seed coat that can be broken by scarifying the seed coat (Baskin and Baskin 1998). This type of dormancy is common in legume species. Chemical dormancy is caused by growth prohibiting chemicals within the surrounding structures of the embryo and the chemicals must first leach out of the seed before germination can occur. Lastly, mechanical dormancy is the result of strong surrounding structures that must be broken to allow radicle protrusion (Baskin and Baskin 1998). Mechanical dormancy is common in fruit species with a hard-woody outer shell.

2.4 Description of Dormancy in *Brassica napus*

Brassica napus is classified as a non-deep physiological dormant species with a high propensity to be induced into secondary dormancy (Baskin and Baskin 1998). Under adverse environmental conditions, a prohibiting mechanism within the embryo prevents radicle protrusion, however, dormancy can easily be broken by a short period of imbibed cold stratification or dry after-ripening. *Brassica napus* seed populations in the seed bank cycle between dormant and non-dormant states and can germinate under a narrow range of conditions known as conditional dormancy or when the seed is fully non-dormant (Baskin and Baskin

1998). This annual cycling pattern coincides with change in seasons and seed not germinating in the current season can persist in the soil for up to 7 years (Beckie and Warwick. 2010), but typically persist for 4-5 years in the soil as found from studies in eastern Canada (Légère et al., 2001; Simard et al., 2002).

Brassica napus has low potential for primary dormancy and fresh seeds are classified as non-dormant and do not require light for germination (Baskin and Baskin 1998; Soltani et al., 2018). It is not known however, if primary dormancy is a prerequisite for secondary dormancy in *B. napus* (Soltani et al., 2018). Significant and non-significant correlations between primary and secondary dormancy have been documented in *B. napus* with *r*-values between the two traits ranging from 0 to 0.62 (Schatzki et al., 2013b; Soltani et al., 2018). The range of results is believed to be due to variation in how the experiment was performed as well as the post-harvest conditions prior to testing the seed. If the seed was 'fresh' but stored for a few months before testing it, there was opportunity for after-ripening to occur which is a primary dormancy breaking condition (Soltani et al., 2018). Another concern when screening for dormancy is the cycling that occurs. Typically, primary dormancy tests are done under a constant temperature in the dark, but these conditions may not break conditional dormancy. A proposed solution for this is to test for primary dormancy under a wide range of temperatures under both light and dark conditions in order to identify seeds that are conditionally dormant (Soltani et al., 2018). Clarifying the relationship between primary and secondary dormancy is important moving forward for breeders to select for both low primary while still preventing precocious germination and secondary dormancy. In other crops such as in wild oat (*Avena fatua* L.), a clear association between primary and secondary dormancy exists and seed cannot become secondarily dormant without some degree of primary dormancy (Symons et al., 1987; Soltani et al., 2018). Furthermore, a positive correlation between primary and secondary dormancy also exists in Arabidopsis, a relative of *B. napus*.

2.5 Factors Contributing to Secondary Dormancy in *Brassica napus*

2.5.1 Genetic Factors Contributing to Secondary Dormancy in *Brassica napus*

A wide distribution of secondary dormancy exists among annual *B. napus* genotypes and have been documented to range from 0-90% dormant (Gulden et al., 2004a). Secondary

dormancy in *B. napus* is a quantitative trait with a large genetic component ranging from 44-82% of phenotypic variability (Gulden et al., 2004a). The reported heritability of winter annual *B. napus* secondary dormancy is extremely high ranging from 0.96 to 0.97 (Weber et al., 2013; Schatzki et al., 2013b). Moreover, five quantitative trait loci (QTL) were identified in a winter annual DH *B. napus* mapping population accounting for 42% of the phenotypic variance in total seed dormancy. Given that such a large portion of secondary dormancy is controlled by genetics, it is theoretically possible for winter annual canola plant breeders to successfully modify the secondary dormancy within the seed through selection. Quantitative trait loci have not been identified in annual *B. napus* populations.

2.5.1.1 Delay of Germination 1 Gene (*DOG1*)

The discovery of the *Delay of Germination 1* (*DOG 1*) gene has provided insight into the genetic control of seed dormancy (Graeber et al., 2014). First found in Arabidopsis using QTL analysis of a natural variation collection, *DOG1* results in increased primary and secondary seed dormancy (Nakabayashi et al., 2012). The gene has been found to be conserved with similar function across many plant species (Graeber et al., 2014). *DOG1* codes for a protein which has an unknown function and is highly expressed during seed maturation, detectable in dry seeds and decreases following seed after-ripening (Bentsink et al., 2007). Quantified by immunoblot analysis and gel electrophoresis, freshly harvested seeds were found to have higher amounts of the *DOG1* protein and greater levels were present when low temperature occurred during seed maturation (Nakabayashi et al., 2012). The mutant, *dog1*, has no dormancy thus, confirming the importance of the gene and protein in dormancy (Nakabayashi et al., 2012; Graeber et al., 2014).

DOG1 activity is independent of ABA, however; both are essential for dormancy control and have parallel pathways (Nakabayashi et al., 2012). High ABA concentration prohibits endosperm weakening and maintains primary dormancy until ABA levels decrease during imbibition. When ABA signalling and *DOG1* expression were studied together, it was concluded that ABA does not have the same prohibiting mechanism against germination that overexpression of *DOG1* alone had (Graeber et al., 2014). However, when an ABA-deficient mutant with the functional *DOG1* was tested for dormancy, it was found that the *DOG1* alone could not induce primary dormancy (Nonogaki. 2014). This finding confirms the interaction between ABA and the presence of *DOG1* (Nonogaki. 2014). Expression of *DOG1*, like ABA

signalling, is temperature-sensitive and determines the optimum temperature for germination to occur. Under cooler temperatures more *DOG1* protein accumulated as seen by a delay in endosperm rupture at 18^0C versus 24^0C in Arabidopsis (Graber et al., 2014). Greater accumulation and storage of the *DOG1* protein in the seed inhibits germination, whereas, lower seed content of *DOG1* protein permits germination to proceed (Graeber et al., 2014). In summary, *DOG1* and its' protein are temperature-sensitive regulators of germination and dormancy working in conjunction with ABA to determine the optimal germination timing through preventing or promoting endosperm rupture.

An association exists in Arabidopsis between the *dog1* mutant and seed longevity with *dog1* mutants showing decreased longevity. This suggests *DOG1* may not only play a role in seed dormancy but other seed characteristics as well (Dekker et al., 2016). Primary metabolites essential for seed storage and survival were less abundant in *dog1* mutants during seed maturation (Dekker et al., 2016). In contradiction to Dekker et al. (2016), Nguyen et al. (2012), found that dormancy and seed longevity have a negative correlation where higher dormancy lines showed decreased longevity. This relationship is proposed to be due to the co-localization of *DOG1* and genes identified for longevity in the specific Arabidopsis population screened (Nguyen et al., 2012).

Most *DOG1* research has been conducted in Arabidopsis, as it is a model species for genetic analysis and expresses both primary and secondary dormancy; however, studies on this gene in *B. napus* are now beginning to arise. Three homologues in *B. napu*s have been identified with high homology to *DOG1* in Arabidopsis and are named *BnaADOG1.a*, *BnaCDOG1.a* and *BnaDOG1.b* (Nee et al., 2015). The combined *BnaDOG1* transcripts are only detected in seed tissues and higher *DOG1* transcript levels and resulting protein are detected after secondary dormancy is induced (Nee et al., 2015). A proposed avenue for screening for genetic secondary dormancy could be examining polymorphisms between high and low secondary dormancy genotypes in the identified *B. napus DOG1* sequences and creating markers for use in marker-assisted breeding (Nee et al., 2015).

2.5.2 Environmental Factors Contributing to Secondary Dormancy in *Brassica napus*

2.5.2.1 Maternal Environment

Secondary dormancy in annual *B. napus* is affected by maternal environmental conditions with the same genotype possessing different absolute dormancy when produced in different environmental locations and/or years (Gulden et al., 2004a). Much is known about the impact of maternal environment on secondary dormancy in winter annual *B. napus*. Ideal environmental conditions producing high yield and high oil content were found to increase secondary dormancy in Germany (Schatzki et al., 2013b). Huang et al. (2016) also determined that *B. napus* seed produced under ideal conditions exhibited higher dormancy than seed produced in stressful environments. In a study by Weber et al. (2013), *B. napu*s cultivars were tested in two different locations in Germany over several years and the absolute dormancy varied across years and locations, but the general ranking of the cultivars remained the same. Maternal environment conditions such as high heat or moisture stress can lower secondary dormancy in winter annual *B. napus* (Weber et al., 2013; Brunel-Mugnet et al., 2015). Hormone balance, age of mother plant and seed position on the mother plant have also been found to impact dormancy in other plant species (Baskin and Baskin 1998). Nitrate also impacts dormancy with higher nitrate levels decreasing dormancy due to the impact on ABA synthesis and degradation (Matakiadis et al., 2009; He et al., 2014). A greenhouse study found a weak inverse correlation between available soil nitrogen and secondary dormancy in annual *B. napus* with higher amounts of soil nitrogen producing seed with lower secondary dormancy (Charles Geddes: personal communication). The correlation was non-significant in the corresponding field study but does warrant further study to examine the possible relationship.

A study on two differing eco-types of Arabidopsis revealed the effect of the maternal environment on primary dormancy (Postma and Âgren. 2015). Arabidopsis ecotypes (genotypes specific to a certain geographical region) with contrasting dormancy were studied to determine the interactions between the maternal environment and potential for dormancy. For the study, the seed production environments included Italy and Sweden. Both ecotypes were also grown in a greenhouse for this experiment. The Italian environment had a hotter environment and shorter days compared to the Swedish environment. The Italian ecotype exhibited high dormancy in Italy and even greater dormancy in Sweden, whereas, the Swedish ecotype exhibited low dormancy in Sweden and no dormancy in Italy. Northern climates have a shortened growing season and therefore, cannot have any delay in germination possibly resulting in the low dormancy observed in the Swedish ecotype in both environments. In a similar study the same

results were found where the seed dormancy increased the further south the seed was produced (Debieu et al., 2013). It should also be noted that field produced seed had higher dormancy than the greenhouse seed, an important consideration when conducting seed dormancy experiments (Postma and Âgren. 2015). The greenhouse likely is a more constant and less stressful environment than the field and as a result may be why the seed produced in it has lower dormancy.

2.5.2.2 Seed bank Conditions

Abiotic stresses within the soil seed bank impacting secondary dormancy of the seed include light, temperature, anoxia and osmotic stress (Momoh et al., 2002; Pekrun et al., 1997). The effect of abiotic stresses on secondary dormancy induction was examined and it was found that osmotic stress and a constant temperature was the most effective way to induce secondary dormancy in *B. napus* seeds (Momoh et al., 2002). The constant temperature was necessary for dormancy induction as fluctuating temperatures are a dormancy-breaking signal within the seed. In the experiments establishing this, all dormancy testing was done in dark conditions as to not break dormancy by light. Flooding or anoxia (low oxygen) were not effective at inducing secondary dormancy as the seed either rotted or germinated immediately once the conditions were removed (Momoh et al., 2002; Pekrun et al., 1997b). Seed lots tested under higher temperatures (20^0C) and osmotic stress were not only artificially induced into dormancy faster, but the seed populations also possessed greater overall dormancy compared to lower temperatures (12^0C) in winter annual accessions (Momoh et al., 2002). The soils in western Canada during early to mid-May are cold, with saturated moisture conditions reducing the propensity of the seed to be induced into secondary dormancy and rather signalling germination or increasing the chance of the seeds rotting due to the low oxygen conditions. Conversely, when seed bank additions occur in the fall, the soil is warm and dry creating ideal secondary dormancy-inducing conditions.

2.5.3 Cultural Practises Contributing to Secondary Dormancy in *Brassica napus*

2.5.3.1 Post-harvest Seed Storage Conditions

Post-harvest seed storage conditions influence secondary dormancy with increased length of storage and high temperatures decreasing secondary dormancy as well as the longevity of *B. napus* seed (Nguyen et al., 2015; Gulden et al., 2003a). Preserving seed dormancy was most effective at a temperature of -70^0C in the Gulden et al. (2004a) study. Commercial canola seed is often stored at low temperatures to preserve longevity, but this practice may also contribute to maintaining secondary dormancy levels. Momoh et al. (2002) tested winter annual seed lots for secondary dormancy from a variety of storage conditions ranging from room temperature to low-temperature storage and observed that the longer the seed lots were stored in cold conditions, the greater the secondary dormancy. While the Momoh et al. (2002) study was not designed to specifically test storage conditions as just the length and type of storage was compared across seed lots, the results are in congruence to what is observed among the species which is that cold storage temperatures maintain degree secondary dormancy.

2.5.3.2 Seed bank Addition

The largest addition of seed into the soil seed bank occurs during the seed maturation stage and seed harvest. As the crop dries down the pods become brittle and prone to shattering which releases the seed into the seed bank. Not all seeds entering the seed bank survive, however and seed loss (prior to harvest and during machine harvesting) of up to 3,000 viable seeds m^{-2}, accounting for 5.9% of the total seed yield has been found leading to large amounts of seed with the potential to persist in the soil seed bank (Gulden et al., 2003b).

Harvest methods commonly practiced in western Canada include swathing the crop at approximately 60% seed colour change then allowing the crop to further dry down in windrows before it is combined (Haile and Shirtliffe. 2014). The second method leaves the crop standing longer in the field to dry down before it is directly combined. Different harvest timings were studied by Haile and Shirtliffe (2014) in western Canada. Seed harvested and tested before full maturity (10-60% seed colour change) was more likely to possess the propensity for primary rather than secondary dormancy while seed tested at maturity (colour change complete) exhibited higher secondary dormancy. Primary dormancy in fresh seed at the early harvest timing ranged from 13-16% but decreased to near zero in the harvested mature seed. It was concluded that seed harvested by swathing then combining ensures the seed is at proper maturity while reducing some of the seed bank input caused by pod shattering if the crop is left in the field longer.

Harvesting the seed any earlier than at 60% seed color change may reduce seed bank inputs, however; the subsequent quality of the seed may be compromised and is not recommended as a way to reduce seed bank input (Haile and Shirtliffe. 2014).

2.5.3.3 Tillage Regime

Tillage regimes have been studied to determine which methods reduce the persistence of *B. napus* seed in the soil. Testing winter annual *B. napus*, Gruber et al. (2005) found in Germany that delayed tillage or zero-till were the most effective methods for seed bank reduction when compared to immediate tillage following harvest. Immediate tillage incorporated the seeds on the soil surface into the soil and resulted in higher volunteer populations in the following years (Gruber et al., 2005). The deeper the seeds were buried, the more likely they were to be induced into secondary dormancy, however; past a depth of 6 cm, the seeds had little chance of survival due to the inability of the seedling to reach the soil surface or the seed remaining dormant. However, if deeply buried dormant seeds were brought back to the surface the seeds could break dormancy and germinate even several years after burial (Gruber et al., 2005). A more recent study also on winter annual *B. napus* in Germany found the timing of tillage had a more significant effect on volunteer occurrence compared to the depth at which the seed was buried (Huang et al., 2018). As stated above, immediate tillage caused higher volunteer populations the following season (Huang et al., 2018). Three weeks post-harvest was the recommended time delay before tillage should be applied in order to reduce the volunteer populations. The depth at which the seed was buried was found to not have a significant effect on volunteer occurrence (Huang et al., 2018).

A study on annual *B. napus* in western Canada concluded contrasting results to Gruber et al. (2005) and Huang et al. (2018), finding that tillage immediately following harvest in early autumn was the most effective method to reduce volunteer populations in subsequent years (Geddes and Gulden. 2017). Post-harvest tillage stimulated the seeds in the seed bank to germinate (seed bank recruitment), however, winter conditions caused winterkill decreasing the seed bank and reducing volunteers the following year. Seed persistence in the seed bank after autumn tillage was reduced by half compared to either zero-till or spring tillage regimes (Geddes and Gulden. 2017). The reasoning for the contrasting recommended tillage timing between Germany and Canada is due to the difference in climatic conditions. In western Canada, the

recruitment of volunteers the same season the seed entered the soil is ideal because the volunteers cannot survive the harsh winters and ultimately die before causing contamination. Whereas, in Europe, the immediate recruitment of volunteers is not ideal because the winters are much less harsh and the volunteers can overwinter and this practice is much like the seeding of winter annual canola in the autumn.

Shifting from focusing solely on tillage regimes, the effect of cultivar choice on volunteer occurrence was examined. Volunteers were identified in farmers' fields and genotyped through polymerase chain reaction and cluster analysis to determine the cultivar (Gruber et al., 2018). Only previously determined high and low dormancy cultivars were used for the analysis and it was found that the majority of the identified volunteers were from high dormancy cultivars (Gruber et al., 2018). Volunteer plant populations of 0-7 plants/m^{-2} of the high dormancy compared to 0-1.3 plants/m^{-2} of the low dormancy cultivars were found (Gruber et al., 2018). The highest volunteer plant populations were seen the year following cultivation. This study concluded that cultivar choice contributed more to volunteer populations via amount of shatter than tillage as low dormancy cultivars had lower volunteer populations compared to high dormancy cultivars. In terms of contributing factors to volunteer occurrence, the environment is not controllable or predictable, however cultivar selection (low dormancy) and tillage timing can be controlled by farmers and are recommended in an effort to reduce volunteer populations (Gruber et al., 2018). Farmers will not screen different *B. napus* cultivars for secondary dormancy thus, selection needs to be performed within breeding programs with the researchers being aware of how their varietal selection for crosses can impact volunteer persistence in future years.

2.5.4 Physiological Characteristics Contributing to Secondary Dormancy in *Brassica napus*

2.5.4.1 Annual versus Winter Annual Growth Types

A marked difference exists between secondary dormancy potential between winter annual and annual genotypes. Twenty-six winter annual and three annual genotypes were screened for secondary dormancy and the annual types had a higher propensity to not only be induced into secondary dormancy but also had higher absolute values when compared to the winter annual types under conditions of osmotic stress and darkness (Momoh et al., 2002). The development

of secondary dormancy in the annuals ranged from 0-85% dormant while the winter annual types ranged from 0-60% dormant (Momoh et al., 2002). Similarly, 75% of the annual genotypes screened by Gulden et al. (2004a) had high dormancy (> 60% dormancy). Such a difference in secondary dormancy between the two growth types is due to selection for differences in physiology between the contrasting growth environments (Momoh et al., 2002). Annual types may exhibit greater dormancy in order to avoid autumn germination when conditions may seem ideal but result in subsequent winterkill. Whereas, winter annual types which germinate following harvest are vernalised and can complete their lifecycle the following spring. Given the difference between the growth types, differences in potential gene flow from volunteers also exist. Gene flow between volunteers and the planted *B. napus* crop can only occur if the volunteers are flowering at similar times as the planted crop (Gruber et al., 2005).

2.5.4.2 Plant Hormones

The hormones abscisic acid (ABA) and gibberellic acid (GA) work antagonistically in the control of seed dormancy and germination; ABA prevents germination and GA promotes germination. Abscisic acid prohibits germination by preventing radicle elongation, cell wall loosening as well as preventing the degradation of seed storage proteins (Schopfer and Plachy. 1985; Bewley. 1997). Gibberellic acid counteracts the effect of ABA and weakens the testa thereby, allowing radicle protrusion and the completion of germination signalled by light and temperature (Weitbrecht et al., 2011; Dekkers et al., 2015).

Crossing experiments in *Arabidopsis* between ABA-deficient and wild-type lines showed maternally inherited tissues such as the endosperm produce ABA. But only ABA produced in the embryo can induce dormancy and once dormancy is induced, ABA is no longer required to maintain a dormant state (Karsen et al., 1983; Baskin and Baskin. 2001). Abscisic acid levels spike during early seed development thereby, preventing germination of the seed while still on the mother plant and decrease as the seed dries down with the lowest concentrations in dry seed (Hilhorst. 1995; Baskin and Baskin. 1998). Low temperatures during seed maturation cause an increase in ABA resulting in greater dormancy (He et al., 2014).

A study between high (AC Excel) and low (DH12075, from which NAM 49 as it is labelled in this thesis was derived) dormancy annual *B. napus* genotypes was conducted to examine the change in gene expression between the genotypes when osmotically stressed (Fei et

al., 2007). Three highly up-regulated genes at the full-size embryo stage were identified in both the high and low dormancy genotypes that are known to play a role in abiotic stress detection (Fei et al., 2007). All three genes are either induced by or responsive to ABA (Fei et al., 2007). A further study on the same genotypes found ABA was three-times higher in the high dormancy genotype when compared to the low dormancy genotype and ABA content of the high dormancy genotype tripled again after dormancy induction (Fei et al., 2009). After induction, 28 genes were commonly up-regulated in both genotypes with the majority of the genes involved in metabolism and abiotic stress detection. Furthermore, 158 genes were solely up-regulated in the high dormancy genotype and involved in primary and secondary metabolism, protein biosynthesis and metabolism compared to only 10 genes in the low dormancy genotype (Fei et al., 2009).

In another study, high dormancy genotypes were found to be more sensitive to ABA application than low dormancy genotypes, after being osmotically treated (Gulden et al., 2004c). Applied ABA did not affect the germination of the low dormancy genotypes but did for the high dormancy genotypes (Gulden et al., 2004c). The total ABA concentration within the osmotically treated seeds of the high dormancy genotype was approximately two-times that of the low dormancy genotype. The reduced sensitivity to ABA of the low dormancy genotype may be attributed to the germination process already being initiated within the seed and the resulting insensitivity to ABA (Schopfer and Placy. 1985; Gulden et al., 2004c). Whereas, the greater dormancy in the high dormancy genotype may be due to increased ABA sensitivity (Gulden et al., 2004c).

2.5.4.3 Seed Sugar and Protein

Seed components have been examined for possible relationships with secondary dormancy and two of interest are sugar and protein. Following induction, higher concentrations of glucose were found in a high dormancy *B. napus* genotype when compared to a low dormancy genotype (Fei et al., 2009). Also post induction, the high dormancy genotype had greater cell wall strength as well as increased sugar movement and metabolism (Finch-Savage and Leubner-Metzger 2006; Fei et al., 2009). In Arabidopsis, exogenously applied glucose prohibited germination at concentrations of 1% (Dekkers et al., 2004). This study was the first to find inhibitory effects of glucose at such low concentrations. The exact reasons behind the inhibitory

effects of glucose remain unknown. No interactions were found between sugar and GA synthesis nor did the glucose cause osmotic stress reducing germination (Dekkers et al., 2004). When the seed coats of the seeds were removed, germination was still restricted indicating that glucose affected the growth potential of the embryo rather than the coat. ABA-deficient mutants were screened in conjunction with glucose application and germination was not affected suggesting that glucose was more prohibitive when ABA is present in seeds. It is believed that glucose affects ABA signalling, but the two pathways are separate (Dekkers et al., 2004).

Brassica napus seed of Canadian Number 1 grade canola in 2018 was composed of approximately 44% oil and 21% protein (CGC. 2019). The protein content of the meal after the oil has been extracted is currently used in the animal feed industry (Schatzki et al., 2014). Within the protein content of the seed the seed storage proteins comprise 80% (Aider and Barbana, 2011; Mieth et al., 1983), with the remaining proteins being mainly oil body and lipid transfer proteins (Schatzki et al., 2014). Seed storage proteins contribute to the germination of the seed and seedling vigour, dormancy and seed longevity (Schatzki et al., 2014; Nguyen et al., 2015). The two main seed storage proteins in *B. napus* are 12S cruciferin, which is known to buffer oxidative stress during seed storage and 2S napin. Both proteins provide nutrients to the germinating seed and seedling before photosynthesis occurs and seedling becomes autotrophic. Cruciferin makes up 60% and napin makes up 20% of the seed storage proteins within the seed (Schatzki et al., 2014). The relative proportions of each protein are highly dependent on environment and genetics and the heritability of each protein ranges from 0.77 to 0.79 (Schatzki et al., 2014). In total, five QTL's have been identified seed storage protein quantity with three associated with napin and two for cruciferin making up 47 and 35% of phenotypic variation, respectively (Schatzki et al., 2014).

A negative correlation between cruciferin and napin exists with higher cruciferin found in modern winter annual *B. napus* genotypes. The higher cruciferin content is due to co-localization between napin content and seed glucosinolate content, thus selection for low glucosinolate varieties in modern canola breeding has indirectly resulted in the reduction in napin content (Schatzki et al., 2014). In a winter annual DH cross between a canola quality and high glucosinolate/ high erucic acid genotype, a significant negative correlation was found for napin content and total seed dormancy with low napin content seed having greater seed dormancy (Schatzki et al., 2014). If this negative correlation between napin content and dormancy is found

across the *B. napus* species, it is expected that modern annual low-glucosinolate canola will have a higher dormancy. Napin and glucosinolates are large sulfur containing components but studies testing if increased sulfur fertilisation resulted in increased protein were not significant. However, in the reverse test, seed production in sulfur limited soil resulted in lower seed protein (Schatzki et al., 2014). When selection for one seed storage protein occurs, the total protein content does not decrease, instead the other seed storage protein compensates to result in the same total protein (Schatzki et al., 2014). An interesting association between napin and dormancy is the production of napin is regulated by the gene *ABI3* which also plays a role in ABA production and ABA is known to promote dormancy (Schatzki et al., 2014). In summary, modern varieties with low napin content due to reduced glucosinolate content may result in higher dormancy due to the negative correlation between napin and dormancy (Schatzki et al., 2014).

2.6 Secondary Dormancy Screening Methodology for *Brassica napus*

Screening for secondary dormancy can take anywhere from 14 up to 46 days to complete, depending on the protocol (Pekrun et al., 1997; Gulden et al., 2004a; Weber et al., 2010). The general procedures are similar across protocols beginning with the induction, followed by germination and viability steps however, the duration of the steps, such as induction and germination, varies. Currently, no standardized method exists for secondary dormancy screening in *B. napus* (ISTA 2008; Weber et al., 2010). The most involved protocol (Pekrun et al..1997) includes an induction period with the osmoticum polyethylene glycol extending for 4 weeks at 20^0C in darkness. Followed by germination testing with water for 14 days at 20^0C, then temperature stratification for any non-germinated seeds and finally another germination test for viability on any remaining non-germinated seeds. Gulden et al. (2004a) followed the same protocol with a tetrazolium test for viability following the temperature stratification period.

The Hohenheim standard dormancy test was developed to reduce the time of the induction period from 4 weeks to 14 days (Weber et al., 2010). The same osmoticum and 20^0C temperature as above were used during the induction for 14 days. Following the induction, a germination test for 14 days at 20^0C in darkness. Any non-germinated seeds following the germination test are tested for viability at alternating temperatures of 30^0C and 3^0C for 12hr/12hr

and alternating light for 12hr/12hr. To further increase the efficiency of screening, the rapid dormancy test was developed from modifications to the Hohenheim standard dormancy test (Weber et al. 2010). The rapid dormancy test reduced the induction and germination period by half to 7 days for each test. When absolute dormancy was compared between the Hohenheim standard dormancy test and rapid dormancy test, a correlation coefficient of 0.96 was found with both tests detecting similar differences in dormancy among a range of genotypes. The Hohenheim standard dormancy test had greater absolute dormancy values compared to the rapid dormancy test but both tests ranked the genotypes in the same order for dormancy. The rapid dormancy test also eliminated the viability screen and found no effect in doing so on the resulting dormancy values. The sample size was also decreased from 100 to 50 seeds, which did result in a significant difference between tests for dormancy. The 50 seed test had lower absolute dormancy, but the ranking of the genotypes between tests remained the same. It was speculated that lower absolute values were due to a lower concentration of polyethylene glycol in the 50 seed test due to fewer seeds up taking water resulting in a more diluted polyethylene glycol solution. Overall, the rapid dormancy test was determined to be the most efficient screening process.

2.7 Seed Vigour, Germination and Precocious Germination in *Brassica napus*

2.7.1 Seed Vigour

Seed vigour is the combination and interactions between many physiological processes and characteristics related to seed germination and plant growth prior to emergence (van de Venter. 2008). Seed vigour officially is defined as, “the sum total of those properties of the seed that determine the potential level of activity and performance of the seed during germination and seedling emergence” (Perry. 1978; Finch-Savage & Bassel. 2016). Germination speed and uniform plant emergence are qualities contributing to the classification of seed vigour. Seed vigour can vary among the same genotype produced in different maternal environments and under different seed storage conditions (van de Venter. 2008; Finch-Savage & Bassel. 2016). Seed vigour is greatest at physiological maturity and shortly thereafter with levels decreasing with subsequent seed handling and storage (Finch-Savage & Bassel. 2016). Factors decreasing vigour include precocious germination, seed age and storage conditions. Crop domestication has

resulted in the selection for fast germinating, large seeded, non-dormant variants of crops, thereby, increasing the vigour of the crop over wild relatives and improving the germination over a wide range of environmental conditions (Finch-Savage & Bassel. 2016).

Seed dormancy spreads germination out over time and if a seed is fully or conditionally dormant, it will not germinate or will do so only under a narrow range of conditions. Thus, differentiation of whether a seed possesses dormancy or it has low vigour is difficult because seeds in both cases have low germination percentages (Finch-Savage & Bassel. 2016). Seed vigour, like seed dormancy, is quantitative in nature with a large environmental influence impacting the phenotype (Finch-Savage & Bassel. 2016).

The most common seed vigour test used for canola is a cold-start test (pre-chill test) under saturated soil-like conditions at 5-7^{0}C for 7 days before testing the germination percentage in a standard germination test (Elliot et al., 2007; AOSA. 2009). Other seed vigour tests include an electroconductivity test and the International Seed Testing Agency (ISTA) standardized test, the controlled deterioration test (ISTA. 2008). The electroconductivity test tests the membrane integrity and seeds with less solute leakage are regarded as more vigourous (AOSA. 2009). The controlled deterioration test accelerates seed ageing under standardized moisture content. Conditions during the controlled deterioration test include a high temperature of 45^{0}C for 24 hours and then a standard germination test (AOSA. 2009). In all of these seed vigour tests, seed lots with higher final germination percentages are considered more vigourous than low germination percentage genotypes and help discern seed lots able to germinate under adverse environmental conditions.

2.7.2 Seed Germination

Germination, a constituent of seed vigour, begins with the rapid uptake of water (imbibition) by a dry seed. Following rehydration of the seed, metabolic activity including increased respiration and protein synthesis resumes. Lastly, cell division and elongation of the embryonic axis cause the radicle to break through the surrounding structures and become visible. Once the radicle is visible, germination is typically classified and subsequent processes are defined as seedling growth however, when the seed is germinating in soil, germination is not scored until the seedling has emerged from the soil (Bewley. 1997).

Several factors influence seed germination with traits such as seed size, coat colour, plant hormones as well as environmental conditions like temperature, available moisture and post-harvest seed storage all playing a complex role in germination (Hatzig et al., 2015; Zhang et al., 2015). The germination of a seed is directly affected by the dormancy and both physiological processes are on the same continuum as the seed can be either dormant, conditionally dormant or germinate (Finch-Savage et al., 2010; Finch-Savage and Bassel. 2016). Under environmental conditions not ideal for germination the seed is dormant but as conditions improve the seed can transition out of dormancy into conditional dormancy. When a seed is conditionally dormant the seed will germinate under a narrow range of environmental conditions and as conditions continue to improve the seed will become fully non-dormant and able to germinate under a wider range of conditions (Baskin and Baskin. 1998). The process of dormancy cycling can occur within a seed over many seasons and directly affects the germination of the seed to ensure germination occurs under the best possible environmental conditions.

Brassica napus has non-deep physiological dormancy meaning that dormancy cycling occurs in this species and impacts the germination of the seed. In addition to the dormancy cycling, high dormancy winter annual types of *B. napus* display slower germination speeds when compared to low dormancy types believed to be due to the depth of dormancy the seed must overcome before germination can occur (Momoh et al., 2002). In addition to seed dormancy, seed size is believed to influence the germination of seeds with larger seeds possessing faster germination times. However, two separate studies in *B. napus* found that seed weight did not play a role in germination time (Schatzki et al., 2014; Zhang et al., 2015).

To better understand germination and the genetics governing it in *B. napus*, a diversity panel of over 200 winter annual genotypes were screened using a high-throughput phenotyping platform. Differences in germination were found to be influenced by the genotype, maternal environment and the genotype x environment interactions (Schatzki et al., 2014). A genomic region of interest influencing time to 50% germination (T50) and final germination rate at 72 hours contained a gene known to also play a role in ABA signalling (Schatzki et al., 2014). Further QTL analysis of germination and seed vigour in relation to plant hormone profiles was tested in a segregating winter annual DH population formed from a cross between a high vigour and a low vigour parent (Nguyen et al., 2018). Germination traits were recorded 7 and 14 days after sowing and profiling of ABA and its metabolites and auxin was performed 5 and 12 days

after sowing. Auxin plays a role in germination and dormancy and was found to maintain dormancy in seeds due to interactions with the ABA pathway (Nguyen et al., 2018). Sixty-four percent of phenotypic variation in seed vigour observed in the DH population could be attributed to genetic influences (Nguyen et al., 2018). The hormone profiles of the DH population were found to be skewed towards the poorer vigour parent in all hormones tested (Nguyen et al., 2018). In total, 12 QTL's overlapped between germination and hormone profiles. Using these identified regions of interest researchers can further investigate and develop molecular markers to select for rapid germination and high vigour breeding lines.

Intense plant breeding for seed quality to reduce erucic acid and glucosinolate content (anti-nutritionals) has occurred over the last 50 years to improve the taste of the meal and health of the oil. However, with such intense breeding, a genetic bottleneck has resulted causing low genetic diversity within the breeding lines. A diversity panel of 215 winter annual *B. napus* genotypes with different seed quality profiles, (low erucic; low glucosinolates, low erucic; high glucosinolates, etc.) were screened for seed vigour traits. It was determined that the breeding for reduced anti-nutritionals within the seed has led to an inadvertent reduction in seed vigour and increase in germination time (Hatzig et al., 2018). Genotypes of canola quality had significantly slower germination time than any of the other quality combinations with varieties released before the 1980s showing quicker germination (Hatzig et al., 2018). The unintentional reduction in germination time resulting from the selection for reduced anti-nutritionals has been caused by linkage drag between the two traits (Hatzig et al., 2018). A QTL identified for mean germination time was found to be in the same region as a minor QTL for both erucic acid and glucosinolates.

2.7.3 Precocious Germination

The opposite to seed being dormant is seed which germinates immediately once physiological maturity has been reached, even while still on the mother plant. Precocious germination (pre-harvest sprouting or vivipary) is the germination of seed while on the mother plant during the maturation phase and can be the result of breeding for fast germinating genotypes with little primary dormancy. *Brassica napus* has a low propensity for primary dormancy and precocious germination but it can be a problem in certain genotypes and environments (Feng et al., 2009). Precocious germination leads to the decrease in seed quality, yield and viability. Precocious germination is quantitative and is affected by both the

environment and genetics (Ren and Bewley. 1998). Environmental conditions leading to greater precocious germination include excess moisture and high humidity (Feng et al., 2009). Lower levels of ABA and changes in osmotic potential are commonly found in precociously germinated seed (Johnson-Flanagan et al., 1991). Heat stress is another environmental factor found to increase precocious germination of seeds likely caused by decrease in ABA:GA ratio resulting in lower dormancy and increased germination (Brunel-Muguet et al., 2015).

Seed development can be classified into two stages, the first is pre-desiccation and the latter is the desiccation stage (Johnson-Flanagan et al., 1991). The transition from pre-desiccation to desiccation occurs around 55% seed moisture, and during this time the seed becomes sensitive to external signals (Johnson-Flanagan et al., 1991). During this transition, moisture levels decease as well as ABA within the seed and in wet environmental conditions, the seed may be more prone to precocious germination (Johnson-Flanagan et al., 1991). In winter annual *B. napus,* five QTL have been identified for precocious germination and in total makeup over 75% of the phenotypic variation with one QTL contributing over 50% of the variation (Feng et al., 2009).

In a study on *B. napus* and *B. rapa* and the subsequent crosses between the two species, higher precocious germination occurrence was found when *B. napus* was the female being crossed (Hauser and Ostergard, 1998). It was determined that precocious germination/ reduced primary dormancy was inherited from the nuclear material from *B. napus*. *Brassica rapa,* a common weed species in Europe, has more pronounced primary dormancy compared to *B. napus* and it is thought to be a survival mechanism; whereas, the higher incidence of precocious germination in *B. napus* is likely due to breeding for rapid germinating material once in the soil.

2.8 Conclusion

A combination of genetic, environmental and cultural factors influence secondary dormancy within the seed. Due to the complexity and interactions between several factors, it is hard to predict dormancy in any given environment. There are many deficits in our understanding of secondary dormancy in *B. napus*. Ultimately, reduction of volunteer populations of canola in cultivated fields is the goal and the major way to do so is to reduce secondary dormancy. Given that such a large amount of the phenotype is controlled by the

genetics, and that a large genetic variation in dormancy has been identified, it is possible to select for reduced dormancy genotypes. However, the association between dormancy and seed germination, vigour and quality traits is unknown; therefore, it is important to determine if any relationships exist before selecting against secondary dormancy in breeding populations. Another factor to consider is good land management strategies to decrease the presence of the seed in the soil entering through pod shattering and then the management of the seed once it is in the seed bank. Low dormancy genotypes in combination with proper agronomic management are a promising way to decrease volunteer populations in western Canadian fields.

This thesis looked firstly at determining the secondary dormancy of a diverse set of annual *B. napus* genotypes produced in different maternal environments. From this screening, the genotypes were categorized for secondary dormancy across environments. Next, seed vigour, seed quality traits and precocious germination occurrence were screened within the population. The associations between secondary dormancy and seed vigour, seed quality traits and precocious germination were examined to help gain a stronger understanding of the biological relationships influencing secondary dormancy.

3.0 Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes in Different Seed Production Environments

3.1 Introduction

The increasing demand for canola oil and meal has resulted in canola being the top crop produced in Canada with 9.2 million hectares planted in 2018 (Statistics Canada. 2018). Canada exports 90% of the canola produced in the country which contributes 26.7 billion dollars annually to the Canadian economy (Canola Council Canada. 2018). With such a large area of the western Canadian provinces dedicated to growing canola, challenges can arise. One persistent issue being the occurrence of volunteer canola caused when canola seed germinates in subsequent years after planting in cultivated crops. Volunteer canola ranked the fourth most prevalent weed on the prairies in the most recent agricultural weed survey in 2015 (Beckie. 2015). Control of volunteer canola can be difficult due to common herbicide tolerance traits within other canola crops as well as in different crops such as herbicide tolerant soybean (Geddes and Gulden. 2015). Furthermore, there is the possibility of trait mixing among canola cultivars from cross-pollination leading to unintended gene combinations in terms of quality requirements and/or herbicide tolerance gene stacking.

Secondary dormancy is the physiological mechanism prolonging the persistence of canola seed in the soil seed bank leading to volunteer canola. Secondary dormancy is induced under adverse environmental conditions in the soil such as low light, moisture and temperature (Baskin and Baskin. 1998). The largest addition of *B. napus* seed into the seed bank results from pod shattering occurring at maturity. This occurs in adverse weather conditions including high wind as well as during machine harvesting (Haile and Shirtliffe. 2014). It is hypothesized that volunteer populations can be reduced by planting genotypes with low secondary dormancy. However, direct selection for low secondary dormancy has not occurred before making the effect of reducing dormancy on other seed traits unknown.

Genotype is the largest contributing factor to secondary dormancy in winter annual and annual *B. napus* (Momoh et al., 2002; Gulden et al., 2004a; Weber et al., 2013; Schatzki et al., 2013a). The reported heritability of secondary dormancy is extremely high with a value ranging from 0.96-0.97 (Weber et al., 2013; Schatzki et al., 2013a). Moreover, genotype contributed 44-82% of the total variation in secondary dormancy among 16 Canadian annual varieties (Gulden et al., 2004a). Commercial Canadian varieties screened in the early 2000s ranged from 0-90% dormant with 75% of the varieties tested ranking as high dormancy (> 60% dormant) (Gulden et al., 2004a). Given that such a large percentage of the commercial varieties tested by Gulden et al. (2004a) possessed high dormancy, it is possible that high dormancy may have been inadvertently maintained given it is prevalent in the breeding material. Screening for secondary dormancy is not widely practised when selecting for future canola varieties because the process is labour intensive and lengthy, an additional factor leading to the maintaining of secondary dormancy in breeding material. Recently, a Nested Association Mapping (NAM) population comprised of diverse parental lines and Recombinant Inbred Lines (RILs) has been created for annual *B. napus*, capturing the diversity among the species from across the globe (Parkin et al., 2017). The parental diversity collection contains a large number of genotypes developed in Canada thus could provide insight into the dormancy dynamics in Canadian breeding material. Given the genetic diversity across the population, low dormancy is likely to be represented.

Although the genetic component is the largest contributing factor to secondary dormancy in *B. napus*, it is highly influenced by the maternal environment in which the seed is produced in. High temperatures and moisture stress during seed development and maturation have been found to decrease secondary dormancy (Brunel-Muguet et al., 2015; Finkelstein et al., 2008). Ideal environmental conditions during flowering and maturity producing high yield and high oil content were found to increase secondary dormancy among winter annual types tested in Germany (Schatzki et al., 2013b). Huang et al. (2016) also determined that winter annual *B. napus* seed produced in optimal conditions had higher dormancy than seed produced in sub-optimal environments. Several studies conducted across different environments and years found that the environment influences the absolute secondary dormancy, but the general ranking of genotypes tended to remain the same (Momoh et al., 2002, Gruber et al., 2009 and Weber et al., 2013).

Taking into account the above mentioned genetic and environmental factors that influence secondary dormancy it may be possible to select for low dormancy genotypes. Ideally, selected canola varieties would have consistently low secondary dormancy when produced in different environments. In order to assess the feasibility of using the annual *B. napus* NAM to develop the low secondary dormancy trait, the parental population needed to be screened for secondary dormancy.

3.2 Objective and Hypothesis

The objective of this research was to determine secondary dormancy of a diverse collection of annual *B. napus* genotypes (NAM) produced in contrasting environments for two purposes. Firstly, to examine genetic and environmental differences among the genotypes and secondly, for use in comparison with results from further experiments analyzing seed germination, vigour and quality traits.

It was hypothesized that a wide range in secondary dormancy would be observed among the diverse collection of annual *B. napus* genotypes (NAM) due, in part, to the different genotypes within the population as well as the contrasting environmental conditions in which the seed was produced in.

3.3 Materials and Methods

3.3.1 Experimental Location and Diversity Collection Seed Production

The genotypes screened were the parental panel of the annual *B. napus* Nested Association Mapping (NAM) population which is comprised of a collection of 51 annual genotypes from around the world that were selected based on genotypic and phenotypic diversity. The origin of the collection included accessions from Canada, Europe, Australia, exotic (N. & S. Korea, China, India, Bangladesh), winter background and other (e.g. Argentina). The genotypes that make up the NAM parental population were all selected for homozygosity of single nucleotide polymorphisms and derived from a single plant (Parkin et al., 2017). Other genotypes were used as the controls in this experiment based on previous results (Gulden et al.,

2004a) and included, AC Excel (high dormancy) and Quantum (mid dormancy). NAM 0 was the reference line for the NAM Recombinant Inbred Lines.

The source seed for the diversity collection was self-pollinated in greenhouse increases in Saskatoon, Saskatchewan at Agriculture and Agri-Food Canada in 2014. From the resulting seed increase, four representative but contrasting environments were selected to produce seed for secondary dormancy screening. In the Saskatoon environments (planted in May and harvested in September 2015 and 2016), 100 seeds of each genotype were planted in 3 metre single rows, divided into three one metre sections and each section covered by a perforated tent prior to flowering. In Chile (planted in November 2015 and harvested in April 2016), 100 seeds of each genotype were planted in three adjacent rows within a 1 m^2 mini-cage. Chilean seed was produced under irrigated conditions while both Saskatoon years were rain-fed (Figure A.1; A.2). The seed was harvested after maturity, hand threshed then placed in envelopes and frozen shortly after at -80^{0}C to preserve the maximum dormancy (Gulden et al., 2004a). Seed produced in Chile was shipped to Saskatoon on dry ice to ensure seed remained frozen before it was immediately placed in -80^{0}C freezers at the Saskatoon Research Center at Agriculture and Agri-Food Canada.

Saskatoon (52^0 N, 106^0 W) was selected as a location for seed production because of the large acreage in the province dedicated to canola production as well as the large concentration of canola breeding research that exists in Saskatoon and surrounding areas. Temuco (38^0 S, 72^0 W) and Los Ángeles (37^0 S, 72^0 W) locations in Chile were selected as much of the contra-season nurseries and increases for Canadian canola breeding programs as well as commercial seed production for Canada occurs in these regions. Saskatoon, on average, had warmer temperatures during the growing season with monthly averages reaching up to 26^{0}C compared to 25^{0}C in Los Ángeles and 22^{0}C in Temuco, Chile. The Los Ángeles environment was expected to be the hottest during flowering based on historical data but that was not what was observed. The day length hours increased to a maximum of 16 hours in Saskatoon and then decreased as the crop matured, whereas, the day length started at around 14 hours and decreased in Chile as the crop matured. Furthermore, the range in altitude varies across Chile when compared to Canada. The altitude of Saskatoon is approximately 482 m above sea level while the altitude of Temuco is 360 metres and 139 metres in Los Ángeles.

In summary, four seed lots for each genotype were produced for this study, one from each environment. When a reference to a seed lot is made it is referring to a specific genotype in a specific environment whereas, a reference to genotype refers to the mean of the four seed lots.

3.3.2 Experimental Procedure

The field experiment for secondary dormancy screening was set up as a spilt-plot randomized complete block design with the main plot factor as the environment and the sub-plot factor as the genotype. Three technical replications were performed for the Saskatoon 2015, Temuco 2016 and Los Ángeles 2016 environments. The Saskatoon 2016 environment was added for the fourth and fifth replications once the seed was harvested from the field.

3.3.2.1 Dormancy Induction

The protocol employed followed the rapid dormancy induction method (Weber et al., 2010). Green light (495-570 nm), a wavelength not affecting the phytochrome of plants but allowing the experimenter to see, was used to carry out the experiment. A solution of polyethylene glycol (PEG) 6000 (Calbiochem®, France) was prepared from 354.37 g dry PEG flakes with 1 L of non-sterilized deionized water and mixed until the flakes were completely dissolved and osmotic potential of -1.5 MPa at 20^0C was reached. Fifty seeds per treatment were placed in a clear polystyrene germination box (11.5 cm x 11.5 cm; Hoffman Manufacturing Inc., Oregon) on top of blue blotter paper (10.16 cm x 10.16 cm; Anchor Paper Company, Saint Paul, Minnesota) and saturated with 9 mL of PEG solution and covered with a lid to prevent evaporation. The clear germination boxes were placed in larger storage boxes (50.5 cm x 42 cm), from now on referred to as location box, to contain all the germination boxes for an environment. Placement of the germination boxes within the location boxes followed a random design. The location boxes were covered completely with aluminium foil and two large black opaque plastic bags to block out any light for the duration of the experiment. The location boxes were then placed in a Conviron Adaptis growth cabinet (Winnipeg, Canada) set to 20^0C in darkness. To account for any temperature gradients in the germination cabinet, the location boxes were rotated each day in a top to bottom fashion. After 7 days, the storage boxes were removed from the cabinet and the next step of the rapid dormancy test was performed.

3.3.2.2 Germination Testing

The location boxes were removed from the germination cabinet and under green light, the germination boxes were removed and the number of non-germinated seeds was counted and recorded. Any decayed seeds were classified as non-viable, discarded and subtracted from the total number of seeds. The non-germinated seeds were transferred to new germination boxes with fresh blotter paper saturated with 9 mL of distilled water. The germination boxes were again randomly arranged and placed in the location boxes and re-covered in aluminium foil and black opaque bags and placed back into the same growth cabinet at 20^0C in darkness for an additional 7 days.

3.3.2.3 Viability Testing

Following the germination testing, the number of non-germinated seeds was counted and recorded (Figure 3.1). Any non-germinated seeds were then tested for viability to determine if the seed was dormant or dead. For this, the ungerminated seeds were transferred to new germination boxes with 9 mL distilled water on top of blotter paper. The temperature regime differed from the Weber et al. (2010) protocol which recommended alternating temperature and light for 12 hours at 30^0C and 12 hours at 3^0C darkness. Faster germination in preliminary testing was observed using the Association of Official Seed Analysts (AOSA) Seed Vigour Testing Handbook guidelines (AOSA. 2009) so the temperature regime was set at 20^0C for 16 hours and 30^0C for 8 hours all in complete darkness for 7 days. After 7 days any non-germinated seeds were considered dead and subtracted from the total number of viable seeds. The final dormancy was calculated as,

(number of dormant seeds/ total viable seeds per replication)……...(3.1)

FIGURE 3.1: PHOTO TAKEN FOLLOWING THE RAPID DORMANCY GERMINATION TEST.
Note: The non-germinated seeds (indicated by arrows) are either dormant or non-viable and the viability test was used to confirm the viability of the seeds.

3.4 Statistical Analysis

The data were found to not conform to normality (non-Gaussian) using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) by testing the Shapiro-Wilk *P*-value (*P*-value < 0.05, data do not follow a normal distribution). Thus, the GLIMMIX procedure was used to analyze the data. The data were count data (germinated seeds/total viable seeds) with only two possible outcomes and values between 0-1, supporting the use of the beta distribution (Bowley. 2015). Values of 0 and 1 were changed to 0.001 and 0.999, respectively because the beta distribution omits values of 0 and 1. The complimentary log-link (*ccll*) function was used to transform the least square mean estimates back to the data scale initially used presenting the estimates as means (proportion). The Laplace method assisted in data convergence as well as aided in stabilizing over-dispersion (Pearson chi-square/degree of freedom = $0 < x < 2$) (Bowley. 2015). Data transformation (arcsine transformed) was not used to generate means and variance partitioning because sufficient examples in the literature provided reasoning that using the GLIMMIX procedure was a more effective way to deal with non-Gaussian germination data (eg. Willenborg et al., 2005). The fixed effects for analysis were the genotype, environment and the genotype x environment interactions while the random effects

were the replications and environment*replication interactions. To determine the percent contribution of each fixed effect, the VARCOMP procedure was used. The type III sum of squares for the fixed effect divided by the corrected total sum of squares was used to determine the percentage value each factor contributed to the phenotype (Gulden et al., 2004a; Botwright-Acuña and Wade. 2012). For the environment correlations, the CORR procedure was used with the Spearman rank function to account for the data not being normally distributed.

For the purpose of this chapter, only genotypes common to all four environments were analyzed. Genotypes in some environments were missing/or did not produce seed and as a result, were not included in the analysis to ensure a balanced dataset.

3.5 Results

3.5.1 Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes across Seed Production Environments

A large range of secondary dormancy was observed among the genotypes examined (Figure 3.2). The collection was divided into three classifications in regards to secondary dormancy: 0.0-10.0%, 10.01-49.0% and 49.1-100% for low, mid and high dormancy, respectively. The classifications were determined by genotype averages significantly different than AC Excel (control genotype previously screened for secondary dormancy) at a level of $P < 0.05$. Genotypes with significantly lower dormancy than AC Excel were classified as 'low dormancy' and genotypes significantly greater than AC Excel were classified as 'high dormancy'. Genotypes not significantly different than AC Excel were classified as 'mid dormancy' along with AC Excel. Of the genotypes screened, 55% were classified as low dormancy, 34% classified as mid dormancy and 11% classified as high dormancy (Figure 3.3). Gulden et al. (2004a) previously classified Quantum, an old commercial variety, as mid dormancy and AC Excel as high dormancy.

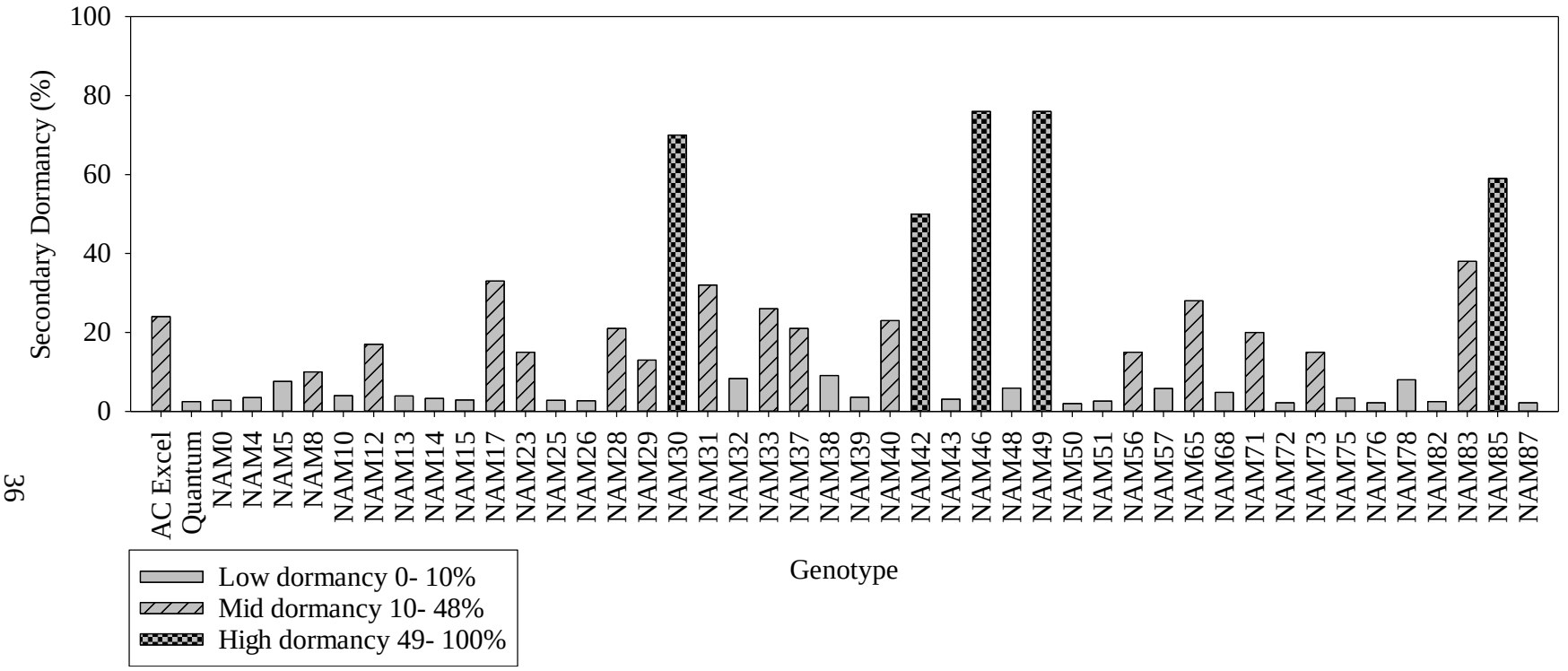

FIGURE 3.2: MEAN SECONDARY DORMANCY OF A DIVERSE COLLECTION OF ANNUAL *BRASSICA NAPUS* GENOTYPES ACROSS FOUR SEED PRODUCTION ENVIRONMENTS (SASKATOON 2015 &2016, LOS ÁNGELES 2016 AND TEMUCO 2016).

Note: The control genotypes are the first three bars on the left of the chart (AC Excel, Quantum and NAM 0). Classifications were determined in relation to AC Excel. Genotypes with lower dormancy than AC Excel were classified as low dormancy, mid dormancy were not significantly different than AC Excel and high dormancy genotypes were significantly different than AC Excel.

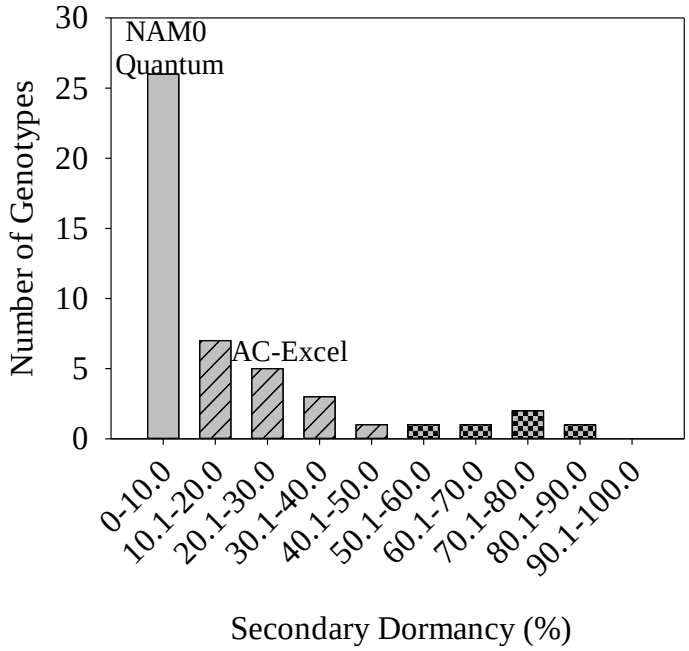

FIGURE 3.3: FREQUENCY DISTRIBUTION OF SECONDARY DORMANCY IN A DIVERSITY COLLECTION OF ANNUAL *BRASSICA NAPUS* SOURCED FROM FOUR SEED PRODUCTION ENVIRONMENTS (SASKATOON 2015 &2016, LOS ÁNGELES 2016 AND TEMUCO 2016).

For the secondary dormancy observed in this diversity collection, the genotype was highly significant ($P<0.001$; Table 3.1). The interactions between the genotype and environment was also highly significant ($P<0.001$). The environment effect alone was not significant ($P > 0.05$) in terms of secondary dormancy however, contributed to the significant interaction effect. The genotype alone contributed almost half of the variation observed for the phenotype, the genotype x environment interactions contributed almost one-third of the variability of secondary dormancy (Table 3.1).

TABLE 3.1: ANOVA TABLE OF FIXED EFFECTS FOR SECONDARY DORMANCY OF A DIVERSE COLLECTION OF ANNUAL *BRASSICA NAPUS* GENOTYPES.

Fixed Effect	*F*-value	*P*-value	% of the total Sum of Squares
Genotype	47.61	<0.001	45.9
Environment	3.52	0.06	4.3
Genotype x Environment	7.67	<0.001	28.3

Note: Percent of the total sum of squares determined by the fixed effect sum of squares divided by the total sum of squares.

A stability analysis of genotype secondary dormancy mean versus variance across environments was conducted to further investigate the significant interactions between genotype and environment (Figure 3.4). The five genotypes classified as high dormancy all had above average variability but only one genotype ranked high dormancy in all four environments (NAM 49). The other four genotypes ranked high dormancy in two or more environments leading to the greater variance distribution for those genotypes (Figure 3.4). The mid dormancy genotypes were more dispersed for variance (along the Y-axis), indicating dormancy varied more depending on the environment in which the seed was produced in compared to the other two classes. AC Excel was the genotype with the greatest variance across environments (highest grey circle along the Y-axis) with a variance value of almost 0.16 compared to the average of 0.03. Dormancy of AC Excel ranged from 3-88% across the four environments (Table 3.3). Of the low dormancy genotypes, 85% were clustered below the average variance line indicating dormancy was consistently low across differing environments.

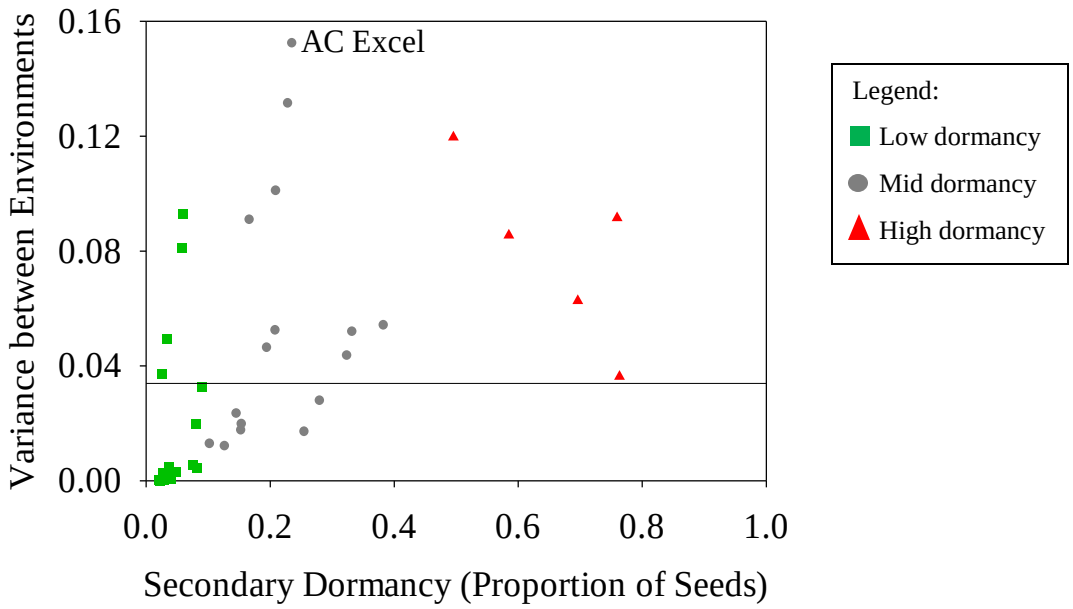

FIGURE 3.4: VARIANCE VERSUS SECONDARY DORMANCY OF A DIVERSITY COLLECTION OF ANNUAL *BRASSICA NAPUS* ACROSS FOUR SEED PRODUCTION ENVIRONMENTS (SASKATOON 2015 &2016, LOS ÁNGELES 2016 AND TEMUCO 2016).

Note: The mean variance was 0.03 (as indicated by the horizontal line) and the mean secondary dormancy was 0.17.

3.5.2 Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes in each of the four Seed Production Environments

The two Saskatoon environments had similar average monthly temperatures during the growing season, however, Saskatoon 2015 had higher maximum temperatures with a temperature of 35.8^0C in July during the peak of flowering in addition to less total precipitation than Saskatoon in 2016 (Figures A.1 and A.2). Seed produced in Saskatoon in 2015 possessed the highest overall secondary dormancy across the environments screened. Los Ángeles was more consistently hot throughout the growing season and produced seed with the lowest overall secondary dormancy. Saskatoon 2016 and Temuco were the two most closely correlated environments with respect to secondary dormancy with a correlation coefficient of 0.64 and had similar average dormancy (Table 3.2). The two Chile environments were the least correlated while still highly significant with a correlation coefficient of 0.45.

TABLE 3.2: CORRELATION BETWEEN SEED PRODUCTION ENVIRONMENTS (SASKATOON 2015 & 2016, LOS ÁNGELES 2016 AND TEMUCO 2016) FOR SECONDARY DORMANCY MEANS.

	Saskatoon 2015	Saskatoon 2016	Temuco 2016	Los Ángeles 2016
Saskatoon 2015		0.53**	0.62**	0.52**
Saskatoon 2016			0.64**	0.59**
Temuco 2016				0.45**

Note: Spearman rank correlations analysis; significance is denoted by an asterisk where '*'- $P< 0.05$; '**'- $P< 0.01$.

There was no significant differences between environments for mean secondary dormancy and environmental means ranged from 17-24% dormant overall (Table 3.3). The secondary dormancy of each seed lot, the secondary dormancy classification and the overall genotype average secondary dormancy was ranked from high to low and colour coded for visualization (Table 3.3).

TABLE 3.3: SECONDARY DORMANCY LEAST SQUARE MEANS ACROSS DIVERSE *BRASSICA NAPUS* GENOTYPES PRODUCED IN SASKATOON 2015 & 2016, LOS ÁNGELES 2016 AND TEMUCO 2016

NAM Founder	2^0 Dorm Class	Average Sec Dorm	Saskatoon 2015	Saskatoon 2016	Los Ángeles 2016	Temuco 2016
			Mean (%)			
Legend	**High dorm**		**Mid dormancy**			**Low dorm**
NAM30	High	70	88	78	72	39
NAM42	High	50	80	15	93	27
NAM46	High	76	94	76	54	75
NAM49	High	76	51	87	77	86
NAM85	High	59	89	62	20	72
AC Excel	Mid	24	2.6	88	72	7.1
NAM12	Mid	17	69	9.0	22	2.8
NAM17	Mid	33	22	19	68	33
NAM23	Mid	15	32	28	5.0	9.1
NAM28	Mid	21	70	3.3	33	17
NAM29	Mid	13	12	16	8.2	16
NAM31	Mid	32	40	52	9.7	50
NAM33	Mid	26	36	40	17	17
NAM37	Mid	21	54	32	3.1	21
NAM40	Mid	23	85	12	7.9	20
NAM56	Mid	15	14	9.1	27	23
NAM65	Mid	28	38	13	39	31
NAM71	Mid	20	1.9	41	39	44
NAM73	Mid	15	8.6	36	10	17
NAM8	Mid	10	29	15	3.1	7.9
NAM83	Mid	38	65	13	58	35
NAM0	Low	2.8	1.9	4.9	3.3	1.9
NAM10	Low	4.0	3.8	2.7	2.3	5.1
NAM13	Low	3.9	2.3	1.9	2.3	8.7
NAM14	Low	3.3	1.9	2.9	4.4	3.0
NAM15	Low	2.9	9.5	1.9	5.3	2.3
NAM25	Low	2.8	6.4	1.9	4.9	2.6
NAM26	Low	2.7	1.9	1.9	1.9	6.6
NAM32	Low	8.3	5.8	21	4.2	7.8
NAM38	Low	9.1	2.3	42	1.9	29

NAM39	Low	3.6	1.9	23	1.9	2.8
NAM4	Low	3.5	3.9	1.9	5.1	3.3
NAM43	Low	3.1	3.4	1.9	2.4	4.1
NAM48	Low	5.9	5.0	3.4	2.4	26
NAM5	Low	7.6	7.0	6.4	5.7	13
NAM50	Low	2.0	2.3	1.9	1.9	1.9
NAM51	Low	2.6	1.9	1.9	6.1	2.3
NAM57	Low	5.8	4.1	1.9	1.9	59
NAM68	Low	4.8	1.9	12	6.6	3.5
NAM72	Low	2.2	1.9	1.9	2.0	2.4
NAM75	Low	3.4	3.1	2.9	6.7	2.8
NAM76	Low	2.2	1.9	2.7	1.9	2.2
NAM78	Low	8.0	24	5.0	5.1	7.2
NAM82	Low	2.5	2.3	3.0	2.6	2.2
NAM87	Low	2.2	2.4	1.9	1.9	2.5
Quantum	Low	2.5	2.3	1.9	1.9	4.7
	Mean	17	24	20	18	19

Note: Genotypes were ranked within environment then colour coded. Shades of blue (favourable outcome) represent lower dormancy and shades of red (less favourable outcome) signify higher dormancy within each environment. Genotypes underlined were the controls (previously determined secondary dormancy by Gulden et al. (2004a).

3.6 Discussion and Conclusion

The objective of this study was to examine secondary dormancy of the diverse annual NAM parental panel produced in contrasting environments for the purpose of further understanding the effects of genotype and environment on the presence of secondary dormancy. The null hypothesis was that, seed from the genotypes produced in different environments would all possess similar secondary dormancy. However, this was rejected and the alternative hypothesis, that a range in secondary dormancy exists among the genotypes and across environments, was supported.

The genotype effect was the largest contributing factor to the variability of secondary dormancy. Genotypic variation alone contributed almost half of the variability in the dormancy observed. The second largest contribution with one-third of the variation in secondary dormancy

was from the genotype x environment interactions. Lastly, the environment alone contributed a much smaller proportion of variability to secondary dormancy at 4%, which was non-significant. Previous studies had similar findings indicating that genetic differences between genotypes were consistently the largest contributing factor to secondary dormancy (Gulden et al., 2004a; Gruber et al., 2009; Weber et al., 2013). This study was the first to look at such a diverse panel of spring *B. napus* genotypes across strategically selected contrasting maternal environments. In this study, the environment effect alone was not significant, but the genotype by environment interactions were highly significant. Reasoning for the non-significance of the environment is due to the large range of secondary dormancy potentials that was observed across the genotypes within an environment which when averaged for the environment resulted in a mean secondary dormancy potential that was similar across each of the four environments. When the genotypes were compared across the environments, the change in secondary dormancy of some genotypes from one environment to another was observed by the significant interaction term. This is in contrast to the results from Schatzki et al. (2013a & b) in which only the genotype and environment effects were significant and not the interactions, likely due to a small number of genotypes screened (28 genotypes). Similar to previous studies on secondary dormancy in *B. napus*, the absolute dormancy of a genotype across years and/or environments was difficult to reproduce in this study (Momoh et al., 2002).

The five genotypes classified as high dormancy (NAM 30, NAM 42, NAM 46, NAM 49 and NAM 85) may not have ranked as high absolute dormancy in their individual environments but had higher dormancy when compared to other genotypes within the same environment. Much more variability was observed in the mid dormancy genotypes. Of the 25 genotypes that changed classifications in one or more environment (e.g. low in Temuco and mid in Saskatoon 2015), 56% of the across environment means resulted in classification of mid dormancy. The majority of the genotypes screened in this study were classified as having low dormancy and as a result low variance across environments. There was no clear relationship between a genotypes' potential for secondary dormancy and the country in which the seed was received from (Table A.1). That being said, it was noted that the eight genotypes with the most extreme differences between environments were of European (NAM 57, NAM 28), Canadian (NAM 48, NAM 71, NAM 12 and AC EXCEL) and Australian (NAM 38, NAM 37) background, but not of Asian background. Two of the five genotypes which were classified as high dormancy originated from

Korea (NAM 46 and NAM 85), however other genotypes were also classified as mid (n=3) and low (n=2) suggesting a wide range of dormancy potential in lines of Korean origin. Only one Canadian genotype was classified as high dormancy and was NAM 49. Four Canadian genotypes were classified as mid dormancy genotypes with three of the four having the common pedigree-parent Westar which was a very popular early maturing variety grown in Canada in the 1980s. Canada again was well represented in the low dormancy grouping followed closely by European origin and lines of Asian descent were scattered throughout all three dormancy classifications.

Two control genotypes (Quantum and AC Excel) were included because they were previously screened by Gulden et al. (2004a) who classified Quantum as mid dormancy (36-60% dormant) and AC Excel as high dormancy (61-100% dormant). The classification system differed between Gulden et al. (2004a) and this study. Using the magnitudes from the Gulden et al. (2004a) classification system, Quantum and AC Excel in this study would both have resulted in both being classified as low dormancy. In this study, Quantum was found to be consistently low, averaging 2.5%. AC Excel was classified as mid dormancy, with an average of 24%; however absolute dormancy for AC Excel across environments was inconsistent ranging from 3-88% across environments with two seed lots from two environments being more than 61% dormant, displaying substantial genotype by environment interactions. AC Excel could be particularly sensitive to environmental conditions during seed development and maturation thus, causing the variability in dormancy classifications. Reasoning for differing dormancy classifications between genotypes screened in this study and other studies are numerous. The environments in which the diversity panel was produced in were deliberately chosen to be more variable and contrasting than environments in previous studies by Gulden et al. (2004a). In this experiment, the seeds were induced for 7 days compared to 4 weeks by Gulden et al. (2004a). Significant differences in dormancy were found to exist between the length of induction time and absolute dormancy in *B. napus* (Weber et al., 2010). It is also impossible to ensure that the seed lots screened traced back to the ones Gulden et al. (2004a) screened. For example, AC Excel is derived from a n F4 composite and genetic drift is more likely when not derived from a highly inbred or DH line. As *B. napus* is an open pollinated species, it is difficult to ensure that the source is genetically pure and not contaminated with pollen from another source.

In the Fei et al. (2007 & 2009) studies, two cultivars were screened for gene expression during seed maturation as well as when induced into secondary dormancy. The cultivars in their study were AC Excel (high dormancy) and DH12075 (NAM 49; low dormancy) and differences in gene expression were observed between the cultivars. More ABA inducible genes were expressed in the high dormancy AC Excel compared to the low dormancy DH12075. A major discrepancy in dormancy classification was identified as Fei et al. (2007 & 2009) classified DH12075 as low dormancy and in this study it was consistently classified as high dormancy. Differences in the dormancy classification between the studies again can be attributed to differences in induction length (four weeks in Fei et al. and two weeks in this study), seed source purity and maternal environment (greenhouse vs field produced seed). The above examples reinforce that it is difficult to compare genotypes across different experiments and environments as many factors may not have been the same, including maternal environment, screening protocol and genetic purity. However, these findings are worth noting to again enforce that there are lots of variation across environments and from this study a new panel of control genotypes have been identified.

An observation from this experiment was that the hottest overall maternal environments produced lower secondary dormancy. The secondary dormancy mean from Los Ángeles was not statistically different from those of the other environments but this environment did have the hottest mean monthly temperatures and produced seed with the lowest overall dormancy. In contrast, Saskatoon 2015 had the highest maximum daily temperature at 35^0C and the highest overall secondary dormancy of the environments (Figure A.1; A.2). The environmental effect cannot be studied due to the small number of environments screened and lack of phenology data from the individual environments but it would be an interesting factor to study further. While high temperatures may cause physiological stress to the maturing seed, it cannot be concluded from this study that high temperature alone is responsible for lower secondary dormancy. Heat stress during the seed filling stage is most detrimental to seed quality and dormancy as large depositions of storage reserves are occurring at this time (Brunel-Muguet et al., 2015). Furthermore, *DOG1* protein accumulation decreases in hot temperatures resulting is lower dormancy seed (Graeber et al., 2014). Moisture stress during seed maturation is another factor known to reduce dormancy and seed quality (Baskin and Baskin. 2001), but neither factor can solely be attributed to influencing dormancy in this experiment, especially since the sites in Chile

were irrigated. It is likely a combination of several environmental conditions including, heat and moisture and that influence the absolute dormancy.

The variable impact the environment had on certain genotypes for secondary dormancy that was observed in this study is an important result for the canola breeding and seed science communities. Absolute secondary dormancy changed from year to year even in the same environment. Optimal growing conditions have been identified as conditions that increase the dormancy of a genotype (Schatzki et al., 2013a; Huang et al., 2016); however, the results from this study showed several consistently low dormancy genotypes regardless of environmental conditions, even if conditions were favourable for increased dormancy. Thus, the results of this study demonstrate that it is possible to identify stable, low dormancy genotypes regardless of the environmental conditions. It is possible given the shortened induction period in this study that the mid and high dormancy genotypes may have been under predicted when compared to the same seed lot induced for four weeks. For future studies it may be beneficial to test the seeds maximum potential for secondary dormancy through longer induction testing.

In terms of volunteer canola reduction on the Canadian prairies, the results from this study corroborate with previous studies concluding that it is possible to reduce secondary dormancy by selecting against the trait. This is due to the genetic control and the stability of low dormancy observed by genotypes expressing low values across different environments. The range in dormancy in the population tested in this study provides a large panel to select from to screen for genetic markers to facilitate selection for low dormancy. The next chapter of this thesis reports the results from seed germination, vigour and quality traits screening of the diversity collection for possible relationships with secondary dormancy. Any association between seed germination, vigour and quality and secondary dormancy is imperative to understand in order to ensure that by reducing dormancy other seed traits are not negatively impacted.

4.0 Seed Vigour, Seed Quality Traits and Precocious Germination of a Diverse Collection of Annual *Brassica napus* Genotypes and the Relationship with Secondary Dormancy

4.1 Introduction

Reducing secondary dormancy in *B. napus* seed has widely been proposed as a method to reduce volunteer populations in cultivated fields (Momoh et al., 2002; Gulden et al., 2004a; Gruber et al., 2009; Weber et al., 2013; Schatzki et al., 2013a & b). The results from the previous chapter confirmed that there is a genetic difference in potential for secondary dormancy in a laboratory setting. The low dormancy phenotype was prevalent in the annual diversity collection with 56% of the genotypes screened classified as low dormancy. The strong genetic component contributed about half of the variation observed for secondary dormancy indicating that selection for low dormancy would be effective. Lastly, the low dormancy phenotype was generally stable in genotypes across environments. However, it is not known whether reducing secondary dormancy impacts other important seed traits such as seed vigour, germination and seed quality-related traits is unknown in annual *B. napus*. In winter annual *B. napus*, seed protein, seed vigour traits including germination time and precocious germination have been found to be associated with secondary dormancy (Momoh et al., 2002; Feng et al., 2009; Schatzki et al., 2014; Hatzig et al., 2015). No correlation between seed size and secondary dormancy has been documented in winter annuals, however, a positive correlation between seed size and secondary dormancy was observed in annual *B. napus* (Gulden et al., 2004a; Schatzki et al., 2013a). A better understanding of any relationships between secondary dormancy and seed vigour, germination time or quality traits in annual populations is required before breeding for reduced secondary dormancy commences.

Seed vigour is defined by the "time from sowing to visible radicle emergence" (Ellis. 1992). Seed vigour is largely genetically controlled but also influenced by the maternal environment and cultural practices including harvest techniques and seed storage (Tesnier et al., 2002). Differences in seed vigour are more discernible in stressful environments where faster time to germination classifies a seed lot as more vigourous. The only recognized seed vigour testfor *B. napus* according to the Association of Official Seed Analysts is the controlled deterioration test (Tesnier et al., 2002; AOSA. 2009). The controlled deterioration tests first standardizes seed moisture content to 20% and then accelerates seed ageing under extreme temperature (45^0C) (Tesnier et al., 2002). Physiological damage to the cell wall results in slower germination and lower final germination percentage making the test a good predictor of field emergence (Tesnier et al., 2002). Another seed vigour test commonly used in seed labs in western Canada is the pre-chill germination test which tests germination under cold, saturated, soil-like conditions (Elliot et al., 2007).

Time to germination, a constituent of seed vigour, is an indicator of vigour and faster germinating seed lots are considered more vigourous (Hatzig et al., 2015). Germination time and secondary dormancy are positively correlated in winter annual *B. napus* with higher secondary dormancy genotypes possessing slower germination times; however, this correlation has not been found in annual *B. napus* genotypes (Momoh et al., 2002). Similarly, Hatzig et al. (2015) examined a diverse set of winter annual *B. napus* genotypes and found germination performance, including traits like mean germination time and germination rate after 72 hours, were dependent on the potential of the seed population for dormancy.

An association has been found to exist between secondary dormancy and seed protein (Schatzki et al., 2014). Within the protein fraction of *B. napus* whole seeds, seed storage proteins make up the majority and contribute to the germination of the seed, seedling vigour, dormancy and seed longevity (Muntz et al., 2001; Schatzki et al., 2014; Nguyen et al., 2015). The two dominant SSPs are cruciferin and napin, with cruciferin making up 60% of the SSPs. Both proteins provide nutrients to the germinating seed and seedling before photosynthesis occurs. Cruciferin and napin are negatively correlated and modern varieties have greater levels of cruciferin (Schatzki et al., 2014). It has been observed that as the cruciferin:napin ratio increases, glucosinolate content decreases due to the co-localization of QTLs for napin and glucosinolate content (Schatzki et al., 2014). Furthermore, seed with lower napin content

possesses greater total seed dormancy (primary + secondary dormancy) (Schatzki et al., 2014). As a result, modern canola varieties, with reduced glucosinolates and consequently lower napin content, may possess greater dormancy.

Precocious germination, otherwise known as vivipary or pre-harvest sprouting, is defined as the premature germination of seed while still on the mother plant. It is the result of breeding for rapid germination by selection against primary seed dormancy (Baskin and Baskin. 2001). Primary dormancy is typically low but maximum levels of primary dormancy up to 26% have been documented on a winter annual panel of 229 bi-parental DH lines (Schatzki et al., 2013b). Precocious germination is under genetic control but largely influenced by the environment. Precocious germination occurs during seed maturation and onwards which coincides with endogenous decreases in ABA during seed maturation (Brunel-Muguet et al., 2015). In cereals, higher dormancy genotypes are less likely to encounter pre-harvest sprouting (Feng et al., 2009). *Brassica napus* has a low propensity for primary dormancy and precocious germination but it can be problematic in certain genotypes and environments (Feng et al., 2009). However, it is not known if an association between seed dormancy and precocious germination exists in *B. napus*.

In conclusion, relationships between several seed traits and secondary dormancy in winter annual populations have been identified, however; the same relationships have not been fully investigated in annual *B. napus* thus, the need for further study. In order for breeders to prioritize secondary dormancy as a breeding objective, which would in turn reduce volunteer canola, the relationship between secondary dormancy and seed vigour traits needs to be explored in annual *B. napus*.

4.2 Hypotheses and Objectives

The hypothesis tested in this chapter was, differences in secondary dormancy are related to differences in other seed characteristics. Specifically, high secondary dormancy genotypes would display slower germination speed, weaker seed vigour, higher protein levels, lower occurrence of precocious germination and higher primary dormancy compared to the genotypes with low secondary dormancy.

The objective of this study was to examine possible relationships between seed vigour, germination time, seed quality traits including protein, precocious germination occurrence and secondary dormancy in a diverse collection of annual *B. napus* genotypes.

4.3 Materials and Methods

4.3.1 Experimental Location

The genotypes screened in the following experiments were the parental panel of the annual *B. napus* Nested Association Mapping population made up of 51 annual genotypes from around the world selected based on genotypic and phenotypic diversity (the experimental location and seed production environments were described in section 3.3.1). NAM 0, the reference parent for the recombinant inbred population, was used as the control genotype for the experiments in this chapter because it has low secondary dormancy and desirable seed quality traits.

For the precocious germination experiment, the same collection of genotypes were tested but produced in different independent, replicated trials. The Saskatoon 2016 trial was located adjacent to the Saskatoon 2016 trial described in section 3.3.1 for production of the seed lots used in dormancy and vigour screening. Given the proximity, these trials were comparable for representation within an environment. All four precocious germination environments were within a 30 km radius of Saskatoon, Saskatchewan (52^0N, 106^0W) in the dark-brown soil zone and planted as a paired-row randomized complete block design. The 2016 environments were located at the Agriculture and Agri-Food Canada Main Farm (seeded May 17th, 2016) (52.15^0N, 106.56^0W) located within Saskatoon city limits and the Llewellyn Road Farm (seeded May 30th, 2016) (52.21^0N, 106.45^0W) north-east of Saskatoon. The 2016 environments were approximately 13 km apart. The 2017 environments were at the Main Farm of Agriculture and Agri-Food Canada in Saskatoon and Moon Lake, southwest of Saskatoon (52.03^0N, 106.74^0W). These two environments were approximately 30 km apart.

When a reference to a seed lot is made it is referring to the mean of a specific genotype in a specific environment whereas, genotype refers to the mean of the four seed lots. The four

different seed lots were tested in each of the experiments in this chapter excluding the precocious germination experiment.

4.3.2 Experimental Procedure

4.3.2.1 Evaluation of Seed Vigour Traits

4.3.2.1.1 Experiment 1: Germination under Contrasting Temperatures

Germination was screened at GEVES (Groupe d'Etude et de contrôle des Variétiés et des Semences) in Angers, France. The detailed description of the image capturing and analysis process was described in Demilly et al. (2014). Briefly, this system allows for precise and detailed seed germination data collection through the use of an automated high-throughput image capturing and analysis platform. The seeds were placed on moist blotter paper on top of the saturated capillary paper on a Jacobsen germination table. Two contrasting and differentiating temperature treatments were selected based on a preliminary experiment (data not shown); one at 20^0C to represent optimal germination conditions and the other treatment at 5^0C simulating cold germination conditions common for canola in western Canada in early to mid-May. The germination tests were completed in darkness. Four PROSILICA AVT GC2450C (Allied Vision, Germany) 5-megapixel cameras mounted above the Jacobsen table captured images every two hours. Four technical replicates (same seed lot tested in replicate) of each seed lot with 25 seeds per replicate (total of 100 seeds per seed lot) were tested using this process.

Fiji, an image processing package based in ImageJ (http://fiji.sc/), was used to process the images and determine the number of germinated seeds at each time point. It had been previously shown on this system that no significant difference in mean germination time was found between the technician counts on the Jacobsen table and the computer algorithm for sunflower (*Helianthus annuus* L.) seed germination (Ducournau et al., 2004). To classify a seed as germinated the software required two specific processes to occur: first, the radicle emergence had to be detected and second was seed movement, however, the order of the detection did not matter (Ducournau et al., 2005). The images were processed by removing the background from the image followed by conversion to black and white. Next, virtual grids were drawn around each seed to separate into individual squares and the two-step germination verification process

was performed. Once the seed was classified as germinated it was removed from the virtual grid and the hour of germination was recorded and exported to a spreadsheet. This classification process was completely automatic but image verification by technical observation was also implemented to validate the accuracy of the computer system at regular intervals. For the purpose of this study, time to 50% germination was the desired parameter because it has been shown to be a good indicator of seed vigour (Hatzig et al., 2015).

4.3.2.1.2 Experiment 2: Controlled Deterioration Test (CDT)

A minimum of 200 seeds were tested in four technical replications for each seed lot in a nested design with technical replicates nested within the environment. The nesting of replicates within the environment was required due to logistics which prohibited randomizing individual technical replicates. First, the initial seed moisture content was determined by weighing out a 5 g sample of seed and recording the weight. Each seed lot was placed in separate aluminium plates and covered tightly with aluminium foil, then placed in a Fisher IsoTemp 300 series oven at 130^0C for 4 hours. The plates were then removed from the oven and quickly weighed. The seed moisture content, or the percentage of dry weight, was determined by (AOSA. 2009),

{(initial seed weight - dry seed weight) / (initial seed weight)}……...(4.1)

Next, the desired moisture content of the seed lots was raised to 20% of the dry weight. This was done by applying the following formula to 300 seeds (weighed to get a new initial seed weight) to get the adjusted seed moisture content in grams,

{(100 – moisture content) / (100 - desired moisture content; 20%) x (new initial seed weight of 300 seeds)}...……..(4.2)

The amount of distilled water to add to the seed sample was calculated by,

(adjusted moisture content – new initial seed weight)……...(4.3)

The adjusted moisture content seed lots were sealed air tight in foil pouches (MarvelSeal 360, Protective Packaging Corporation, Carrollton, TX) using an impulse sealer which melted the two pieces of the bag together. The bag was then shaken vigorously for 30 seconds and laid down flat on a countertop, each pouch was shaken for 30 seconds every 30 minutes for the next four hours to ensure even water distribution throughout the seeds. As stated above, all four technical replicates were tested in unison because the moisture adjustment was made for the seed lot for an environment all at once to ensure even moisture levels. All the technical replicates for a given environment were completely randomized on the thermal gradient plate at the Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada. The thermal gradient bench is a 97-cell unit with the capability of maintaining extremely tightly controlled temperature ranges of within 0.1^0C for each individual cell (Figure 4.1) (McLaughlin et al., 1985). Each cell was covered in a thick concave glass lid to help regulate the temperature while still allowing light from the florescent light bars mounted above to pass through. Each individual cell fit up to four petri dishes.

Following the moisture adjustment, the seeds were equilibrated for 24 hours at 10^0C then subjected to the deterioration treatment for 24 hours at 45^0C on the thermal gradient plate (AOSA. 2009). Following the deterioration, for each seed lot, the pouches were opened and fifty seeds were counted for each technical replicate. The seeds were then transferred to a petri dish moistened with 9 mL of distilled water and moved to the thermal gradient plate for 7 days with a temperature of 20^0C and 16 hours light/ 8 hours darkness. Petri dishes were rotated daily to account for any slight temperature gradients that might exist within the cell. The germination counts were performed on day seven. The controlled deterioration germination was calculated as,

(number of germinated seeds/ total number of viable seeds per replicate)……..…….(4.4)

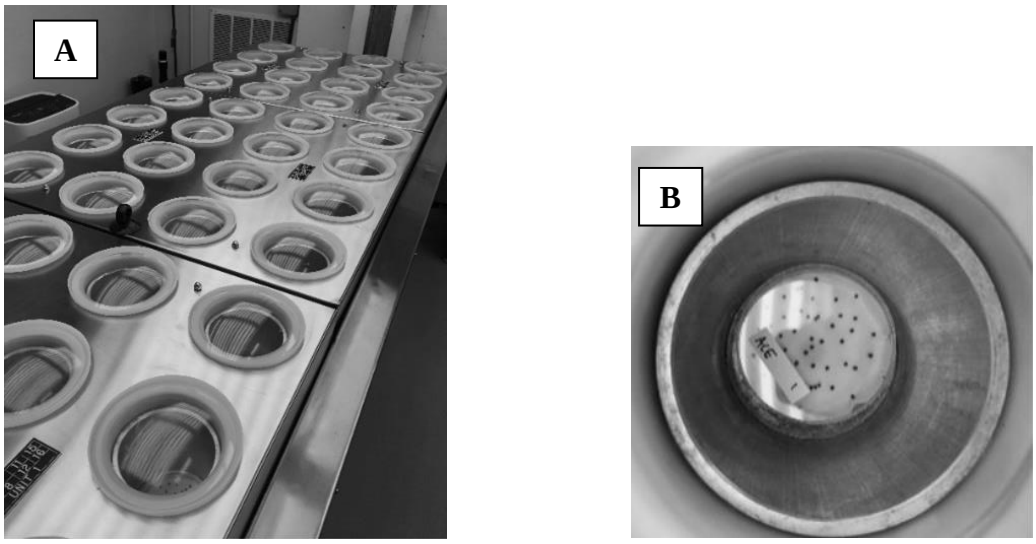

FIGURE 4.1: A) THE THERMAL GRADIENT PLATE AT THE SASKATOON RESEARCH AND DEVELOPMENT CENTRE, AGRICULTURE AND AGRI-FOOD CANADA. B) AN INDIVIDUAL CELL ON THE THERMAL GRADIENT PLATE.

4.3.2.1.3 Experiment 3: Pre-chill Germination Test (PCT)

The pre-chill germination test assesses seed vigour under saturated soil-like conditions at low temperature. A split-plot randomized complete block design was used with the environment as the whole plot factor and the genotypes as the sub-plot factor. The protocol followed Elliot et al. (2007) and was conducted in a Conviron Adaptis (Winnipeg, Canada) growth cabinet. Approximately 70-80 g of growth mix [3 parts Sunshine #4 (Agawam, MA): one-part sand] was measured out and placed in a clear germination box (11.5 cm x 11.5 cm; Hoffman Manufacturing Inc., Oregon). The mix was saturated with 20 mL of distilled water and mixed until saturated, followed by compression creating a flat, even surface. Fifty seeds were then placed on top of the mix followed by an additional layer of growth mix on top of the seeds which was re-compressed to create a flat surface to ensure all seeds had contact with the mix and moisture. The temperature for the first 7 days was 5^0C in complete darkness, followed by raising the temperature regime for the next 5 days to 25^0C/15^0C for 8 hours light/16 hours darkness. Germination was visually assessed as seedling emergence on day 12 after the temperature was raised. The pre-chill germination for each seed lot was calculated as,

(total number of seeds germinated/ total number of seeds)……...(4.5)

4.3.2.2 Evaluation of Primary Dormancy and Precocious Germination

4.3.2.2.1 Experiment 4: Primary Dormancy Screening

The primary dormancy screening protocol consisted of a germination test with a minimum of 200 seeds per genotype tested in two technical replications (AOSA. 2009). The temperature regime was 20^0C in complete darkness for 7 days in a Conviron Adaptis growth cabinet (Winnipeg, Canada) (Haile and Shirtliffe. 2014). A split-plot randomized complete block design was used with environment as the main plot factor and the genotype as the sub-plot factor. Seeds not germinated after 7 days and with no signs of mould (viable) were classified as displaying primary dormancy. The primary dormancy was calculated as,

(number of un-germinated seeds/ total number of viable seeds)……...(4.6)

4.3.2.2.2 Experiment 5: Precocious Germination Occurrence Screening

Equivalent sources of seed for each of the genotypes were used for seeding the precocious germination field trials as was used for all the other experiments (refer to section 3.3.1). Agronomic notes included, total emergence, days to flower and days to maturity were recorded within all trials. The trials were set up as a randomized complete block design with four replications and within each experimental paired-row plot, three random plants were sampled for the main raceme at maturity (>80% seed colour change). The samples were placed in brown paper bags and dried at 40^0C for 72 hours. Following drying, five pods per raceme were removed, opened up and the germinated and total seeds were counted (~200 seeds/plant). Precociously germinated seeds were counted as seeds with the radical or cotyledons protruded from the seed coat. The data was recorded as,

(precocious germinated seed/ total seeds examined)…….(4.7)

4.3.2.3 Evaluation of Seed Quality Traits

Routine seed quality screening was performed on the seed lots from each environment. Seed traits quantified included total protein, total oil, glucosinolate content, acid detergent lignin, whiteness index, seed size and thousand kernel weight. The protein content, measured by nitrogen content, was determined using the Dumas method on a LECO (Saint Joseph, MI) machine. The seed lot samples were burned at high temperature in the presence of oxygen gas resulted in carbon dioxide, water and nitrogen. Water and carbon dioxide was filtered out and the nitrogen was turned into pure nitrogen gas when pushed through a combustion chamber containing copper. The resulting nitrogen gas was quantified on a dry seed matter basis presented as a percentage, N x 6.25 (Jones. 1941). Seed oil content (% dry weight basis) was quantified using nuclear magnetic resonance, which uses magnetic frequencies to vibrate the hydrogen atoms within the seed. From the vibrations, the oil content can be determined using pre-determined calibrations from seed oil extraction using hexane (Raney et al., 1999).

Near-infrared reflectance spectroscopy (NIRs) was used to estimate the glucosinolate content (µmol g^{-1}), acid detergent lignin (% dry weight basis) and whiteness index (a proxy for seed coat colour) (Foss NIR Systems 6500 Series). The NIR machine works by radiating light in the near infrared region of the electromagnetic spectrum (780-2500 nm) on the seed sample. The quantification of quality properties in the sample of interest is determined by measuring the reflectance of light from the sample and then referencing that value to predetermined sample reflectance values for a specified content of (oil, protein, etc.). Glucosinolate content calibrations were developed based on gas chromatography (Theis. 1977; Heaney and Fenwick. 1980). An Ankom 2000 fibre analyzer (A2000, Macedon, NY, USA) was used to determine acid detergent lignin values used for calibrating the NIR and whiteness index was based on L*a*b colour index (CIE. 1976) readings.

Seed size was determined using a series of eight sieves ranging in diameter from 1.2-2.6 mm secured to a mechanical shaker (Haver & Boecker Seed Shaker Model: RX-812). Approximately 8-10 g of seed was poured onto the smallest sieve and shaken at 231 oscillations per minute for two minutes. After such time, the number of seeds in each sieve was counted and weighed resulting in a distribution of seed diameter for each seed lot. Lastly, thousand kernel weight was determined by weighing 1000 seeds in three replicates from each seed lot.

4.4 Statistical Analysis

4.4.1 Time to 50% Germination Analysis

The germination data was analyzed using the NONLIN procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) as non-linear regression is a common way to analyze germination data because no linear relationship exists. Using a three parameter logistic regression, time to 50% germination was determined. The logistic regression equation was,

Total germination= M/(1 + exponent(a*(-hour +b)))…….(4.8)

M was the final germination percent; a was the slope; b was time to 50% germination.

The genotype, environment and the genotype x environment interactions were considered fixed, while the random effects were the technical replicates and environment*technical replicates interactions. To determine the percent contribution of each fixed effect, the VARCOMP procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used. The type III sum of squares for the effect divided by the corrected total sum of squares was used to determine the percentage value each effect contributed to the phenotype (Gulden et al., 2004a; Botwright-Acuña and Wade. 2012).

4.4.2 Controlled Deterioration, Pre-chill Germination, Precocious Germination and Primary Dormancy Analyses

The primary dormancy, controlled deterioration, pre-chill germination and precocious germination data were all found to not conform to normality (non-Gaussian) using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) and referencing the Shapiro-Wilk W-value (P-value < 0.05). Thus, the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used to analyze the data. The data were count data, (germinated seeds/total viable seeds) with only two possible outcomes and values between 0-1, supporting the use of the beta distribution (Bowley. 2015). Values of 0 and 1 were changed to 0.001 and 0.999, respectively because the beta distribution omits those values. The complimentary log-link function was used to transform the least square mean estimates back to the data scale initially used presenting the estimates as means (proportion). The Laplace method assisted in data convergence as well as stabilizing over-dispersion (Pearson chi-square/degree of

freedom = $0 < x < 2$) (Bowley. 2015). Data transformation (arcsine transformed) was not used to generate means because sufficient examples in the literature provided reasoning that the GLIMMIX procedure was a more effective way to deal with non-Gaussian germination data. (Willenborg et al., 2005).

For analysis, the genotype, environment and the genotype x environment interactions were considered fixed while the random effects were the technical replicates and environment*technical replicates interactions. Exceptions for random effects was the CDT test where the technical replicates were nested within the environment as well as the precocious germination experiment where the blocks were nested within the environment to compare multiple site years. To balance the data sets, seed lots with missing seed or values were not included. To determine the percent contribution of each fixed effect, the VARCOMP procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used. The type III sum of squares for the effect was divided by the corrected total sum of squares was to determine the percentage value each effect contributed to the phenotype (Gulden et al., 2004a; Botwright-Acuña and Wade. 2012).

4.4.3 Correlation and Partial Least Square Regression Analysis

To determine the association among variables, a correlation analysis was performed using the CORR procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The Spearman rank function accounted for the non-Gaussian distribution. The precocious germination results were not included in the correlation analysis or partial least squares analysis because different seed lots were tested. A separate Spearman correlation was performed for secondary dormancy and precocious germination in Saskatoon 2016 because the fields with the two trials were adjacent to each other.

The partial least squares analysis is a multiple linear regression analysis and was performed using the SIMPLS procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). To determine the number of significant factors in the model the 'CV= one' function was used. Variables used in the partial least squares model to explain secondary dormancy included primary dormancy, controlled deterioration germination, pre-chill germination, germination time and 5^0C and 20^0C, thousand kernel weight, mode seed size, total protein, total oil, total glucosinolates, acid detergent lignin, whiteness index and geographical origin of the genotypes.

4.5 Results

4.5.1 Seed Vigour Traits

4.5.1.1 Experiment 1: Time to 50% Germination of a Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

A large range in time to 50% germination (T50) was observed across the genotypes and temperatures with a minimum of 26 and maximum of 43 hours at 20^0C and a minimum of 113 and a maximum of 183 hours at 5^0C (Table 4.2). The average germination time at 20^0C was 33 hours compared to 163 hours, or 4.5 times longer at 5^0C. The genotype and environment effects alone were both highly significant for time to 50% germination (*P*- value <0.001) (Table 4.1). Highly significant genotype x environment interactions (*P*-value <0.001) for T50 at both temperatures was also found (Table 4.1). At 5^0C, the genotype by environment interactions was slightly larger based on the percent sum of squares (Table 4.1), suggesting that the phenotypes exhibited under stressful conditions were more affected by the interaction between the genotype and environment. Although highly significant, the proportion of variation of the environment effect alone at both temperatures was small in terms of T50 based on the percent sum of squares. Substantial residual variance was not explained by the fixed effects for germination at either temperature. Approximately half of the variance for T50 at 5^0C was not explained but the fixed effects and around one-third of the variance not explained for T50 at 20^0C.

In regard to the individual environments screened, seed produced in Saskatoon in 2015 displayed the lowest T50 overall. Saskatoon in 2016 and Temuco were not statistically different and displayed the highest T50 at 5^0C (Table 4.2). Furthermore, the two Saskatoon production environments (2015 and 2016) were weakly correlated while none of the other environments were found to be correlated at 5^0C (Table A.1). Similarly, at 20^0C differences in overall T50 were observed between the seed production environments with Saskatoon 2015 again displaying quicker germination. Saskatoon 2016 and Los Ángeles produced seed were not different from each other in terms of germination time and displayed moderate germination time. Temuco exhibited the slowest overall germination time of all the environments (Table 4.2). At 20^0C, germination of seed produced in all the environments were moderately significantly correlated to each other with the exception of Saskatoon 2015 and Temuco, the fastest and slowest

germination environments (Table A.1). Final germination percentages from the data generated at GEVES were high for all seed production environments at 20^0C (data not shown). Saskatoon 2015, Los Ángeles and Saskatoon 2016 had 99% germination overall with specific seed lots ranging from 83-100% germination. The final germination percentage for Temuco was 98% with seed lots ranging from 88-100% final germination.

TABLE 4.1: ANOVA TABLE FOR T50 AT 5^0C, T50 AT 20^0C, CONTROLLED DETERIORATION TEST, PRE-CHILL TEST, PRIMARY DORMANCY AND PRECOCIOUS GERMINATION FOR A DIVERSE COLLECTION OF ANNUAL *BRASSICA NAPUS* GENOTYPES.

	T50 @ 5^0C			T50 @ 20^0C			Controlled Deterioration Test		
Effect	**F-value**	***P*-Value**	**%**	**F-value**	***P*-Value**	**%**	**F-value**	***P*-Value**	**%**
Geno	3.1	<0.001	16	11	<0.001	30	16	<0.001	20
Enviro	15	<0.001	6	60	<0.001	15	250	<0.001	22
G*E	2.1	<0.001	28	2.8	<0.001	22	10	<0.001	46
Residual			50			33			12

	Pre-Chill Test			Primary Dormancy			Precocious Germination		
Effect	**F-value**	***P*-Value**	**%**	**F-value**	***P*-Value**	**%**	**F-value**	***P*-Value**	**%**
Geno	8.2	<0.001	29	2.1	0.003	18	8.6	<0.001	21
Enviro	33	<0.001	6	2.4	0.24	6	2.6	0.11	1
G*E	4.3	<0.001	38	2.4	<0.001	54	1.3	0.04	18
Residual			27			22			60

Note: G*E- Genotype by environment interactions. The percent contribution (%) was determined by dividing the 'effect' sum of squares by the total sum of squares.

TABLE 4.2: MEAN TIME TO 50% GERMINATION AT 20^0C AND 5^0C OF SEED LOTS OF EACH GENOTYPE GROWN AT SASKATOON 2015 & 2016, LOS ÁNGELES 2016 AND TEMUCO 2016.

				20^0C				5^0C			
				SK 2015	SK 2016	LA 2016	TEM 2016	SK 2015	SK 2016	LA 2016	TEM 2016
NAM Founder	2^o Dorm Class	Mean T50 @ 20^0C	Mean T50 @ 5^0C	Mean (hours)							
Legend	High germ time					Avg germ time					Low germ time
NAM30	High	40.0+	163	36.3+	33.1	42.0+	48.7+	164	.	162	.
NAM42	High	38.9+	162	35.9+	41.8+	37.0	40.8+	150	187	158	154
NAM46	High	25.8	140	.	23.9	29.5	24.1	.	143	149	126
NAM49	High	35.1+	167	33.9	31.8	32.7	42.1+	160	174	166	.
NAM85	High	.	.	.	.	.	.	.	.	.	.
AC Excel	Mid	33.5+	173	32.6	39.3	32.6	29.4	181	171	172	168
NAM12	Mid	34.0+	185	32.2	30.3	37.3	36.3+	171	182	173	217+
NAM17	Mid	30.9	171	26.9	30.6	35.3	30.7	150	188	166	180+
NAM23	Mid	31.5	168	28.4	32.3	30.0	35.4+	149	177	172	173+
NAM28	Mid	29.0	154	27.2	29.8	30.5	28.6	146	163	150	156
NAM29	Mid	.	.	.	.	.	.	.	.	.	.
NAM31	Mid	32.3	137	29.7	28.5	35.6	35.2+	123-	145	168	110-
NAM33	Mid	33.6	162	.	30.4	35.1	35.2	.	165	161	161
NAM37	Mid	33.7+	154	29.4	36.5	29.5	39.5+	152	169	137	159

NAM40	Mid	29.3	158	26.6	27.7	29.8	33.3+	149	151	163	171+
NAM56	Mid	31.9	162	28.0	33.0	29.7	36.9+	134	176	155	182+
NAM65	Mid	32.7	180	33.3	33.2	27.5-	36.7+	159	197	172	191+
NAM71	Mid	36.2	167	25.7	36.3	.	46.6	164	168	.	171+
NAM73	Mid	35.0+	170	28.9	33.3	31.2	46.4+	152	192	170	168+
NAM8	Mid	43.4+	183	37.9+	36.8	46.4+	52.4+	176	189	194	174+
NAM83	Mid	31.1	156	28.6	29.4	30.8	35.6+	117-	184	169	154
NAM0	Low	29.9	151	28.5	30.9	35.3	25.0	165	.	153	135
NAM10	Low	30.7	149	28.3	31.2	.	32.7	141	178	.	128-
NAM13	Low	33.7+	154	29.1	36.9+	34.2	34.4+	145	161	155	152
NAM14	Low	26.4-	146	21.8-	24.8-	24.5-	34.7+	128-	174	149	133-
NAM15	Low	27.9	161	26.4	30.0	26.6-	28.8	164	162	168	149
NAM25	Low	36.9+	189	35.0+	31.5	40.3	40.7+	195	217	156	.
NAM26	Low	34.2+	172	26.3	34.7	42.5+	33.1+	187	180	128	191+
NAM32	Low	34.0+	149	30.4	32.7	35.6	37.2+	137	156	161	140
NAM38	Low	32.8	142	31.6	33.1	29.6	37.0+	154	140	146	130-
NAM39	Low	33.2+	189	28.1	32.2	32.5	40.2+	175	180	201+	200+
NAM4	Low	39.4+	171	28.1	43.1+	37.0	49.5+	135	198	180	170+
NAM43	Low	36.9+	155	30.7	32.9	44.3+	39.7+	160	147	163	149
NAM48	Low	27.7	155	25.8	28.4	26.5-	30.3	157	144	141	180+
NAM5	Low	35.3+	166	30.1	32.2	41.0	37.8+	166	189	144	165-
NAM50	Low	25.9-	134	26.3	25.8	25.9-	25.8	128-	142	139	127
NAM51	Low	30.5	160	31.3	31.0	31.7	28.0	175	190	152	124

NAM57	Low	29.4	156	30.2	26.5	25.3-	35.7+	147	153	139	186+
NAM68	Low	28.4	169	24.7	29.8	27.7-	31.5+	150	149	187+	189+
NAM72	Low	30.6	173	26.0	32.9	27.5-	36.1+	148	190	168	187+
NAM75	Low	37.5+	177	34.9+	38.0+	38.1	38.8+	161	194	165	189+
NAM76	Low	31.1	158	26.5	32.7	31.0	34.3+	144	177	169	144
NAM78	Low	33.8+	167	27.2	32.3	36.0	39.7+	159	173	158	177+
NAM82	Low	43.1+	164	28.2	51.5+	42.6+	50.2+	140	182	170	.
NAM87	Low	35.3	180	32.9	40.5	.	32.6	160	195	.	183+
Quantum	Low	30.3	169	31.8	31.5	27.5-	30.6	179	.	167	161
	Mean	33	163	29.6 A	32.9 B	33.4 B	36.6 C	152 A	170 C	161 B	167 C

Note: Genotypes were ranked within environment then colour coded. The darker shades of red and blue represent the extremes with red (less favourable outcome) identifying genotypes with greater time to 50% germination and blue (favourable outcome) identifying genotypes with faster time to 50% germination. Means are LSmeans. "-" indicates significantly less than NAM 0 at $P < 0.05$. "+" indicates significantly greater than NAM 0 at $P < 0.05$. Different letters in the mean row at the bottom of the chart denote significant difference at $P < 0.05$. NAM 0 was not screened at 5^0C in Saskatoon 2016, therefore, the comparison could not be made for that environment or for the mean T50 @ 5^0C. SK15- Saskatoon 2015; SK16- Saskatoon 2016; LA- Los Ángeles 2016; Tem- Temuco 2016.

4.5.1.2 Experiment 2: Controlled Deterioration Germination (CDT) of a Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

The genotype, environment and genotype x environment interactions were found to significantly affect germination in the CDT test (*P*-value < 0.001). The genotype x environment interactions contributed twice as much variation compared to either the genotype or environment alone which were very similar (Table 4.1). The CDT tested the vigour of the seed lot by determining the germination after being subjected to high heat. Across the genotypes screened, a small range in germination existed (average values ranging from 80.4 to 99.0%), but in general, most genotypes had high germination after being stressed at 45^0C (Table 4.3).

The environment that produced seed with the lowest CDT germination and thus vigour was Temuco and was congruent to what was observed in the germination time experiment at 20^0C. Saskatoon 2015 and Los Ángeles had similar CDT germination while Saskatoon 2016 had significantly higher germination than the other environments. The two Saskatoon (2015 and 2016) environments were significantly correlated, similar to what was observed in the germination time experiments under both temperatures, while none of the other environments was correlated (Table A.2).

TABLE 4.3: MEAN CONTROLLED DETERIORATION GERMINATION OF SEED LOTS OF EACH GENOTYPE GROWN AT SASKATOON 2015 & 2016, LOS ÁNGELES 2016 AND TEMUCO 2016.

NAM Founder	2° Dorm Class	Average CDT Germ (%)	Saskatoon 2015	Saskatoon 2016	Los Ángeles 2016	Temuco 2016
			Mean (%)			
Legend	**High vigour**		**Average vigour**			**Low vigour**
NAM30	High	98.5	98.0	98.6	98.9+	98.5
NAM42	High	94.1-	97.9	98.9	94.1	72.8-
NAM46	High	98.2	99.2	99.2	99.2+	90.2-
NAM49	High	98.0	98.6	98.6	98.6+	95.0-
NAM85	High	.	.	.	.	.
AC Excel	Mid	97.5	98.6	99.2	98.5+	88.9-
NAM8	Mid	96.9	98.9	99.2	88.6	95.4-
NAM12	Mid	97.4	98.9	98.9	97.4+	90.7-
NAM17	Mid	97.3	98.6	99.2	86.1	98.6
NAM23	Mid	97.9	97.7	99.2	95.1	98.4
NAM28	Mid	98.1	97.9	96.5-	99.2+	98.0
NAM29	Mid	.	.	.	.	.
NAM31	Mid	97.0	95.9	99.2	98.6+	90.6-
NAM33	Mid	98.8+	97.8	99.2	98.9+	99.2
NAM37	Mid	96.6	98.3	98.9	98.9+	79.4-
NAM40	Mid	97.9	98.5	98.9	94.9	98.3
NAM56	Mid	97.7	98.6	98.0	93.5	98.9
NAM65	Mid	99.0+	98.8	99.2	98.9+	99.0
NAM71	Mid	97.5	98.5	98.9	98.5+	90.4-
NAM73	Mid	93.1-	97.7	99.2	97.8+	52.8-
NAM83	Mid	94.0-	98.9	92.3-	99.2+	67.2-
NAM0	Low	97.8	98.6	98.9	89.9	99.2
NAM4	Low	95.9-	98.9	98.2	97.8+	78.3-
NAM5	Low	97.5	99.2	98.6	97.8+	90.6-

NAM10	Low	.	.	.	.	.
NAM13	Low	97.0	97.2	99.2	96.8+	92.3-
NAM14	Low	98.8+	98.9	99.2	99.2+	97.3
NAM15	Low	96.1-	87.9-	98.9	98.1+	94.6-
NAM25	Low	99.0+	98.9	98.9	99.2+	98.9
NAM26	Low	93.6-	98.9	99.2	95.1	58.4-
NAM32	Low	90.0-	93.9+	98.5	97.1+	49.4-
NAM38	Low	92.6-	98.2	98.4	91.2	67.8-
NAM39	Low	97.9	98.2	99.2	99.2+	90.9-
NAM43	Low	98.8+	97.8	99.2	99.0+	99.1
NAM48	Low	92.7-	91.9+	89.6-	98.3+	86.6-
NAM50	Low	97.7	98.9	99.2	87.0	98.9
NAM51	Low	97.7	98.9	99.2	98.3+	89.3-
NAM57	Low	95.8-	98.9	98.9	97.2+	75.0-
NAM68	Low	97.2	87.4-	98.6	98.2+	98.9
NAM72	Low	80.4-	60.7-	93.1-	88.6	73.0-
NAM75	Low	94.8-	98.9	98.9	95.1	70.9-
NAM76	Low	98.1	99.2	98.5	98.9+	94.0-
NAM78	Low	94.1-	98.6	99.2	98.3+	53.3-
NAM82	Low	98.1	98.9	99.2	98.9+	92.0-
NAM87	Low	98.2	98.5	98.9	98.9+	95.1-
Quantum	Low	98.4	98.4	98.6	97.5+	98.8
	Mean	97.7	97.9 B	98.7 A	97.4 B	91.1 C

Note: Genotypes were ranked within environment then colour coded. Shades of red (less favourable outcome) represent lower germination and shades of blue (favourable outcome) signify higher germination. Means are LSmeans. "-" indicates significantly less than NAM 0 at $P < 0.05$. "+" indicates significantly greater than NAM 0 at $P < 0.05$. Different letters in the mean row at the bottom on the chart denote significant difference at $P < 0.05$.

4.5.1.3 Experiment 3: Pre-chill Germination Test (PCT) Germination for a Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

In the PCT, similar to the CDT, the higher the germination value, the better the vigour of the seed lot but for the PCT test, the assay simulated the seed lot's germination under cold, saturated moisture conditions. A wider range in PCT germination was observed compared to the CDT test with average values ranging from 67-97% making differences in vigour more apparent (Table 4.4). The genotype, environment and the interactions were all highly significant effects for the germination in the PCT test (Table 4.1). Similar to the CDT, the genotype x environment interaction was the largest contributor to variability to PCT germination (Table 4.1). The results in the PCT differ from the CDT as the magnitude of genotype x environment interactions and environmental variation was lower in the PCT.

Similarly to the previous seed vigour and germination time experiments, Temuco again had significantly lower germination and therefore, vigour. Saskatoon 2016 and Los Ángeles were similar in terms of germination and had the highest germination percentages (Table 4.4). Seed lots produced in all environments were significantly correlated with the exception of Saskatoon 2015 and Temuco and Los Ángeles and Temuco (Table A.3). The moderate correlation between seed lots from the two Saskatoon environments was consistent across the different experiments.

TABLE 4.4: MEAN PRE-CHILL GERMINATION OF SEED LOTS OF EACH GENOTYPE GROWN AT SASKATOON 2015 &2016, LOS ÁNGELES 2016 AND TEMUCO 2016.

			Saskatoon 2015	Saskatoon 2016	Los Ángeles 2016	Temuco 2016
NAM Founder	2^{O} Dorm Class	PCT Average Germ (%)	Mean (%)			
Legend	High vigour		Average vigour			Low vigour
NAM30	High	94.5	92.1	93.1	97.0	95.0
NAM42	High	90.1	89.6	92.9	94.2	81.2-
NAM46	High	96.0	99.2	96.9	91.3	92.9
NAM49	High	89.7	92.9	79.8	98.9+	75.3-
NAM85	High	.	.	.	.	.
AC Excel	Mid	90.1	88.0-	95.4	94.2	78.3-
NAM8	Mid	84.8-	89.0	92.0	79.9	75.7-
NAM12	Mid	93.2	96.8	90.0	92.9	91.9
NAM17	Mid	94.7	91.3	96.1	92.1	97.5
NAM23	Mid	95.2	88.0-	93.9	98.8+	96.0
NAM28	Mid	91.7	84.3-	96.0	93.1	91.1
NAM29	Mid	.	.	.	.	.
NAM31	Mid	93.5	75.3-	98.2+	95.8	95.6
NAM33	Mid	89.1	74.0-	91.3	97.3	86.8
NAM37	Mid	96.5+	96.8	98.9+	94.5	94.2
NAM40	Mid	91.0	93.8	91.9	88.6	89.2
NAM56	Mid	90.6	80.6-	97.0	87.0	92.9
NAM65	Mid	93.4	97.7	90.8	95.0	87.1
NAM71	Mid	92.0	96.3	97.0	91.1	76.6
NAM73	Mid	90.3	91.7	89.0	96.8	79.5-
NAM83	Mid	88.7	91.8	93.9	97.0	60.0-
NAM0	Low	93.2	96.1	90.8	91.1	93.8
NAM4	Low	92.5	96.7	92.9	96.4	77.3-

NAM5	Low	92.9	88.3	92.3	97.3	91.3
NAM10	Low	76.6-	73.4-	88.4	69.3-	73.2-
NAM13	Low	90.4	95.0	93.3	91.8	77.4-
NAM14	Low	90.5	70.3-	97.0	97.0	86.9
NAM15	Low	91.0	71.9-	93.9	96.8	93.8
NAM25	Low	95.8	90.0	95.0	98.8+	96.4
NAM26	Low	92.5	92.9	97.7+	87.2	88.6
NAM32	Low	90.3	92.3	96.3	93.9	71.7-
NAM38	Low	90.9	98.9	79.7	91.7	84.3
NAM39	Low	96.9+	95.8	98.9+	98.8+	90.0
NAM43	Low	94.1	88.6	90.6	98.9+	93.6
NAM48	Low	67.4-	50.8-	70.3-	78.6-	69.3-
NAM50	Low	93.9	96.3	89.2	84.8	98.8+
NAM51	Low	85.9-	95.8	74.3-	87.7	80.6-
NAM57	Low	89.7	98.9	91.1	82.3	75.7-
NAM68	Low	85.3-	78.2-	85.6	86.3	89.9
NAM72	Low	71.9-	53.0-	76.8-	78.2-	78.6-
NAM75	Low	92.7	93.6	97.0	96.5	76.6-
NAM76	Low	87.2-	89.1	78.8-	95.0	82.3-
NAM78	Low	89.5	95.6	90.3	93.9	71.7-
NAM82	Low	93.5	89.1	98.8+	90.6	91.3
NAM87	Low	91.3	91.8	95.0	94.2	81.2-
Quantum	Low	91.1	98.9	81.2	87.2	89.2
	Mean	92	90.7 B	92.6 A	93.2 A	86.5 C

Note: Genotypes were ranked within environment then colour coded. Shades of red (less favourable outcome) represent lower germination and shades of blue (favourable outcome) signify higher germination. Genotypes are organized according to secondary dormancy. Means are LSmeans. "-" indicates significantly less than NAM 0 at $P < 0.05$. "+" indicates significantly greater than NAM 0 at $P < 0.05$. Different letters in the mean row at the bottom of the chart denote significant difference at $P < 0.05$.

4.5.2 Primary Dormancy and Precocious Germination Occurrence

4.5.2.1 Experiment 5: Primary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

Primary dormancy had low occurrence among the genotypes assessed ranging from less than 1% to 3% (Table 4.5). The genotype and the genotype x environment interactions were both significant with *P*-values of 0.004 and <0.001, respectively. The interactions contributed over half of the variability in primary dormancy with genotype also contributing to the differences observed but to a lesser extent (Table 4.1).

TABLE 4.5: MEAN PRIMARY DORMANCY OF SEED LOTS FOR EACH GENOTYPE GROWN AT SASKATOON 2015 & 2016, LOS ÁNGELES 2016 AND TEMUCO 2016.

			Saskatoon 2015	Saskatoon 2016	Los Ángeles 2016	Temuco 2016
NAM Founder	2O Dormancy	Primary Dormancy Average (%)	Mean (%)			
Legend	High Primary Dormancy		Average Primary Dormancy			Low Primary Dormancy
NAM30	High	0.91	0.53	1.14	1.23	0.94
NAM42	High	1.49	1.17	0.87	3.59	1.36
NAM46	High	0.80	0.83	0.87	0.42	1.36
NAM49	High	0.68	0.53	0.53	0.42	1.74
NAM85	High	2.55+	0.53	0.87	5.65+	15.32+
AC Excel	Mid	0.79	0.68	1.01	0.42	1.36
NAM12	Mid	0.50	0.53	0.53	0.42	0.54
NAM17	Mid	0.50	0.53	0.53	0.42	0.54
NAM23	Mid	0.50	0.53	0.53	0.42	0.54
NAM28	Mid	0.86	0.53	0.53	1.23	1.56
NAM29	Mid	.	.	.	.	.
NAM31	Mid	0.93	0.53	6.15+	0.42	0.54
NAM33	Mid	0.74	1.17	1.14	0.42	0.54
NAM37	Mid	1.55	0.53	2.99	0.42	8.40+
NAM40	Mid	0.86	0.53	0.53	1.78	1.11
NAM56	Mid	0.74	0.83	0.53	1.23	0.54
NAM65	Mid	.	.	.	.	.
NAM71	Mid	.	.	.	.	.
NAM73	Mid	.	.	.	.	.
NAM8	Mid	1.91+	4.29+	0.53	4.01	1.43
NAM83	Mid	.	.	.	.	.
NAM0	Low	0.63	0.53	0.45	1.23	0.54

NAM10	Low	.	.	.	.	.
NAM13	Low	0.58	0.53	0.53	0.42	0.94
NAM14	Low	.	.	.	.	.
NAM15	Low	0.50	0.53	0.53	0.42	0.54
NAM25	Low	.	.	.	.	.
NAM26	Low	1.03	0.53	0.53	0.42	9.29+
NAM32	Low	0.97	2.86+	0.53	0.42	1.36
NAM38	Low	.	.	.	.	.
NAM39	Low	0.60	0.53	0.53	0.42	1.09
NAM4	Low	0.63	0.53	0.53	0.42	1.36
NAM43	Low	0.58	0.53	0.53	0.42	0.94
NAM48	Low	1.57+	0.53	0.53	0.42	.
NAM5	Low	0.66	0.53	0.53	1.23	0.54
NAM50	Low	0.84	0.95	0.53	1.78	0.54
NAM51	Low	0.94	0.53	0.53	1.07	2.60
NAM57	Low	1.94+	0.53	0.53	1.78	25.17+
NAM68	Low	0.82	2.02	0.87	0.42	0.60
NAM72	Low	1.10	0.53	0.53	1.78	2.89
NAM75	Low	.	.	.	.	.
NAM76	Low	.	.	.	.	.
NAM78	Low	.	.	.	.	.
NAM82	Low	.	.	.	.	.
NAM87	Low	0.64	0.53	0.53	1.07	0.54
Quantum	Low	0.64	0.68	0.53	0.42	1.09
	Mean	0.94	0.69 A	0.68 A	0.76 A	1.45 A

Note: Genotypes were ranked within environment then colour coded. Shades of red (less favourable outcome) represent lower germination and shades of blue (favourable outcome) signify higher germination. Genotypes are organized according to secondary dormancy. Means are LSmeans. "-" indicates significantly less than NAM 0 at $P < 0.05$. "+" indicates significantly greater than NAM 0 at $P < 0.05$. Different letters in the mean row at the bottom of the chart denote significant difference at $P < 0.05$.

4.5.2.2 Experiment 5: Precocious Germination Occurrence in the Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

Overall, the precocious germination observed was low with percentages ranging from 0-4% when averaged across environments (Table 4.6). The genotype and the genotype x environment interactions were significant while the environment effect alone was not (Table 4.1). The genotypic effect as well as the genotype by environment interactions contributed similar variability with both contributing about one quarter of the variability in precocious germination observed. Occurrence was highest in 2017, possibly due to late season precipitation creating moist conditions ideal for precocious germination in early September as the pods were maturing. Of the locations screened, Llewellyn 2016 had the lowest precocious germination occurrence of all the environments and Saskatoon 2017 had the greatest.

TABLE 4.6: MEAN PRECOCIOUS GERMINATION (PG) OCCURRENCE OF SEED LOTS FOR EACH GENOTYPE GROWN AT SASKATOON 2016, LLEWELLYN 2016, SASKATOON 2017 AND MOON LAKE 2017.

NAM Founder	2^0 Dorm	PG Average Germ Pot (%)	Saskatoon 2016	Llewellyn 2016	Saskatoon 2017	Moon Lake 2017
			Mean (%)			
Legend	**High PG**		**Average PG**			**Low PG**
NAM30	High	0.34	0.30	0.24	0.84	0.24
NAM42	High	0.74	0.69	0.86	1.11	0.45
NAM46	High	0.48	0.75	0.17	0.75	0.55
NAM49	High	0.83	0.08	.	1.23	0.43
NAM85	High	0.66	0.30	.	0.54	1.06
AC Excel	Mid	0.52	.	0.60	0.33	0.58
NAM8	Mid	0.44	0.83	0.21	0.70	0.29
NAM12	Mid	0.15	0.09	0.10	0.10	0.63
NAM17	Mid	0.88	0.53	0.59	1.80	1.06
NAM23	Mid	0.21	0.08	0.10	0.36	0.67
NAM28	Mid	0.50	0.45	0.40	0.51	0.67
NAM29	Mid	2.36	0.14	.	3.74	2.65
NAM31	Mid	0.26	0.10	0.15	0.63	0.49
NAM33	Mid	0.17	0.10	0.14	0.32	0.20
NAM37	Mid	0.81	0.56	0.67	1.32	0.85
NAM40	Mid	0.46	0.56	0.55	0.37	0.41
NAM56	Mid	0.40	0.28	0.26	0.46	0.75
NAM65	Mid	2.28+	1.49	2.27+	4.63+	1.71
NAM71	Mid	0.31	0.16	0.25	0.85	0.29
NAM73	Mid	0.17	0.09	0.19	0.22	0.22
NAM83	Mid	0.34	0.00	.	0.42	0.50
NAM0	Low	0.56	0.50	0.34	0.93	0.61
NAM4	Low	0.32	0.54	0.10	0.45	0.43

NAM5	Low	0.48	0.62	0.35	0.27	0.91
NAM10	Low	0.22	0.09	0.22	0.17	0.67
NAM13	Low	0.45	4.58+	0.16	0.30	0.18
NAM14	Low	0.21	0.39	0.10	0.23	0.22
NAM15	Low	2.70+	2.23	2.16+	3.47+	3.19+
NAM25	Low	0.55	1.98	0.15	0.53	0.56
NAM26	Low	0.32	0.19	0.20	0.50	0.59
NAM32	Low	0.55	0.78	0.41	0.71	0.39
NAM38	Low	0.52	0.77	0.30	1.11	0.27
NAM39	Low	0.42	0.09	0.45	1.03	0.72
NAM43	Low	0.41	0.51	0.27	0.39	0.53
NAM48	Low	3.20+	1.85	.	4.00+	3.42+
NAM50	Low	1.40	0.55	.	2.58	0.67
NAM51	Low	0.20	0.11	0.10	0.30	0.53
NAM57	Low	0.38	0.10	0.57	1.01	0.36
NAM68	Low	0.31	0.11	0.59	0.56	0.27
NAM72	Low	4.05+	1.92	4.10+	7.56+	4.49+
NAM75	Low	2.50+	0.80	1.14	3.01+	13.62+
NAM76	Low	0.42	1.15	0.37	0.30	0.24
NAM78	Low	0.32	0.43	0.48	0.39	0.14
NAM82	Low	0.44	0.64	0.32	0.64	0.28
NAM87	Low	0.46	0.11	0.22	0.60	3.02+
Quantum	Low	0.09	.	0.07	0.14	0.06
	Mean	0.48	0.39 A	0.33 A	0.64 A	0.57 A

Note: Genotypes were ranked within environment then colour coded. Shades of blue (favourable outcome) represent lower precocious germination and shades of red (less favourable outcome) signify higher precocious germination. Genotypes are organized according to secondary dormancy (previous chapter). Means are LSmeans. "+" indicates significantly greater than NAM 0 at $P < 0.05$. Different letters in the mean row at the bottom of the chart denote significant difference at $P < 0.05$.

4.5.3 Experiment 6: Seed Quality Traits of a Diverse Collection of Annual *Brassica napus* Genotypes Produced in Different Environments

The seed quality traits of the diversity collection were measured using routine oilseed screening methods. The total oil content within the seed ranged from 32.4-47.1%, total protein content ranged from 24.6-30.3%, glucosinolate content ranged from 7.6-98.1 µmol/g, acid detergent lignin (fibre) content ranged from 1.4-10%, mode seed size ranged from 1.3-2.7 mm, thousand kernel weight ranged from 2.3-3.2 g and lastly, the whiteness index (seed coat colour) ranged from -21.6- 5.2 where the more negative the number, the yellower the seed (data not shown).

4.5.4 Correlation Matrix with all Examined Traits

All the possible correlations between all traits measured were compared (Table 4.7). Precocious germination was not included due to seed lots being produced in different environments compared to the other experiments. That being said, adjacent fields housed seed lot production and the precocious germination trial in 2016 thus, these values were assessed for correlation in a separate comparison. Within the Saskatoon 2016 environment, no correlation was detected between secondary dormancy and precocious germination (r= -0.2, P>0.05). It was observed that more precocious germination occurred in the low and mid dormancy genotypes rather than the high dormancy genotypes. Moving forward, only significant correlations will be mentioned in the results section and trends among other traits will be further explored in the discussion section.

Secondary dormancy was positively correlated to total protein content and negatively correlated with total oil content. Secondary dormancy was also found to be positively correlated to seed coat colour (whiteness index), with darker seed coat genotypes having greater secondary dormancy than lighter seeded genotypes. Primary dormancy was negatively correlated to both the PCT and CDT vigour tests, with lower primary dormancy occurrence being associated with greater germination and therefore, greater vigour. The PCT test was positively correlated to both seed fibre (acid detergent lignin) and seed coat colour (whiteness index), indicating low fibre, yellow seeded genotypes may be less vigourous. The other seed vigour test, the CDT test, was also positively correlated to seed fibre (acid detergent lignin) but negatively correlated to T50 at 5^0C suggesting, lower vigour genotypes had greater T50, as expected. Seed size and thousand

kernel weight were strongly positively correlated as were seed size and glucosinolate content. Seed size and thousand kernel weight were both positively correlated to oil and acid detergent lignin. Time to 50% germination at 20^0C was positively correlated to many of the seed quality traits including, thousand kernel weight, glucosinolate content, acid detergent lignin and whiteness index; however, these relationships were absent at T50 at 5^0C. Glucosinolate content was positively correlated to total protein, acid detergent lignin and whiteness index and negatively correlated to total oil. Total protein was highly negatively correlated to total oil and positively correlated to whiteness index. Lastly, the whiteness index was negatively correlated to total oil content and positively correlated to acid detergent lignin.

Weak correlation between environments were observed for the majority of the traits measured in this chapter. Seeds from the two Saskatoon environments were moderately correlated to each other in all traits measured except primary dormancy. In the time to 50% germination at 20^0C and the pre-chill test, both Saskatoon environments were significantly correlated to LA, and the two Chilean environments were correlated to each other. Lastly, Saskatoon 2016 was correlated to Temuco. The magnitude of environmental correlations between traits in this chapter differ from the previous chapter where all the environments were moderately correlated for secondary dormancy. The reasoning for this is that the interactions were the major contributing factor for the seed vigour traits measured in this chapter as opposed to the genotypic effect being the greatest contributing factor for secondary dormancy occurrence.

TABLE 4.7: CORRELATION BETWEEN SECONDARY DORMANCY, SEED GERMINATION, VIGOUR AND QUALITY TRAITS.

	T50 @ 5^0C	T50 @ 20^0C	CDT	PCT	PD	SS	TKW	GLS	PRO	OIL	WI	ADL
SD	0.03	0.17	-0.09	0.1	0.12	-0.15	-0.16	0.1	**0.34*** **	**-0.24***	**0.22***	-0.07
T50 @ 5^0C		0.17	**-0.21***	-0.11	0.05	0.02	-0.06	0.11	-0.04	0.02	-0.07	-0.02
T50 @ 20^0C			-0.09	0.17	0.06	**0.29****	**0.30****	**0.41*****	0	-0.08	**0.31****	**0.43*****
CDT				**0.53*****	**-0.30****	0.07	0.14	-0.07	-0.06	0.15	0.16	**0.21***
PCT					**-0.30****	0.03	0.13	0.15	0.02	-0.07	**0.37****	**0.38****
PD						-0.04	0.04	0.05	-0.02	0.04	-0.1	-0.17
SS							**0.80*****	**0.21***	-0.17	**0.27****	-0.09	**0.29****
TKW								0.17	-0.11	**0.27****	-0.03	**0.21***
GLS									**0.33****	**-0.50*****	**0.55*****	**0.41*****
PRO										**-0.73*****	**0.27****	-0.19
OIL											**-0.49*****	-0.02
WI												**0.36****

Note: Number denotes *r*-value. '*'- $P< 0.05$; '**'- $P< 0.01$; '***'- $P< 0.001$. **SD**-Secondary Dormancy; **T50 @ 5^0C**- Time to 50% Germination at 5^0C; **T50 @ 20^0C**- Time to 50% Germination at 20^0C; **CDT**- Controlled Deterioration Test; **PCT**- Pre-Chill Test; **PD**- Primary Dormancy; **SS**- Seed Size; **TKW**- Thousand Kernel Weight; **GLS**- Glucosinolates; **PRO**- Total Protein; **OIL**- Total Oil; **WI**- Whiteness Index; **ADL**- Acid Detergent Lignin.

4.5.5 Partial Least Squares Regression

A partial least squares regression analysis was performed to determine which traits screened in this set of experiments had an influence on secondary dormancy. Only three models were found to significantly contribute to secondary dormancy and contributed collectively 35% and 33% of the model and dependant (secondary dormancy) variability (Table A.4). Within the first model, the total oil content was the largest negative influencing factor on secondary dormancy followed closely by total protein content (positive) whiteness index (positive). Lesser contributing factors to secondary dormancy within the first model included seed size, winter annual background which both negatively interacted where lack of winter annual background and larger seeds were associated with more secondary dormancy.

4.6 Discussion and Conclusion

Overall, the traits measured in this chapter had highly significant genotype x environment interactions which contributed the greatest amount of variability to the observed phenotype. The two exceptions were time to 50% germination at 20^0C and precocious germination, where the genotypic contribution alone was marginally greater. The traits with large genotypic control are more predictable when grown across different environments. Whereas, when a significant interactions occurs, a larger number of environments need to be screened for a more accurate representation of the phenotype across environments. The genotype effect alone also had a highly significant effect but to a lesser extent on all traits measured in this chapter with values ranging from one quarter to one-third of the amount of variability observed in the phenotype. Lastly, the environment effect was also highly significant in all traits measured excluding primary dormancy and precocious germination, with contributions being less than the other two effects. Previous studies have documented both primary dormancy and precocious germination are largely environmentally influenced. Reasoning for the non-significance of the environment in this study may be due to the low absolute values observed for both traits ranging from 0-3% for primary dormancy and 0-4% for precocious germination which when averaged for an environment had similar values to the other environment averages.

4.6.1 Seed Vigour Traits

4.6.1.1 Time to 50% Germination and Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes

The detailed germination data provided insight into annual *B. napus* germination time under ideal and adverse conditions. The 5^0C temperature represented field conditions more closely than the 20^0C temperature, as canola is planted in early to mid-May into cold soil temperatures typically around 6-8^0C (Canola Council of Canada. 2018). Collectively, nine genotypes were identified with faster T50 than average (< 33 hours at 20^0C and < 153 hours at 5^0C) under both temperatures. Of these genotypes, five were low dormancy, three were mid dormancy and one high dormancy. Moreover, for the slower than average T50, nine genotypes were identified under both temperatures. Low dormancy genotypes dominated the identified genotypes for slow T50; however, two genotypes were identified as high dormancy. The hypothesis for this experiment was that genotypes displaying slower T50 would have higher dormancy and based on the T50 experiment, this hypothesis was rejected as the majority of the genotypes identified as slow germinating were low dormancy genotypes. It can be concluded that selection against secondary dormancy will not affect germination time.

Further evidence of a weak/no association between germination time and secondary dormancy was provided in the correlation matrix and partial least square analysis, where neither of the temperatures tested for germination time had a significant correlation with secondary dormancy or were a significant model term in the partial least squares. There was no association between T50 at 5^0C and secondary dormancy or between secondary dormancy and T50 at 20^0C. The correlation coefficient for T50 at 20^0C trended towards the hypothesis, however, was not significant.

Momoh et al. (2002) determined high secondary dormancy genotypes displayed slower germination time in winter annual *B. napus* with correlation between the two traits ranging from 0.63-0.76 ($P < 0.01$); contrary to what was observed in the present study which studied a larger, more diverse panel of annual *B. napus* genotypes. Storage length was also noted by Momoh et al. (2002) which may have contributed to the significant correlation between secondary dormancy and germination time. They found longer storage periods maintained secondary

dormancy but increased germination time. Seed lots dated from 1993 to 2000 in varying and inconsistent storage environments and the slower germination time could have been more related to seed viability as opposed to seed dormancy. Hatzig et al. (2015) also suggested germination time was related to a genotypes propensity to become dormant with slower germinating and lower vigour genotypes presumed to have greater secondary dormancy.

In rice (*Oryza sativa* L.), four QTL have been identified for germination time with one of the QTLs identified on the same chromosome region as a seed dormancy QTL, suggesting germination time and seed dormancy may be under the same genetic control (Li et al., 2011). Rice, like canola, has different ecotypes (*japonica* and *indica*) with noticeably different germination times with *japonica* adapted to colder climates and possessing slower germination speeds (Li et al., 2011). The differences in germination time in rice are attributed to different adaptive mechanisms including temperature for germination between the ecotypes (Li et al., 2011). The same relationship may be observed between the annual and winter annual type in *B. napus* for germination time however, this study did not directly compare annual and winter annual types.

Much of the variance was not attributed to the fixed effects for T50 in particular at 5^0C. The genotype, environment and genotype x environment interactions contributed 50% of the variance observed for T50 with the other 50% of variance unexplained. This unexplained or residual variance could be due to variation between replications and other experimental error. It could also be speculated that within-environment variation affecting genotypes with phenological differences would account for much of the residual variance observed; however, without information on flowering and maturation times of each of the genotypes within the maternal environments, this can not be tested.

4.6.1.2 Controlled Deterioration, Pre-chill Germination Test and Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes

The correlation between the CDT and PCT experiments had a significant correlation coefficient of 0.53 (P <0.001), indicating that both tests were detecting similar vigour differences among the seed lots across different environments. No relationship was detected between either vigour test and secondary dormancy in the correlation matrix or partial least square analysis. The hypothesis stating low vigour genotypes would exhibit higher dormancy was rejected from

the results in this study, as no significant correlation was found between the two traits. The AOSA handbook supports the use of the CDT to distinguish low germination (low vigour) from high germination (high vigour), however, this test cannot decipher smaller differences in vigour (AOSA. 2009). The results of this experiment were aligned with these established limitations of this test. From the results in this experiment and in conjunction with the results from the germination time experiment, it can be concluded that the direct selection of secondary dormancy will not affect the vigour of the seed.

Interestingly, both vigour tests had significant negative correlations with primary dormancy with higher vigour genotypes having lower primary dormancy (r =-0.3; P <0.01). The correlation states, the greater the primary dormancy the lower the vigour of the genotype. The primary dormancy levels observed in this study was low and ranged from less than 1% to 3%, while germination from the vigour tests was high, close to 100%. The inverse correlation may reflect the method of screening for primary dormancy which was a dark germination test, and seeds that did not germinate were classified as possessing primary dormancy. The non-germinated primary dormant seeds may be more prone to deterioration from a vigour test (lower germination) thus, resulting in the negative relationship.

4.6.2 Primary Dormancy and Precocious Germination Occurrence

4.6.2.1 Primary and Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes

The occurrence of primary dormancy has been tested in several studies in *B. napus* and generally is low (Baskin and Baskin 1998; Momoh et al., 2002; Gruber et al., 2004). Primary dormancy is highest in freshly harvested seed and decrease as the seed is after-ripened (Haile and Shirtliffe. 2014; Schatzki et al., 2013b; Huang et al., 2016). A strong correlation between primary and secondary dormancy during early seed development exists but this decreases as the seed matures (Schatzki et al., 2013b; Huang et al., 2016). Significant correlations between primary and secondary dormancy have been documented in *B. napus* with *r*-values ranging up to 0.62 (Schatzki et al., 2013b; Soltani et al., 2018). The range in results is believed to be due to how and when the seed was tested. If the seed was ‘fresh’ but stored for a few months before testing it can be argued that the seed was after-ripened, a primary dormancy breaking condition,

and therefore would not express any primary dormancy (Soltani et al., 2018). It is not known, however, if primary dormancy is a prerequisite for secondary dormancy (Soltani et al., 2018).

For this study, it was hypothesized high secondary dormancy genotypes would also possess high primary dormancy levels. Primary dormancy was measured in fully mature seed and no correlation between primary and secondary dormancy was found (r-value = 0.12; P >0.05). This is similar to what was found by Huang et al. (2016) where primary and secondary dormancy correlations coefficients decreased by half from 0.58 at 50 days after flowering to 0.09 when the seed was fully mature. The maternal environment played a significant role in primary dormancy in the Huang et al. (2016) study. Given the significant interaction term in the current study it can be also be concluded that the environment had an impact of primary dormancy. The low absolute values observed for primary dormancy across the environments resulted in the individual maternal environments not being a significant factor to primary dormancy, however, the affect of the maternal environment, as stated above, is reflected in the significant genotype x environment interaction.

4.6.2.2 Precocious Germination Occurrence and Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes

The hypothesis stating, high secondary dormancy genotypes would have lower precocious germination occurrence, was rejected from the initial analysis of the Saskatoon 2016 environment as no correlation existed between the two traits (r= -0.20; P >0.05). It was not possible to examine the association in any of the other environments without precocious germination data from the seed lots screened for secondary dormancy. Furthermore, because of the large genotype x environment interactions observed for both traits comparison would be too convoluted with other factors to provide any clarity. The occurrence of precocious germination is highly dependent on the environmental conditions present during seed maturation and in order to get an accurate depiction of a genotypes propensity for precocious germination, several years of data in differing environments would be beneficial under different conditions such as greenhouse with higher humidity and irrigation during maturity. Among the environments tested in this study, there were differences in precocious germination occurrence. In 2017, the propensity for precocious germination at both environments was greater than in 2016 likely due to greater rainfall during the maturation period in 2017.

From previous studies, high secondary dormancy genotypes were less likely to have precocious germination when compared to genotypes with low secondary dormancy (Gruber et al., 2005; Feng et al., 2009). Given that QTLs have been identified for precocious germination which cumulatively account for 75% of the phenotypic variation, it is possible to identify markers and study the interactions between secondary dormancy and precocious germination further for more conclusive results (Feng et al., 2009).

4.6.3 Seed Quality Traits

4.6.3.1 Seed Quality Traits and Secondary Dormancy of a Diverse Collection of Annual *Brassica napus* Genotypes

Secondary dormancy had weak, but significant correlations with three seed quality traits including, total protein (r= 0.34; P <0.001), total oil (r= -0.24; P <0.05) and whiteness index (r= 0.22; P <0.05). The hypothesis stating, high dormancy genotypes possess greater protein was not rejected. This study looked at total protein content as a percent of seed dry weight but did not examine the individual protein fractions such as seed storage proteins. Regardless of this, a relationship could be detected. In terms of overall protein content, Saskatoon 2015 was the environment with the greatest overall secondary dormancy levels and also the environment with the greatest overall protein content.

Within the protein fraction in the seed, cruciferin (60%) and napin (20%) make up the majority of the seed storage proteins (Schatzki et al., 2014). Seed storage proteins contribute to the germination, seedling vigour, dormancy and longevity of the seed (Muntz et al., 2001; Schatzki et al., 2014; Nguyen et al., 2015). Cruciferin and napin are negatively correlated and the cruciferin:napin ratio is negatively correlated to glucosinolate content (r =0.81). Schatzki et al. (2014) found a significant negative correlation also existed between napin and total seed dormancy with low napin seed having greater seed dormancy in winter annual *B. napus.* Given the tight genetic linkage of napin and glucosinolate content and the breeding for low glucosinolates in modern breeding, it is hypothesized that more recent double-low/canola varieties have increased dormancy compared to previous, higher glucosinolate varieties. In selecting for reduced glucosinolate content, the ratio between cruciferin:napin has shifted independent of changes in total protein content (Schatzki et al., 2014; Brunel-Muguet et al., 2015). Genetic pathways for napin and ABA expression are both signalled by the gene *ABI3*

further suggesting napin may be involved in seed dormancy (Brocard-Gifford et al., 2003; Schatzki et al., 2014). In the seed lots studied, the individual seed storage proteins were not quantified, but doing so would be of great value in further understanding the relationship between protein and secondary dormancy in the genotypes tested. Future work may lead to the indirect selection for reduced dormancy by quantifying napin which could in turn reduce secondary dormancy.

A negative correlation between oil content and secondary dormancy existed in this study (r= - 0.24; P <0.05). Schatzki et al. (2013a) observed ideal growing conditions produced seed with higher oil content, higher yield and higher dormancy. However, it was only an observation and not statistically supported as they had no correlation (r= -0.10; P > 0.05), similar to the results in this study. The negative correlation found in this study is believed to be a result of the established negative association between protein and oil and as the protein content associated with greater dormancy increases, the oil content decreased.

The last statistically significant correlation with secondary dormancy was whiteness index (r= 0.22; P < 0.05), which measured the seed coat (testa) colour where more negative values indicated lighter seed colour. All genotypes were dark seeded except for two which were noticeably yellow (NAM 48 and NAM 72). The two yellow seeded genotypes also had very low fibre content in comparison to the rest of the diversity collection and the yellow seed colour was consistent across the different seed lots of the two genotypes. It is worth noting that the two yellow seeded genotypes are not naturally occurring and have been selected to also have low glucosinolates. When the two yellow seeded genotypes were removed from the correlation analysis to determine if the extreme negative whiteness index values were skewing the results, the correlation coefficient decreased slightly to 0.17 and the correlation was no longer significant (P-value > 0.05; data not shown). Whiteness index was still the third largest contributor in the partial least squares analysis when the yellow seed genotypes were removed but the value did decrease, similarly to the correlation analysis (data not shown). It is likely the that yellow seeded genotypes were skewing the results and it can be concluded that no relationship exists between the seed colour and secondary dormancy in this study.

The role of the seed coat is to protect the seed from biotic and abiotic stresses and play a role in the control of germination and dormancy (Auger et al., 2010). Darker seed coats have

more pigmentation from the flavonoids to protect the seed from attack from external factors and therefore, prolong the existence of the seed (Auger et al., 2010). Whereas the lack of pigmentation and thin seed coat are responsible for the yellow seed colour in *B. napus* (Nguyen et al., 2016). Yellow *B. napus* seeds deteriorate faster, have higher rates of solute leakage from the seed leading to increased microbial attack and finally, are more prone to precocious germination (Auger et al., 2010). A yellow-seeded (derived from an interspecific cross between *B. rapa* and *B. oleracea*), poor vigour line was crossed to a black seeded, normal vigour line to create a DH population of 166 homozygous lines (Nguyen et al., 2016). Hormone profiling of the population including the parents revealed elevated amounts of ABA and auxin, two dormancy promoting hormones, caused by physiological defects within the yellow seeded line. Observations in the current study were that the yellow seeded genotypes had poorer vigour and more precocious germination occurrence likely due to thinner seed coats and fewer flavonoids for protection against external stresses. However, the positive correlations between germination time, seed colour and fibre would indicate differently. The lower fibre, lighter seeded lines displayed faster germination time and vigour.

In Arabidopsis, mutants with transparent seed coats had reduced primary dormancy compared to the dark, wild-type seed coat genotypes (Debeaujon et al., 2000). Similarly, reduced dormancy in red seed coat compared to black seed coat seeds in *Sinapsis arvensis* L. (wild mustard) is documented (Duran and Retamal. 1989; Debeaujon et al., 2000). In general, the seed coat provides protection to the embryo in many ways including, from microbial attack, preventing imbibition by acting as an impermeable barrier as well as providing mechanical resistance restricting the embryo from breaking through.

There were no relationships between secondary dormancy and the other seed quality traits tested including, total kernel weight, glucosinolate content and acid detergent lignin. Similar seed quality traits (thousand kernel weight, acid detergent lignin and glucosinolate content) were analyzed for correlation with secondary dormancy in Schatzki et al. (2013a&b) on different winter annual and annual *B. napus* genotypes and, similar to this study, no relationships were found. Gulden et al. (2004a) tested secondary dormancy levels of sized seed fractions within a seed lot and found a relationship to exist where the larger seed fractions had greater dormancy. The Schatzki et al. (2013a&b) study and this study examined relationships between seed size averaged across a seed lot and the corresponding dormancy level and both found no

correlation to exist, suggesting that average seed lot size is not a factor in secondary dormancy in *B. napus* across the species.

The seed traits most closely associated with secondary dormancy identified in the partial least square analysis were also the traits identified to have significant correlations with secondary dormancy and in both cases the correlations and effect on the dependant variable (secondary dormancy) were low. The highest correlated traits for the correlation analysis were protein, oil and seed colour (whiteness index). The largest contributing traits from the partial least square regression were similar with only protein and oil changing ranking. The partial least squares regression is a linear regression analysis identifying factors (quantitative and qualitative) that contribute to the dependent variable (secondary dormancy). The partial least squares analysis is a valuable analysis for examining the response variable (secondary dormancy) in relation to many collinear predictor variables which in this case were seed germination, vigour and quality traits (Sawatsky et al., 2015). This test is the next step after a correlation analysis as the collinear variables are separated and made independent using linear transformations and lastly, attributing a percent value influencing the dependent variable (secondary dormancy) (Boulesteix and Strimmer. 2006).

As for the associations identified in this study between secondary dormancy and quality traits, more research is needed to provide definitive answers however, the correlations are weak which simplifies the process. The end use of the seed needs to be considered when selecting to reduce secondary dormancy and caution is required as to not disrupt the quality requirements the seed must meet for registration of new varieties. The selection goals over past 40 years may have affected secondary dormancy as it is prevalent in the species thus, justifying a closer look into protein and oil relationships with secondary dormancy.

4.6.4 Relationship between Germination and Glucosinolate Content

An interesting correlation identified from the correlation matrix was between T50 at 20^0C and glucosinolate content (r=0.41; P-value <0.001). Genotypes with low glucosinolates took less time to reach 50% germination at 20^0C; however, there was no observed correlation at 5^0C. Current canola varieties have low glucosinolates due to intensive breeding to reduce levels making the meal fit for animal feed by reducing the unpalatable taste.

Contrasting results regarding the relationship between germination time and anti-nutritionals was identified by Hatzig et al. (2018). Their study found genotypes of canola quality (double-low) possessed slower germination times under constant temperature of 20^0C than genotypes with higher erucic acid and higher glucosinolates. In addition, the more recently developed genotypes showed slower germination times than genotypes developed before the major shift in quality occurred (Hatzig et al., 2018). This is believed to be due to the linkage between anti-nutritionals and germination speed related genes and the intense breeding efforts that have occurred to reduce the anti-nutritionals within the seed creating a genetic bottleneck (Hatzig et al., 2018). The genotypes tested in this study included those that were canola quality as well as those with seeds high in glucosinolates and erucic acid and were all annual types whereas, the genotypes tested by Hatzig et al. (2018) were all winter annual types with differing glucosinolate and erucic acid content. The most probable explanation for the contrasting results is due to the difference in growth types tested between the two studies and the seed-bed environment under which selection for canola-quality would have occurred. Annual *B. napus* must germinate soon after planting in cold soil temperatures of 4 to 6^0C (Canola Council of Canada. 2017) in order to ensure that the plant can complete the reproductive lifecycle before a killing frost arrives. Whereas, winter annual crops germinate in warm summer temperatures and extend their lifecycle over three seasons. The stark difference in germination temperatures and the selection for annual genotypes to germinate in cooler conditions may underline the differences in germination speeds.

Time to 50% germination at 20^0C was positively correlated with other important seed quality traits including, seed size, thousand kernel weight, seed fibre and seed colour whereas, T50 at 5^0C was not significantly correlated to any seed quality traits screened in this experiment. This suggests that selection for seed quality traits in optimal conditions (20^0C) may result in not selecting for germination time in sub-optimal conditions (5^0C), a great concern given that seed germination in western Canada rarely occurs under ideal conditions and may be a contributing factor to the low stand establishment farmers are observing.

A positive correlation between seed size and glucosinolate content was observed (r=0.21; P-value <0.05) similar to results found in Schatzki et al. (2014) on a large DH diversity panel in winter annual *B. napus*. No significant relationship between thousand kernel weight and glucosinolate content was observed as in the Schatzki et al. (2014) paper but the thousand kernel

weight and seed size were highly correlated in this study (r=0.88; P-value <0.001). In this study larger seeds with increased glucosinolates did not necessarily weight more, reflected by the non-significant relationship between thousand kernel weight and glucosinolates.

4.6.5 Conclusion

The objective of this study was to screen for seed vigour, seed quality traits and precocious germination occurrence in the annual *B. napus* diversity collection to examine if relationships existed with secondary dormancy of the same seed lots. The first hypothesis that higher dormancy genotypes would have slower T50 was rejected as no association between higher dormancy genotypes and germination time at either temperature was observed. The second hypothesis that high dormancy genotypes would have decreased seed vigour traits was rejected from the results in this study as no association between seed vigour and secondary dormancy existed. Taking the first two hypotheses together, it can be concluded that selection against secondary dormancy will not affect the seed vigour and selection for seed vigour will not indirectly select for or against secondary dormancy.

The hypothesis that greater protein content exists in higher dormancy genotypes was supported by the results from this study. Significant but weak correlations between total protein, total oil and whiteness index were found with secondary dormancy. It is hypothesized that the relationship between total protein and seed dormancy is due to seed storage proteins. Seed glucosinolate content is genetically linked to the content of the seed storage protein napin and as glucosinolates are decreased so does napin content. Thus, selection against glucosinolate content has likely indirectly led to increased dormancy due to reduction in napin (protein) and consequently dormancy. The secondary dormancy and total oil correlation is believed to be due to the previously established negative association between oil and protein. As oil content is increased within the seed, the secondary dormancy decreases whereas, when protein is selected for, secondary dormancy increases. Lastly, seed colour (whiteness index) was correlated to secondary dormancy with greater dormancy being found in darker seed coats. Yellow-seeded *B. napus* genotypes have been found to deteriorate faster, have higher rates of solute leakage from the seed leading to increased microbial attack, and are prone to precocious germination which could result in less secondary dormancy induction (Auger et al., 2010). However, when the yellow seeds were removed from the analysis, no relationship was found within the dark-seeded

genotypes meaning that the extreme yellow seeds were skewing the relationship and no relationships exist within natural variants of *B. napus*.

Results from this study suggest a potential for indirect selection of secondary dormancy through determining the total protein content and the seed storage protein ratio within the seed. However, determining seed storage protein ratios is not a routine screening procedure in canola research companies and therefore, may not lead to wide adoption. The potential for indirect selection resulting from this study may be premature however, as the relationship with protein content identified is weak and the relationship with seed storage protein rations has not yet been tested in these seed lots.

The traits, secondary dormancy included, that were examined in this study were all found to have a highly significant genetic factor controlling the traits, indicating selection will be effective within breeding programs. There was also highly significant interactions effects therefore, prediction and selection may be difficult due to influence by the maternal environment. Overall, the reduction of secondary dormancy in commercial canola varieties as an avenue to reduce volunteer canola populations is promising as relationships to other important seed properties including, seed vigour and germination time were not found. The seed quality traits, namely oil and protein content may pose a challenge given that registration of canola varieties has a strict standard for those traits, however, the relationships were weak and may not have a large impact on the end breeding goals if suitable breeding designs are employed.

5.0 General Discussion and Future Objectives

Volunteer canola is a persistent issue in western Canadian fields. Recent technological advances like herbicide tolerance have made the control of volunteer canola more difficult particularly as crop rotations tighten. The increasing incorporation of herbicide-tolerant soybeans to rotations on the prairies will bring added challenges as common herbicide tolerance (glyphosate) are popular for both crops. Thus far, reactive methods have been practised including spraying herbicide and tillage after the appearance of volunteers. The question about the 'cost of volunteers' has arisen and whether it is sufficient to control the volunteers once they have germinated in fields; or if breeding companies should focus their efforts on reducing shattering and/or secondary dormancy of new varieties. Breeding for shatter-tolerant varieties is occurring as a method to reduce volunteers. Another possibility is the reduction of secondary dormancy within *B. napus* seed to reduce seed persistence in the soil seed bank. Low dormancy seed may still enter the seed bank through harvest losses, but would not persist in the soil. The range in secondary dormancy and large genetic control among annual genotypes screened in this study confirmed reduction is possible; however, the effect of reducing secondary dormancy on other seed traits like seed vigour and quality was unknown prior to this study. Use of the annual *B. napus* diversity collection screened in this project has provided insight into the relationships between secondary dormancy and other seed traits. It has been shown here that the reduction of secondary dormancy will not adversely affect other seed germination or vigour traits. Likewise, secondary dormancy is not solely responsible for poor emergence of the crop due to no association between vigour and dormancy. From this study, associations between secondary dormancy and protein and oil content were found.

The first objective included screening for secondary dormancy in the diversity collection and revealed that a wide range of dormancy existed among the genotypes and across different environments, ranging from 0-77% dormant. These results confirmed the first hypothesis

stating, that a large range in secondary dormancy would be observed across genotypes and environments. The genotypic effect alone contributed half of the variability to the phenotype and the significant genotype x environment interactions contributed the second largest amount of variability at just over a quarter. Absolute secondary dormancy values changed from year to year when grown at the same location due to the genotype by environment interactions. Optimal growing conditions have been identified as conditions that increase the dormancy of a genotype. However, the results from this study showed that low dormancy genotypes were consistently low dormancy regardless of maternal environment, even if conditions were favourable for increased dormancy. In addition, the majority of genotypes screened in this study were classified as low dormancy indicating that low dormancy is prevalent within the annual *B. napus* breeding lines.

The second part of the study examined if associations between seed vigour, seed quality, precocious germination and secondary dormancy existed. The concern with modifying secondary dormancy was that it would inadvertently affect other seed traits similar to what has been documented in winter annual *B. napus*. From the results in this study, it can be concluded that it is unlikely for germination and seed vigour to be affected by selection for lower secondary dormancy. Thus, the hypothesis stating that higher dormancy genotypes would have weaker seed vigour and slower germination was not supported. The speculation from the agronomic community that the poor emergence of canola was due to secondary dormancy can be rebutted. Other seed trait(s) must be influencing the poor emergence and not secondary dormancy. The data sets developed in this project can be further mined to explore other seed traits in terms of vigour and plant emergence in the way this study has done so with secondary dormancy.

A relationship between secondary dormancy and seed quality traits were found to exist, albeit the relationships were weak. Three seed quality traits were found to be significantly correlated to secondary dormancy and included, total protein (positive), total oil (negative) and seed colour (positive). The hypothesis stating, higher dormancy genotypes would possess greater total protein content was confirmed in the annual population. The association between protein and secondary dormancy was believed to be due to seed storage proteins and the negative correlation between dormancy and the seed storage protein napin. Quantification of the seed storage proteins and the corresponding secondary dormancy would be beneficial to support the results found in this study. It is believed that the negative relationship between oil and secondary dormancy was due to the pre-existing correlation between protein and oil and as the protein

content (higher dormancy) increases the oil content decreases. Increased oil content has been directly and indirectly selected for many years. However, given the increased interest and demand in canola protein, while still maintaining current oil contents, secondary dormancy of the varieties released targeting protein markets may inadvertently be increased. Protein content within the seed varies from year to year depending on environmental factors, and as such the variation in secondary dormancy from different seed production (genotype by environment interactions) environments may be caused by this. This was observed in this study as the environment with the greatest overall secondary dormancy was also the environment with the greatest overall protein content. The modification of the oil and protein content over past 40 years may have affected secondary dormancy in *B. napus* as it is prevalent in the species thus, justifying a closer look into protein and oil relationships with secondary dormancy.

The relationship with whiteness index is of interest and the correlation indicated that the higher the secondary dormancy, the darker the seed. However, when the yellow seeded genotypes were removed from the analysis, no association existed. Darker seed coat genotypes have been shown to be better suited to resist biotic and abiotic stresses due to increased flavonoid concentration, while yellow seeded canola deteriorates faster and is more prone to precocious germination. It is possible that these characteristics of yellow seeded genotypes result in reduced capacity for secondary dormancy induction. Differences in seed health were observed similar to what was stated above between the yellow and the darker seeded genotypes, however, there is not enough evidence to believe the yellow or lighter seeded genotypes impact secondary dormancy in *B. napus*.

Overall, the results of this study conclude that secondary dormancy and seed germination or vigour traits are not associated. Seed quality traits, namely protein, in relation to secondary dormancy still require investigation to better understand the relationship, but the relationships that were found in this study were weak. Successful selection for low dormancy is promising given that a strong genetic component for secondary dormancy exists. Furthermore, within the panel screened in this study, selection for low dormancy is also possible given that the majority of the genotypes screened possessed low dormancy. A reduction in volunteer canola presence subsequent years after planting due to the planting of low dormancy canola with maintained germination and vigour properties appears to be a viable option to reduce volunteers in the field. From the associations identified in this study, there may be a possibility to screen for secondary

dormancy more efficiently, thus, increasing the possibility of breeding companies screening for the trait. In closing, the results from this study provide many avenues for continued study but one challenge will be the adoption of screening due to the additional resources required and in turn reduction in secondary dormancy in commercial breeding companies.

5.1 Future Objectives

The results from this study bring into question the use of 'standard' vigour tests as an indicator of germination and seed vigour under conditions of stress given no correlation between germination time at 20^0C and 5^0C was observed. Correlations between standard seed quality traits and germination at 20^0C existed but not at 5^0C, indicating that screening for seed quality traits at optimal temperature may not be indicative of what happens under sub-optimal conditions. The standardized test in this study was the controlled deterioration and the pre-chill test was added as another common test. These tests exclude other stressful conditions that are likely to occur in the field such as salinity and drought. Either a more inclusive vigour test should be recommended or various stressor tests can be performed with scores assigned to each genotype and then summed for an overall rank. Furthermore, bigger seed was more vigourous at 20^0C than at 5^0C, indicating that the notion that larger seeds are more vigourous may only be true under ideal conditions. The poor stand establishment that is observed in this crop may also be due to the vigour tests not being indicative of field conditions. No association between seed vigour and secondary dormancy existed with the vigour tests performed in this study but that is not to say that other tests under different conditions are not related to secondary dormancy and poor stand establishment in the field.

The range in dormancy found in the panel of genotypes screened in this study provides a platform to identify genetic markers for this trait. By screening the NAM Recombinant Inbred Lines (RILs) for secondary dormancy and associating with linkage maps through QTL analysis, potential genetic markers for favourable alleles in genes of interest can be identified. The RIL population consists of over 2500 lines with each line founder line (ie. panel for this project) reciprocally crossed to the reference NAM 0. NAM 0 was found to have low dormancy in this study supporting effective used of the NAM population to study this trait. The greater distribution in secondary dormancy levels that is expected in the RIL population will provide more information on what is happening to the high dormancy genotypes as the parental

population only identified a small number of high genotypes.

Furthermore, quantifying the cruciferin and napin content in the founder seed will also provide some insight into the relationship between seed storage proteins and secondary dormancy and many be a possible avenue for indirect selection. A deeper examination into whether a relationship between precocious germination and secondary dormancy in the founder genotypes will need to be conducted in order to determine if this association is worth pursuing in the RILs. This will be done by screening the founder seed lots for both precocious germination and secondary dormancy over the multiple site years. In order to observe higher precocious levels, one possibility may be to screen for it in greenhouse produced seed where the humidity levels are higher and/or can be modified. Further exploration of the relationship between seed coat colour and secondary dormancy is warranted. This could be done by crossing a yellow seeded, low dormancy line to a black seed coat, high dormancy line and examining the DH population for the relationship.

Along with the relationship between seed storage proteins and dormancy, the *DOG1* protein of unknown function has been linked to the presence of dormancy (Graeber et al., 2014). More recently, three copes of this gene in *B. napu*s have been identified and are named *BnaADOG1.a*, *BnaCDOG1.a* and *BnaDOG1.b* (Nee et al., 2015). Another proposed avenue for screening for secondary dormancy is through identifying sequence polymorphisms within these genes between high and low secondary dormancy lines to identify molecular markers for selection against dormancy (Nee et al., 2015). Interesting relationships have been identified in this project with secondary dormancy. As a result it may lead to wider adoption of commercial screening as the current methods are time-consuming and therefore, not widely practised. Further research into scalable screening protocols will enable decreasing the volunteer canola populations through selection in breeding programs.

Appendix A

TABLE A1: COMMON NAME, ORIGIN, PEDIGREE, YEAR OF REGISTRATION, TUKEY-KRAMER LEAST SQUARE MEAN LETTER AND SECONDARY DORMANCY CLASSIFICATION OF EACH NAM FOUNDER GENOTYPE

NAM Founder	Name	Origin	Pedigree	Year of Registration	Letters	Secondary Dormancy
NAM46	Dong Hae 21	S. Korea			A	
NAM49	DH12075	Canadian	Cresor/Westar		A	
NAM30	Egra	European			AB	High
NAM85	Dae Chosen	S. Korea			ABC	
NAM42	PI432392	Bangladesh			BCD	
NAM83	SWU Chinese 9	China			CDE	
NAM17	N00-C3661sp2	Canada	N89-53/Dunkeld//N89-53/Range		DEF	
NAM31	Wase Chousen	Korea			DEF	
NAM65	Puma	Sweden or Denmark	Seln from /SV751406 x SV74465	1986	DEFG	
NAM33	Dong Hae 3	S. Korea			EFG	
AC Excel	AC Excel	Canada	Karat/Westar	1990	EFGH	
NAM40	Mlochowski	Poland	'Local'		EFGH	
NAM28	Topas	Sweden	Hermes/Bronowski x Gulle/	1981	EFGHI	Mid
NAM37	Wesroona	Australia	Norin20/Tower	1980	EFGHI	
NAM71	ACS N22	Canada	Norin11/Regent//Westar		EFGHI	
NAM12	46A65	Canada	Pioneer Hi-Bred	1996	FGHIJ	
NAM56	Mazowiecki	Poland	'Local'	1945	FGHIJ	
NAM73	Optima	Denmark	Line/Tower	1985	FGHIJ	
NAM23	Daichousen	N. Korea			GHIJ	
NAM29	PSA12	Resynthesized *B. napus*			GHIJK	

NAM8	86004	Unknown			HIJKL	
NAM38	Wesreo	Australia	Major/Oro	1978	IJKLM	
NAM32	Dong Hae 2	S. Korea			IJKLMN	
NAM78	Unknown	Canada	N89-53/YN9592		IJKLMN	Low
NAM5	BN-1	India	Tata Energy Research Institute		IJKLMNO	
NAM48	DH27298	Canada	Range/YN979-262//Ebony/YN97-262///Dunkeld/YN97-262//Magnum/YN97-262	2005	JKLMNOP	
NAM57	Mozart	Denmark		2000	JKLMNOP	
NAM68	Lirawell	Germany		1986	KLMNOP	
NAM10	Global	Sweden or Denmark		1985	LMNOP	
NAM13	Campino	Germany		2004	LMNOP	
NAM39	Nakate Chousen	Korea			LMNOP	
NAM4	Wesway	Australia	Ramses/Oro	1979	LMNOP	
NAM75	Magnum	Canada		1995	LMNOP	
NAM14	Svalöf's Gulle	Sweden		1985	MNOP	
NAM43	PI432395	Bangladesh			MNOP	
NAM15	N01D-1330	Australia			MNOPL	
NAM25	Nolza 541	Argentina			MNOPL	
NAM0	N99-508	Canada	Quantum/LG3260		NOP	
NAM26	Nolza 531	Argentina			NOP	
NAM51	AC Elect	Canada	Topas/Westar, Karat/Westar, Rabo/Westar	2005	NOP	
NAM82	Tribune	Australia	Karoo/Surpass400		NOP	
Quantum	Quantum	Canada	Maluka/88-53473	1995	NOP	
NAM72	YN03-C656	Winter Background	Express/3/N93-P1526////Rsyn2-11		OP	
NAM76	Ebony	Canada		1995	OP	
NAM87	Tanto	France		1990	OP	
NAM50	N01-C113	Winter Background	Express/3/N93-P1526		P	

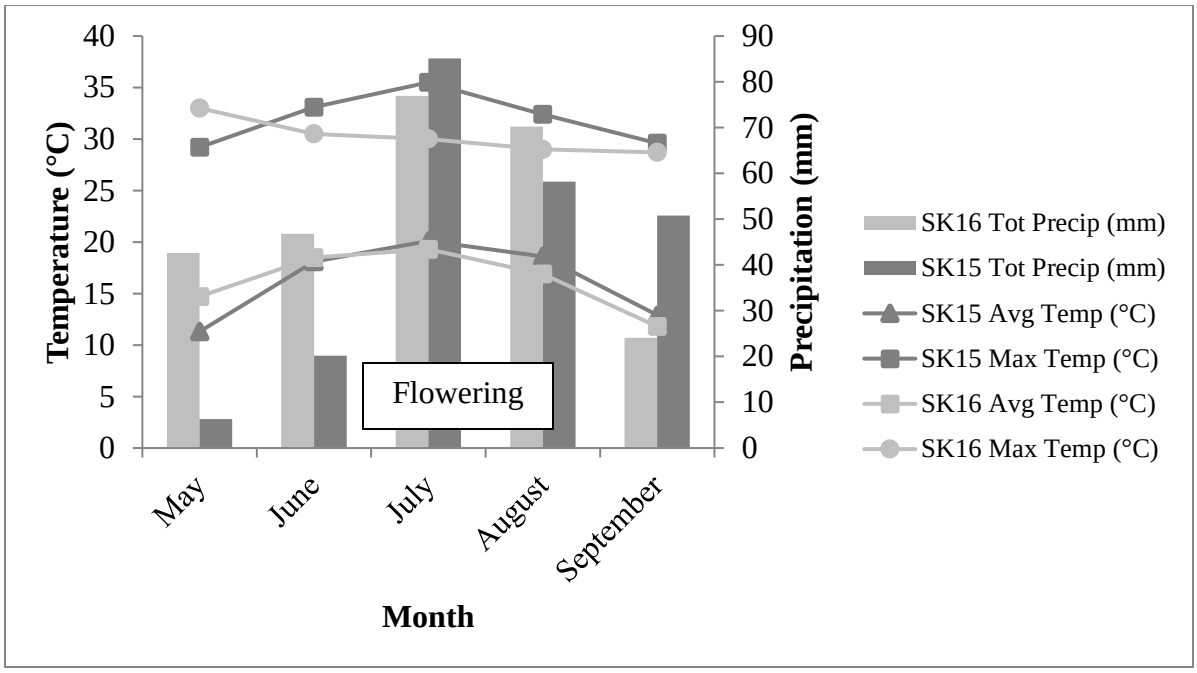

FIGURE A.1: CLIMATIC CONDITIONS IN SASKATOON, SK IN 2015 AND 2016.

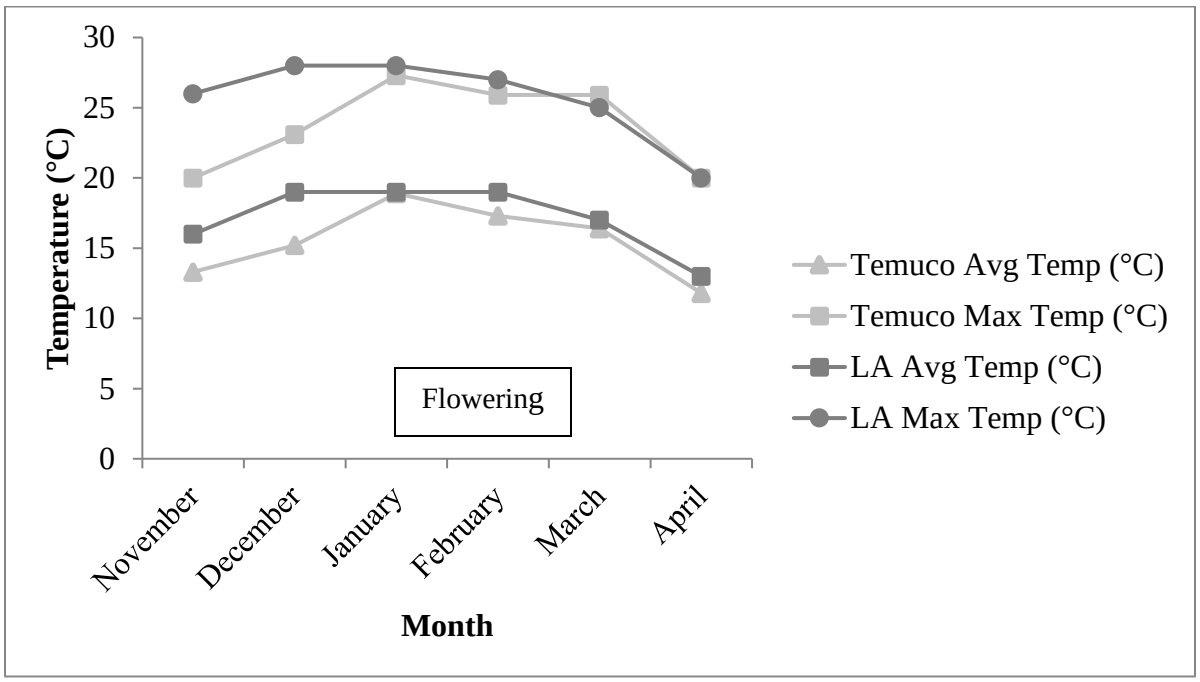

FIGURE A.2: CLIMATIC CONDITIONS IN TEMUCO AND LOS ÁNGELES, CHILE IN FALL 2015 TO SPRING 2016.

Appendix B

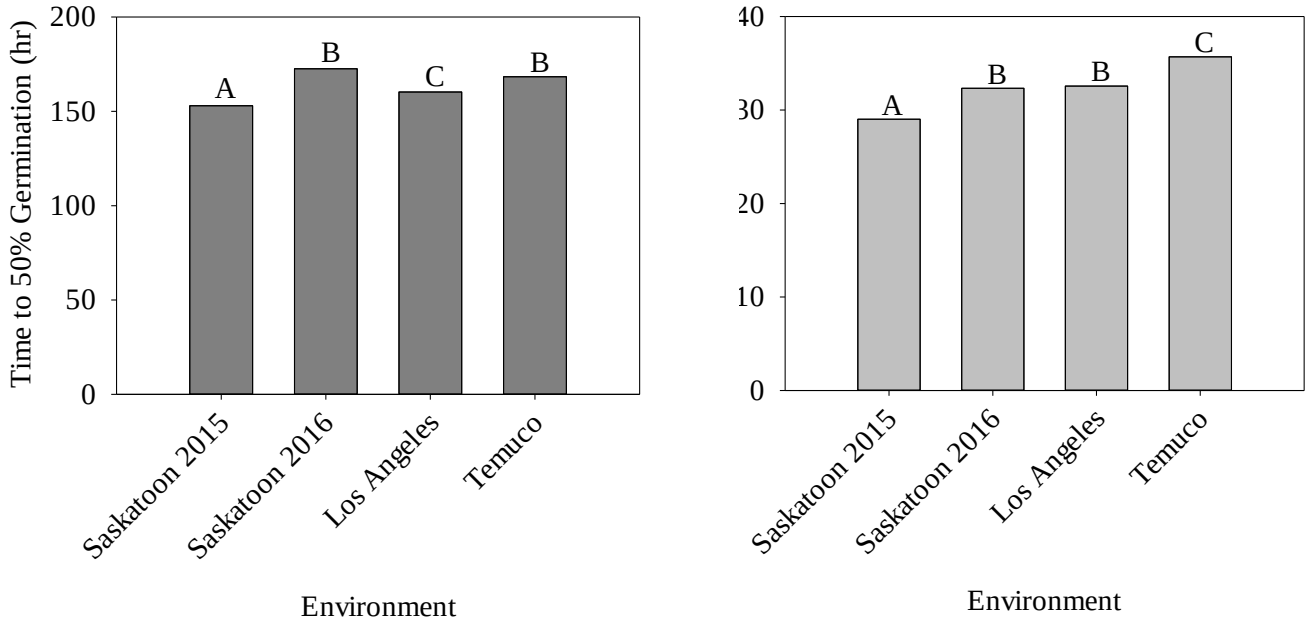

FIGURE B.1: MEAN TIME TO 50% GERMINATION FOR SEED PRODUCTION ENVIRONMENTS AT 5^0C (LEFT) AND 20^0C (RIGHT).

Note: Letters denote significance at $P< 0.05$.

TABLE B.1: CORRELATION BETWEEN SEED PRODUCTION ENVIRONMENTS FOR TIME TO 50% GERMINATION AT 5^0C AND 20^0C.

5^0C	Saskatoon 2015	Saskatoon 2016	Temuco 2016	Los Ángeles 2016
Saskatoon 2015		0.30*	0.20	0.05
Saskatoon 2016			0.07	0.32
Temuco 2016				0.25

20^0C	Saskatoon 2015	Saskatoon 2016	Temuco 2016	Los Ángeles 2016
Saskatoon 2015		0.51**	0.32	0.47**
Saskatoon 2016			0.52**	0.46**
Temuco 2016				0.40*

Note: Numbers denotes correlation coefficients. '*'- $P< 0.05$; '**'- $P< 0.01$.

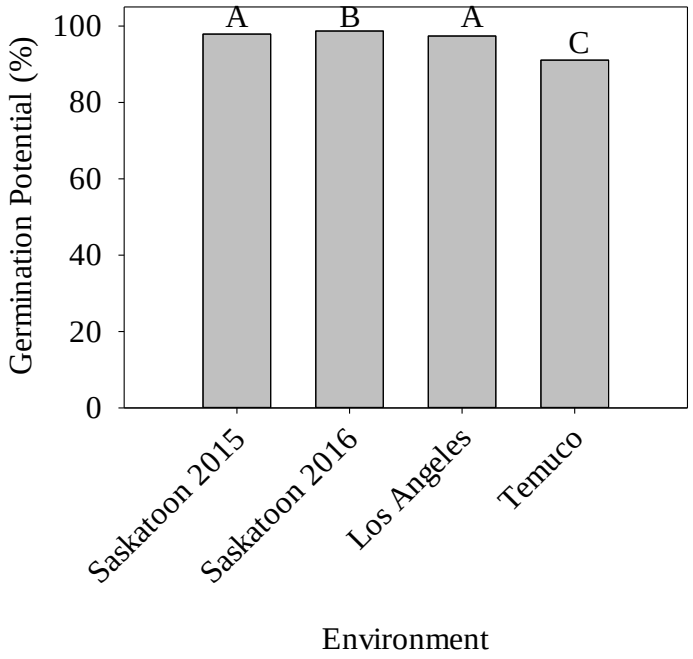

FIGURE B.2: MEAN CONTROLLED DETERIORATION GERMINATION POTENTIAL OF EACH SEED PRODUCTION ENVIRONMENT.

Note: Letters denote significance at $P< 0.05$.

TABLE B.2: CORRELATION BETWEEN SEED PRODUCTION ENVIRONMENTS FOR CONTROLLED DETERIORATION GERMINATION.

	Saskatoon 2015	**Saskatoon 2016**	**Temuco 2016**	**Los Ángeles 2016**
Saskatoon 2015		0.48**	0.13	0.26
Saskatoon 2016			0.17	0.02
Temuco 2016				0.0

Note: Number denotes correlation coefficient. '*'- $P< 0.05$; '**'- $P< 0.01$.

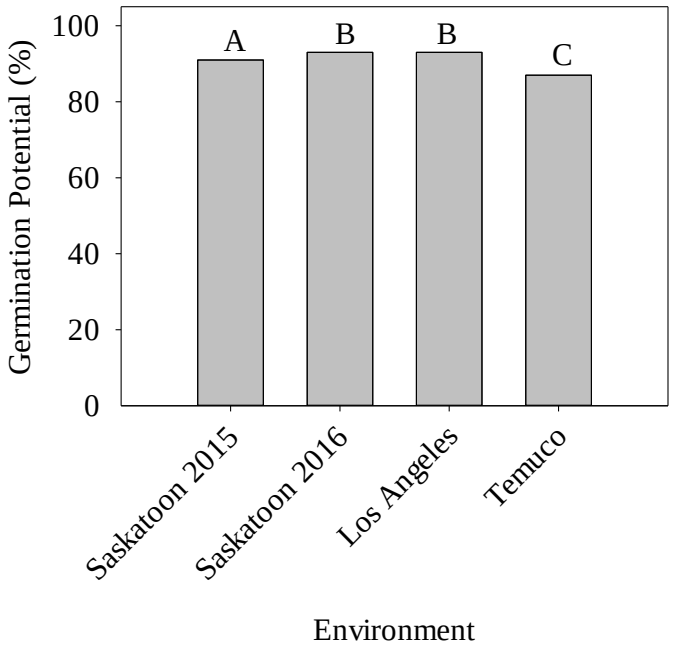

FIGURE B.3: MEAN PRE-CHILL GERMINATION POTENTIAL OF EACH SEED PRODUCTION ENVIRONMENT.

Note: Letters denote significance at $P < 0.05$ and different letters denote environments are significantly different.

TABLE B.3: CORRELATION BETWEEN SEED PRODUCTION ENVIRONMENTS FOR PRE-CHILL GERMINATION.

	Saskatoon 2015	**Saskatoon 2016**	**Temuco 2016**	**Los Ángeles 2016**
Saskatoon 2015		0.31*	0.10	0.35*
Saskatoon 2016			0.31*	0.39**
Temuco 2016				0.25

Note: Number denotes correlation coefficient. '*'- $P< 0.05$; '**'- $P< 0.01$; '***'- $P< 0.001$.

TABLE B.4: PARTIAL LEAST SQUARE REGRESSION ANALYSIS OF SECONDARY DORMANCY AND SEED GERMINATION, VIGOUR AND QUALITY TRAITS.

	Model Effects		Dependant Variable (Secondary Dormancy)	
Significant Model	**Current**	**Total**	**Current**	**Total**
1	12.05	12.05	24.56	24.56
2	13.80	25.85	5.66	30.21
3	9.00	34.83	2.90	33.12

Literature Cited

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity – A practical and critical review. *Trends in Food Science & Technology*. 22: 21-39. doi:10.1016/j.tifs.2010.11.002

AOCS. American Oil Chemists' Society. 2005. Determination of oil, moisture and volatile matter, and protein by near-infrared reflectance. AOCS Standard Procedure Am 1-92.

AOSA. 2009. Seed Vigor Testing Handbook. Contribution No. 32. Association of Official Seed Analysts. Lincoln, NE.

Auger, B., Marnet, N., Gautier, V., Maia-Grondard, A., Leprince, F., Renard, M., Guyot, S., Nesi, N., Routaboul, J.M. 2010. A Detailed Survey of Seed Coat Flavonoids in Developing Seeds of Brassica napus L. *Journal of Agricultural and Food Chemistry*. 58: 6246-6256. doi:10.1021/jf903619v

Baskin, C., Baskin, J. 1998. Types of Seed Dormancy. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Elsevier Inc. p: 27-47.

Beckie, H.J., Warwick, S.I., Nair, H., S**é**guin-Swartz, G. 2003. Gene flow in commercial fields of herbicide-resistance canola (*Brassica napus*). *Ecological Applications*. 13:1276-1294. doi: 10.1890/02-5231

Beckie, H.J., Warwick, S. I. 2010. Persistence of an oilseed rape transgene in the environment. *Crop Protection*. 29: 509-512. doi:10.1016/j.cropro.2009.11.013

Beckie, H. J., Harker, N., Légère, A., Morrison, M., Seguin-Swartz, G., Falk, K. 2011. GM Canola: The Canadian Experience. *Farm Policy Journal*. Vol. 8 Num. 1. Autumn Quarter 2011.

Beckie, H. J. 2015. State of weed resistance in Western Canada and future outlook. (PDF slides). Accessed February 10, 2016 from http://www.weedsummit.ca/images/stories/Presentations/2_Hugh%20Beckie%20-%20HRsummit16-beckie.pdf

Bentsink, L., Jowett, J., Hanhart, C.J., Koornneef, M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*. 45: 17042-17047. doi: 10.1073/pnas.0607877103

Bentsink, L., Soppe, W., Koornneef, M. 2007. Genetic Aspects of Seed Dormancy, in Annual Plant Reviews Volume 27: Seed Development, Dormancy and Germination. Blackwell Publishing Ltd. p. 113-132.

Bewley, J.D. 1997. Seed Germination and Dormancy. *The Plant Cell*. 9: 1055-1066. doi: 10.1105/tpc.9.7.1055

Botwright-Acuña, T.L., Wade, L.J. 2012. Use of genotype x environment interactions to understand rooting depth and the ability of wheat to penetrate hard soils. *Annals of Botany*. 112: 359-368. doi:10.1093/aob/mcs251

Boulesteix, A., Strimmer, K. 2006. Partial least squares: a versatile tool for the analysis of high0dimensional genomic data. *Briefing in Bioinformatics*. 1:32-44. doi:10.1093/bib/bbl016

Bowley, S., 2015. A Hitchhiker's Guide to Statistics in Biology: Generalized Linear Mixed Model Edition. Plants et al. *Inc*. Kincardine, Ontario, Canada.

Brocard-Gifford, I.M., Lynch, T.J., Finkelstein, R.R. 2003. Regulatory networks in seeds integrating developmental, abscisic acid, sugar, and light signaling. *Plant Physiology*. 131:78-92. doi:10.1104/pp.011916

Brunel-Muguet, S., D'Hooghe, P., Bataille, M.P., Larré, C., Kim, T.H., Trouverie, J., Avice, J.C., Etienne, P., Dürr, C. 2015. Heat stress during seed filling interferes with sulfur restriction on grain composition and seed germination in oilseed rape (*Brassica napus* L.). *Frontiers in Plant Science*. 6:213. doi:10.3389/fpls.2015.00213

Bus, A., Körber, N., Snowdon, R. J., Stich, B. 2011. Patterns of molecular variation in a species wide germplasm set of *Brassica napus. Theoretical and Applied. Genetics.* 123*:*1413—1423. doi:10.1007/s00122-011-1676-7

Canola Council of Canada. 2017. Canola Encyclopedia: Time of Seeding. Accessed June 13, 2019 from https://www.canolacouncil.org/canola-encyclopedia/plant-establishment/time-of-seeding/

Canola Council of Canada. 2018. Canola Encyclopedia: History of Varietal Development. Accessed February 14, 2019 from https://www.canolacouncil.org/canola-encyclopedia/crop-development/history-of-varietal-development.

CGC. 2019. Quality of western Canadian canola 2018. Canadian Grain Commission. Accessed April 10, 2019 from https://www.grainscanada.gc.ca/en/grain-research/export-quality/oilseeds/canola/2018/02-summary.html.

CGSA. 2019. Canadian Regulations and Procedures for Pedigreed Seed Crop Production-Circular 6. Canadian Seed Growers' Association. February 1, 2019. Accessed May 27, 2019 from https://seedgrowers.ca/seed-growers/regulations/

CIE. 1976. International Commission on Illumination. Accessed June 12, 2019 from http://www.cie.co.at/

Debeaujon, I., Leon-Kloosterziel, K.M., Koornneef, M. 2000. Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. *Journal of Plant Physiology*. 122: 403-413. doi: https://doi.org/10.1104/pp.122.2.403

Debieu M., Tang C., Stich B., Sikosek T., Effgen S., Josephs E., et al. 2013. Co-Variation between Seed Dormancy, Growth Rate and Flowering Time Changes with Latitude in *Arabidopsis thaliana*. *PLoS ONE* 8: e61075. doi:10.1371/journal.pone.0061075

DeBonte, L., Iassonova, D., Lui, L., Loh, W. 2012. Commercialization of high oleic canola oils. *Lipid Technology*. 24: 175- 177. doi: 10.1002/lite.201200214

Dekkers, B., Schuurmans, J., Smeekens, S. 2004. Glucose delays seed germination in Arabidopsis thaliana. *Planta*. 218: p 579-588. doi:10.1007/s00425-003-1154-9

Dekkers, B.J.W., He, H., Hanson, J., Willems, L., Jamar, D.C.L., Cueff, G., Rajjou, L., Hilhorst, H., Bentsink, L. 2016. The Arabidopsis *DELAY OF GERMINATION 1* gene affects ABSCISIC ACID INSENSTIVIE 5 (ABI5) expression and genetically interacts with ABI3 during Arabidopsis seed development. *The Plant Journal*. 85: 451-465. doi: 10.1111/tpj.13118.

Demilly, D., Ducournau, S., Wagner, M. H., Dürr, C. 2014. Digital imaging of seed germination. Plant Image Analysis: Fundamentals and Applications. Chapter 7. CRC Press, Boca Raton, FL. pp 147-163.

Downey, R.K., Stefansson, B.R., Stringam, G.R., McGregor, D.I. 1975. Breeding rapeseed and mustard crops. In: Harapuik, J.P. (ed) Oilseed and Pulse Crops in Western Canada: A Symposium. Western Cooperative Fertilizers, Canada. Pg 157-183.

Downey, R.K., Rimmer, S.R. 1993. Agronomic improvements in oilseed Brassica. *Advances in Agronomy*. 50: 1-66.

Ducournau, S., Feutry, A., Plainchault, P., Revollon, P., Vigouroux, B., Wagner, M.H. 2004. An image acquisition system for automated monitoring of the germination rate of sunflower seeds. *Computers and Electronics in Agriculture*. 44:189-202. doi:10.1016/j.compag.2004.04.005

Ducournau, S., Feutry, A., Plainchault, P., Revollon, P., Vigouroux, B., Wagner, M.H. 2005. Using computer vision to monitor germination time course of sunflower (*Helianthus annuus* L.) seeds. *Seed Science and Technology*. 33: 329-340. doi: 10.15258/sst.2005.33.2.06

Duncan, R.W., McVetty, P.B.E., Duguid, J.L., Fox, S.L., Paulmann, W., Sass, O., Fernando, W.G.D., Li, G. 2017. HYHEAR 3 Round Ready®, high erucic acid, low glucosinolates hybrid summer rape. *Canadian Journal of Plant Science*. 97: 1185-1187. doi: 10.1139/cjps-2016-0229

Duran, J.M., Retamal, N. 1989. Coat structure and regulation of dormancy in *Sinapsis arvensis* L. seeds. *Journal of Plant Physiology*. 135: 218-222. doi: 10.1016/S0176-1617(89)80180-4

Elliot, R.H., Mann, L.W., Johnson, E.N., Brandt, S., Vera, C., Kutcher, H.R., Lafond, G., May, W.E. 2007. Vigor Tests for Evaluating Establishment of Canola under Different Growing Conditions and Tillage Practices. *Seed Technology*. 29: 21-36.

Ellis, R. 1992. Seed and seedling vigour in relation to crop growth and yield. *Plant Growth Regulation*. 11(3): 249-255. doi: 10.1007/BF00024563

Fei, H., Tsang, E., Cutler, A. 2007. Gene expression during seed maturation in *Brassica napus* in relation to the induction of secondary dormancy. *Genomics*. 89: 419-428. doi: 10.1016/j.ygeno.2006.11.008

Fei, H., Ferhatoglu, Y., Tsang, E., Huang, D., Cutler, A. 2009. Metabolic and hormonal processes associated with the induction of secondary dormancy in *Brassica napus* seeds. *Botany*. 87: 585-596. doi: 10.1139/B09-022

Feng, F., P., Liu, Hong, D., G., Yang. 2009. A major QTL associated with pre harvest sprouting in rapeseed (*Brassica napus* L.). *Euphytica.* 169: 57-68. doi: 10.1007/s10681-009-9921-8

Finch-Savage, W., Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. *New Phytologist*. 171: 501-523. doi: 10.1111/j.1469-8137.2006.01787.x

Finch-Savage, W.E., Bassel, G.W. 2016. Seed vigour and crop establishment: extending performance beyond adaption. *Journal of Experimental Botany*. 67: 567-591. doi:10.1093/jxb/erv490

Finkelstein, R., Reeves, W., Ariizumi, T., Steber, C. 2008. Molecular aspects of seed dormancy. *Annual Review of Plant Biology*. 78: 687-415. doi:10.1146/annurev.arplant.59.032607.092740.

Geddes, C. M., Gulden, R.H. 2015. Managing seed return of volunteer canola in soybean production. Conference paper. Accessed September 24, 2018 from https://www.researchgate.net/publication/285056759_Managing_Seed_Return_of_Volunteer_Canola_in_Soybean_Production

Geddes C. M., Gulden, R.H. 2017. Early autumn soil disturbance decreases persistence of volunteer summer- annual. oilseed rape (*Brassica napus*). *Weed Research* 57: 182-192. doi: 10.1111/wre.12248

Graeber, K.A.I., Nakabayashi, K., Miatton, E., Leubner-Metzger, G., Soppe, W.J.J. 2012. Molecular mechanisms of seed dormancy. *Plant, Cell and Environment*. 35: 1769-1786. doi: 10.1111/j.1365-3040.2012.02542

Graeber, K., Linkies, A., … Leubner-Metzger, G. 2014. *DELAY OF GERMINATION 1* mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *Proceedings of the National Academy of Sciences of the United States of America*, *111*: E3571–E3580. doi.org/10.1073/pnas.1403851111

Gruber S., Pekrun, C., Claupein, W. 2005. Life cycle and potential gene flow of volunteer oilseed rape in different tillage systems. *Weed Research*. 45: 83–93. doi: 10.1111/j.1365-3180.2004.00422.x

Gruber, S., Emrich, K., Claupein, W. 2009. Classification of canola (*Brassica napus*) winter cultivars by secondary dormancy. *Canadian Journal of Plant Science*. 89: 613-619. doi: 10.4141/CJPS08190

Gruber S., Weber, E.A., Thöle, H., Möhring, J., Dietz-Pfeilstetter, A., Claupein, W. 2018. Impact of cultivar on survival of volunteer oilseed rape populations in fields is more important than field management. *Weed Research*. 58: 89-98. doi: 10.1111/wre.12285.

Gulden, R.H., Shirtliffe, S.J., Thomas, A.G. 2003a. Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. *Weed Science*. 51: 904-913. doi: 10.1614/P2002-170

Gulden, R.H., Shirtliffe, S.J., Thomas, A.G. 2003b. Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. *Weed Science*. 51: 83-86. doi: 10.1614/0043-1745(2003)051[0083:HLOCBN]2.0.CO;2

Gulden, R.H., Thomas, A.G., Shirtliffe, S.J. 2004a. Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). *Weed Research*. 44: 97-106. doi: 10.1111/j.1365-3180.2003.00377.x

Gulden, R.H., Thomas, A.G., Shirtliffe, S.J. 2004b. Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. *Weed Science*. 52: 382-388. doi: 10.1614/WS-03-123R1

Gulden, R., Chiwocha, S., Abrams, S., McGregor, I., Kermode, A., Shirtliffe, S.J. 2004c. Response to abscisic acid application and hormone profiles in spring *Brassica napus* seed in relation to secondary dormancy. *Canadian Journal of Botany*. 82:1618-1624. doi: 10.1139/B04-119

Jones, D.B., 1941. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. US Department of Agriculture- circ. 183. Washington, DC.

Haile, T., Shirtliffe, S.J. 2014. Effect of Harvest Timing on Dormancy Induction in Canola Seeds. *Weed Science*. 62: 548-554. doi: 10.1614/WS-D-13-00178.1

Harker K. N., Clayton G. W., Blackshaw R. E., O'Donovan J. T., Stevenson F. C. 2003. Seeding rate, herbicide timing and competitive hybrids contribute to integrated weed management in canola (*Brassica napus*). *Canadian Journal of Plant Science*. 83: 433-440. doi: 10.4141/P02-073

Harker, K., O'Donovan, J., Smith, E., Johnson, E., Peng, G., Willenborg, C., Gulden, R., Mohr, R., Gill, K., Grenkow, L. 2015. Seed size and seeding rate effects on canola emergence, development, yield and seed weight. *Canadian Journal of Plant Science*. 95: 1-8. doi: 10.4141/cjps-2014-222.

Hatzig, S.V., Frisch, M., Breuer, F., Nesi, N., Ducournau, S., Wagner, M.H., Leckband, G., Abbadi, A., Snowdon, R. 2015. Genome-wide association mapping unravels the genetic control of seed germination and vigor in *Brassica napus*. *Frontiers in Plant Science*. 6: 221. doi: 10.3389/fpls.2015.00221

Hatzig, S., Breuer, F., Nesi, N., Ducournau, S., Wagner, M.H., Leckband, G., Abbadi, A., Snowdon, R. J. 2018. Hidden effects of seed quality breeding on germination in oilseed rape (*Brassica napus* L.). *Frontiers in Plant Science*. 9: 419. doi:10.3389/fpls.2018.00419

Hauser, T. P., Ostergard H. 1998. Precocious germination of Brassica rapa x B. napus seeds within pods. *Hereditas*. 130: 89-93. doi: 10.1111/j.1601-5223.1999.00089.x

He, H., de Souza Vidigal, D., Basten Snoek, L., Schnabel, S., Nijveen, H., Hillhorst, H., Bentsink, L. 2014. Interaction between parental environment and genotype affects plant and seed performance in Arabidopsis. *Journal of Experimental Botany*. 65: 6603-6615. doi: 10.1093/jxb/eru378

Heaney, R.K., Fenwich, G.R. 1980. The analysis of glucosinolates in Brassica species using gas chromatography. Dirst determination of the thiocyanate ion precursors, glucobrassicin and neoglucobrassicin. *Journal of the Science of Food and Agriculture*. 31: 593-599. doi: doi.org/10.1002/jsfa.2740310612

Hilhorst, H.W. M. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research*. 5: 61-73. doi: 10.1017/S0960258500002634

Hilhorst, H.W.M. 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. *Seed Science Research*. 8: 77-90. doi:10.1017/S0960258500003974.

Hilhorst, H.W.M. 2007. Definitions and hypotheses of seed dormancy. Seed development, dormancy and germination. Blackwell Publishing. P 50-72.

Huang, S., Gruber, S., Stockmann, F., Claupein, W. 2016. Dynamics of dormancy during seed development of oilseed rape (*Brassica napus* L.). *Seed Science Research*. 26: 245-253. doi: 10.1017/S0960258516000118

Huang, S., Gruber, S., Claupein, W. 2018. Timing and depth of post-harvest soil disturbance can reduce seedbank and volunteers of oilseed rape. *Soil and Tillage Research*. 175: 187-193. doi: 10.1016/j.still.2017.09.005

Hwang, S.F., Ahmed, H.U., Zhou, Q., Strelkov, S.E., Gossen, B.D., Peng, G., Turnbull, G.D. 2012. Assessment of the impact of resistant and susceptible canola on *Plasmodiophora brassicae* inoculum potential. *Plant Pathology*. 61: 945-952. oi: 10.1111/j.1365-3059.2011.02582.x

ISTA. 2008. International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

Johnson-Flanagan, A. M., Thiagarajah, M.R., Pomeroy, M. K. 1991. The impact of freezing during maturation on storage products in canola seeds. *Physiologia Plantarum*. 81: 301-308. doi: 10.1111/j.1399-3054.1991.tb08736.x

Karsen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E., Koornneef, M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: Studies on abscisic acid deficient genotype of *Arabidopsis thaliana* (L.) Heynh. *Planta*. 157: 158-165. doi: 10.1007/BF00393650

Lamichhane, J.R., Debaeke, P., Steinberg, C., You, M.P., Barbetti, M.J., Aubertot, J.N. 2018. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant and Soil*. 432: 1-28. doi:10.1007/s11104-018-3780-9

Légère, A., Simard, M. J., Thomas, A. G., Pageau, D., Lajeunesse, J., Warwick, S. I., Derksen, D. A. 2001. Presence and persistence of volunteer canola in Canadian cropping systems. Pages 143–148 *in* Proceedings of the Brighton Crop Protection Conference—Weeds. Farnham, Great Britain: British Crop Protection Council.

Li, M., Sun, P., Zhou, H., Chen, S., Yu, S. 2011. Identification of quantitative trait loci associated with germination using chromosome segment substitution lines of rice (*Oryza sativa* L.). *Theory of Applied Genetics*. 123: 411-420. doi: 10.1007/s00122-011-1593-9

Linkies A., Müller, K., Morris, K., et al. 2009. Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. *The Plant Cell*. 21: 3803–3822. doi: doi.org/10.1105/tpc.109.070201

Matakiadis T., Alboresi, A., Jikumaru, Y., Tatematsu, K., Pichon, O., Renou, J.P., Kamiya, Y., Nambara, E., Troung. H.N. 2009. The Arabidopsis abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. *Plant Physiology*. 149: 949-960. doi: 10.1104/pp.108.126938

McLaughlin, N.B., Bowes, G.R., Thomas, A.G., Dyck, F.B., Lindsay, T.M., Weiss. R.F. 1985. A new design for a seed germinator with 100 independently temperature controlled cells. *Weed Research*. 25: 161-173. doi: 10.1111/j.1365-3180.1985.tb00632.x

Mieth, G., Schwenke, K., Raab, B., Bruckner, J. 1983. Rapeseed: constituents and protein products. Part 1. Composition and properties of proteins and glucosinolates. *Die Nahrung*. 27: 675-697. doi: 10.1002/food.19830270713

Momoh, E.J.J., Zhou, W.J., Kristiansson, B. 2002. Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. *Weed Research*. 42: 446-455. doi: 10.1046/j.1365-3180.2002.00308.x

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

Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R, Seo M, Soppe W. 2012. The time required for dormancy release in Arabidopsis is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. *Plant Cell* 24: 2826–2838. doi: 10.1105/tpc.112.100214

Nee, G., Obeng-Hinneh, E., Sarvari, P., Nakabayashi, K., Soppe, W. 2015. Secondary dormancy in *Brassica napus* is correlated with enhanced BnaDOG1 transcript levels. *Seed Science Research*. 25: 221-229. doi: 10.1017/S0960258514000427

Nguyen, T.C.T., Keizer, P., van Eeuwijk, F., Smeekens, S., Bentsink, L. 2012. Natural variation for seed longevity and seed dormancy are negatively correlated in Arabidopsis. *Plant Physiology*. 160: 2083-2092. doi: 10.1104/pp.112.206649

Nguyen, T.C.T., Cueff, G., Hegedus, D., Rajjou, L., Bentsink, L. 2015. A role for seed storage proteins in Arabidopsis seed longevity. *Journal of Experimental Botany*. 66: 6399-6413. doi: 10.1093/jxb/erv348

Nguyen, T.C.T., Obermeier, C., Friedt, W., Abrams, S.R., Snowdon, R.J. 2016. Disruption of germination and seedling development in *Brassica napus* by mutations causing severe seed hormonal imbalance. *Frontiers in Plant Science*. doi: 10.3389/fpls.2016.00322

Nguyen, T.C.T., Abrams, S. R., Friedt, W., Snowdon, R. J. 2018. Quantitative trait locus analysis of seed germination, seedling vigour and seedling-regulated hormones in *Brassica napus. Plant Breeding*. 137: 388-401. doi: 10.1111/pbr.12576

Nonogaki, H. 2014. Seed dormancy and germination- emerging mechanisms and new hypotheses. *Frontiers in Plant Science*. 5: 233. doi:10.3389/fpls.2014.00233

Parkin, I., S. Vail, S. Robinson. 2017. Development of a germplasm resource to dissect complex traits in *Brassica napus*. ADF Project Final Report Project #20110155. Accessed January 30, 2019 from https://www.saskcanola.com/quadrant/System/research/reports/CGDP_SCDC_ADF_2011-155_Parkin_Short_Report-1.pdf

Pekrun, C., Lutman, P., Baeumer, K. 1997. Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. *European Journal of Agronomy*. 6: 245-255. doi: 10.1016/S1161-0301(96)02051-5

Perry, D. 1978. Report of the vigour test committee 1974-1977. *Seed Science Technology*. 6: 159-181.

Postma, F.M., Âgren, J. 2015. Maternal environment affects the genetic basis of seed dormancy in *Arabidopsis thaliana*. *Molecular Ecology*. 24: 785-797. doi: 10.1111/mec.13061

Rakow, G., Woods, D. L. 1987. Outcrossing in rape and mustard under Saskatchewan prairie conditions. *Canadian Journal of Plant Science*. 67: 147-151. doi: 10.4141/cjps87-017

Rakow, G. 2004 (ed.). I. 1 Species origin and economic importance of Brassica. Springer-Verlag Berlin Heidelberg.

Raney, J.P., Rakow G.F.W. 1999. Selection for increased seed mucilage content in yellow mustard. Contribution #79 *in*: New Horizons for an Old Crop, Proceedings of the GCIRC 10th International Rapeseed Congress, 26–29 September 1999, Canberra, Australia. 6 pp. (CD ROM).

Ren, C., Bewely, J.D. 1998. Seed development, testa structure and precocious germination of Chinese cabbage (*Brassica rapa* subsp. Pekinensis). *Seed Science Research*. 8: 385-398. doi: 10.1017/S0960258500004311

Schopfer, P., Placy. C. 1985. Control of seed germination by abscisic acid. III. Effect of embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. *Plant Physiology*. 77: 676-686. doi : 10.1104/pp.77.3.676

Schatzki, J., Allam, M., Kloppel, C., Nagel, M., Borner, A., Mollers, C. 2013a. Genetic variation for secondary seed dormancy and seed longevity in a set of black-seeded European winter oilseed rape cultivars. *Plant Breeding*. 132: 174-179. doi: 10.1111/pbr.12023

Schatzki, J., Schoo, B., Ecke, W., Herrfurth, C., Feussner, I., Becker, H. C., Mollers, C. 2013b. Mapping of QTL for seed dormancy in a winter oilseed rape doubled haploid population. *Theoretical and Applied Genetics.* 126: 2405-2415. doi: 10.1007/s00122-013-2144-3.

Schatzki, J., Ecke, W., Becker, H., Mollers, C. 2014. Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. *Theoretical and Applied Genetics*. 127: 1213-1222. doi: 10.1007/s00122-014-2292-0

Sawatsky, M.L., Clyde, M., Meek, F. 2015. Partial least squares regression in the social sciences. *Quantitative Methods for Psychology*. 11: 52-62.

Simard, M. J., Légère, A., Pageau, D., Lajeunesse, J., Warwick, S. 2002. The frequency and persistence of volunteer canola (*Brassica napus*) in Quebec cropping systems. *Weed Technology*. 16: 433-439. doi: 10.1614/0890-037X(2002)016[0433:TFAPOV]2.0.CO;2

Slinkard, A.E., Knott, D.R. 1995. Harvest of Gold: The history of crop breeding in Canada. University Extension Press. University of Saskatchewan. Pg 140-152.

Soltani, E., Baskin, J.M., Baskin, C.C. 2018. A review of the relationship between primary and secondary dormancy, with reference to the volunteer weed oilseed rape (*Brassica napus*). *Weed Research*. doi: 10.1111/wre.12342

Statistics Canada. 2018. Principle field crop areas, June 2018. Accessed February 14, 2019 from https://www150.statcan.gc.ca/n1/daily-quotidien/180629/dq180629b-eng.htm

Strelkov, S.E., Hwang, S.F. 2014. Clubroot in the Canadian canola crop: 10 years into the outbreak. *Canadian Journal of Plant Pathology*. 36: 27-36. doi:10.1080/07060661.2013.863807

Symons, S.J., Simpson, G.M., Adkins, S.W. 1987. Secondary dormancy in *Avena fatua*: effect of temperature and after-ripening. *Physiologia Plantarum*. 70: 419-426. doi: 10.1111/j.1399-3054.1987.tb02838.x

Swanson, E.B., Herrgesell, M.J., Arnoldo M., Sippell, D.W., Wong, R.S.C. 1989. Microspore mutagenesis and selection: canola plants with field tolerance to the imidazolinones. *Theory of Applied Genetics*. 78: 525-530. doi: 10.1007/BF00290837

Tan, S., Evans, R.R., Dahmer, M.L., Singh, B.K. Shaner, D.L. 2005. Imidazolinone-tolerant crops: history, current status and future. *Pest Management Science*. 61: 246-257. doi: 10.1002/ps.993

Thies, W. 1977. Analysis of glucosinolates in seeds of rapeseed (*Brassica napus* L.): Concentration of glucosinolates by ion exchange. *Z. Pflanzenziichtg*. 79: 331-335.

Tesnier, K., Strookman-Donkers, H.M., Van Pulen, J.G., Van Der Geest, A.H.M., Bino, R.J., Groot, S.P.C. 2002. A controlled deterioration test for *Arabidopsis thaliana* reveals genetic variation in seed quality. *Seed Science and Technology*. 30: 149-165.

van de Venter, A. 2008. What is seed vigour? *Journal of New Seeds*. 2: 67-72. doi: 10.1300/J153v02n04_06

Wang, N., Qian, W., Suppanz, I., Wei, L., Mao, B., Long, Y., Meng, J., Muller, A., Jung, C. 2011. Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the FRIGIDA homologue BnaA.FRI.a. *Journal of Experimental Botany*. 62: 5641-5658. doi: 10.1093/jxb/err249

Weber, E.A., Frick, K., Gruber, S., Claupein, W. 2010. Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. *Seed Science and Technology*. 38: 298-310. doi: 10.15258/sst.2010.38.2.03

Weber, E.A., Gruber, S., Stockmann, F., Claupein, W. 2013. Can low-dormancy oilseed rape (*Brassica napus*) genotypes be used to minimize volunteer problems? *Field Crops Research*. 147: 32-39. doi: 10.1016/j.fcr.2013.03.017

Weitbrecht, K., Muller, K., Leubner-Metzger, G. 2011. First off the mark: early seed germination. *Journal of Experimental Botany*. 62: 3289-3309. doi: 10.1093/jxb/err030

Willenborg, C., Wildeman, J., Miller, A., Rossnagel, B., Shirtliffe, S. 2005. Oat germination characteristics differ among genotypes, seed sizes, and osmotic potentials. *Crop Science*. 45: 2023-2029. doi: 10.2135/cropsci2004.0722

Zhang, J., Hu, L., Redden, B., Yan, G. 2015. Identification of fast and slow germination accessions of Brassica napus L. for genetic studies and breeding for early vigour. *Crop and Pasture Science*. 66: 481-491. doi: 10.1071/CP14269