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CANOLA: A MODERN CROP FOR A MODERN ERA

by

Kenneth James Roché

A Doctoral Document

Presented to the Faculty of

The College of Agricultural Sciences and Natural Resources

In Partial Fulfillment of Requirements

For the Degree of Doctor of Plant Health

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Under the Supervision of Professor Gary L. Hein

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CANOLA: A MODERN CROP FOR A MODERN ERA

Kenneth James Roché

University of Nebraska, 2015

Advisor: Gary L. Hein

The University of Nebraska-Lincoln Doctor of Plant Health program is a professional doctorate program with a comprehensive approach to plants and agriculture. The program emphasizes a broad interdisciplinary education across all plant-related disciplines, practical learning, research, and experience through internships. For my final required internship, I worked as a senior agricultural research intern with Research Designed for Agriculture (RD4AG) in Montana. RD4AG is a contract research organization based in Yuma, AZ with over thirty-years of experience. During my three month internship at RD4AG in Montana, a large portion of my responsibilities centered on managing regulated canola trials that were undertaken in Montana for industry sponsors. I was responsible for gathering, monitoring, and collating, all the raw data as per the standard operating procedures outlined by the study director and sponsors and in accordance with Good Laboratory Practice standards (Title 40 CFR Part 160 FIFRA Good Laboratory Practice Standards). This internship allowed me to experience the intricacies of private sector research and product development prior to commercial release of a novel technology or hybrid cultivar.

Globally, canola products are known to shoppers and grocers alike as a healthy edible vegetable oil and to livestock producers as a nutritious protein meal cake for

animal diets. However, many people may be surprised to learn that the plant we know as canola did not exist more than forty years ago. This document examines the historical context regarding the domestication of *Brassica* crops, the transition of rapeseed to canola, and the breeding techniques, such as half seed breeding, protoplast fusion, introgression, and resynthesis, used to develop canola from traditional rapeseed species, i.e., *B. napus*, *B. rapa*, and *B. juncea*. The document has a special emphasis on the production requirements for canola in North America that include planting, fertility, water, weed, insect, and disease management. The document also provides details on blackleg disease (*Leptosphaeria maculans* (Tul. & C. Tul) Ces. & Not.) and the insect pest, the crucifer flea beetle (*Phyllotreta cruciferae* Goeze) in canola production.

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CHAPTER 1

FROM RAPESEED TO CANOLA

Globally, canola products are well known to shoppers and grocers alike as a healthy edible vegetable oil and to livestock producers as a nutritious protein meal cake for animal diets. However, many people may be surprised to learn that the plant we know as canola did not exist more than forty years ago. This chapter will review the circumstances that gave rise to the creation of canola, the domestication and development of *Brassica* crops, the important compositional components of canola oil, and the economics of canola production across its global markets.

THE DOMESTICATION OF BRASSICA CROPS

Canola, previously known prior to the 1970s as oilseed rape, is placed within the mustard family Brassicaceae Burnett (formerly Cruciferae) and in the genus *Brassica* L. (Woodland 2000). The genus *Brassica* contains approximately one-hundred species (FAO 2002) and the domestication of *Brassica* crops dates back to antiquity. Seeds of *Brassica rapa* L. (previously *B. campestris* L., commonly known as turnip rape) have been unearthed at Neolithic sites in Switzerland (Reiner et al. 1995, Prakash et al. 2011). The presence of the turnip rape in the Fertile Crescent can be traced back to 1800 BCE in ancient Assyrian cuneiform documents (Reiner et al. 1995, Prakash et al. 2011). In Asia the growth of rapeseed was recorded in ancient Sanskrit writings dated 2000-1500 BCE (Khachatourians et al. 2001), and it was believed to be introduced to China and Korea from Northern Europe ca. 2000 BCE (Raymer 2002). The Tollund Man, a 4th century Scandinavian mummified corpse, contained seeds of *B. rapa* within his stomach (Prakash

et al. 2011). Further, in the Mediterranean and Europe, *Brassica nigra* - Koch (black mustard), *Brassica napus* L. (rapeseed and root forming rutabaga) and *Brassica rapa* (turnip and Chinese cabbage) were known to the Greeks (e.g., Theophrastus ca. 370-285 BCE) and the Romans (e.g., Pliny the Elder ca. 25 BCE) (Bell 1982, Reiner et al. 1995, Livarda and Van der Veen 2008, Prakash et al. 2011). Evidently, *Brassica* species were a practical oil substitute for European countries that could not cultivate olive and poppy oils.

European countries made the transition from using *Brassica* species as food and fodder crops to including the cultivation and production of an edible oil, lamp oil, and soap made from the seeds of *Brassica* species during the Middle ages (Appelqvist and Ohlson 1972, Khachatourians et al. 2001, Raymer 2002, Prakash et al. 2011). However, the cultivation and production of oil from *B. napus* has been a relatively new occurrence. European records indicated the cultivation of *B. napus* rapeseed for oil production began to appear around the 15th century (Prakash et al. 2011). Shortly after the invention of the steam engine that heralded the dawn of the industrial age in the 18th century, it was discovered that rapeseed oil had unique properties that allowed it to adhere to metal parts in the presence of water, thus making it an efficient marine lubricant (Appelqvist and Ohlson 1972, Bell 1982, Prakash et al. 2011). As the industrial age spread throughout the civilized world, so did the cultivation and production of rapeseed oil derived from the *Brassica* crop complex.

In general, the *Brassica* crop complex consists of six species (Fig. 1.1); *B. nigra* (black mustard), *B. juncea* (L.) Czern. (mustard greens), *B. rapa* (three groups, oleiferous, leafy, and turnip/root forming), *B. napus* (oilseed rape, and root forming/rutabaga), *B.*

oleracea L. (cole crops, i.e., leaf, stem, and flower vegetable crops), and *B. carinata* Braun (Ethiopian mustard). Morinaga (1934) was the first to elucidate the genomic relationships among the six *Brassica* species. Later this was verified and diagrammatically represented as the The Triangle of U by Nagaharu (1935) (Fig. 1.1). Three species were found to be monogenomic, *B. nigra* ($2n = 16$, BB), *B. rapa* ($2n = 20$, AA), and *B. oleracea* ($2n = 18$, CC). The natural occurring hybridization of *B. nigra* x *B. rapa*, *B. rapa* x *B. oleracea*, and *B. oleracea* x *B. nigra* resulted in the digenomic, amphidiploid hybrids, i.e tetraploids *B. juncea* ($2n = 36$, AABB), *B. napus* ($2n = 38$, AACC), and *B. carinata* ($2n = 34$, BBCC), respectively (Fig. 1.1). Of these *B. oleracea*, *B. rapa*, *B. juncea*, and *B. napus* are highly polymorphic, i.e., each includes vegetable, root, and oilseed crops (Raymer 2002, Prakash et al. 2011).

CREATING CANOLA

Prior to World War II, many European countries were producing edible oils from rapeseed. European production represented approximately 7.7% of global production; however, Asia produced represented approximately 74.5% of the global production (Khachatourians et al. 2001). Since the 18th century, forage rape had been grown in Canada, but the earliest record of rapeseed production in Canada was in 1936 and credited to a migrant farmer from Poland, a Mr. Fred Solvoniuk at Shellbrook, Saskatchewan (Bell 1982, Khachatourians et al. 2001). The rapeseed Mr. Solvoniuk brought with him from Poland was later identified as *B. rapa* L. (Polish type).

The military blockades of World War II severely limited Canada's access to Asian and European sources and supplies of rapeseed oil. This drove increased interest in

research and significantly increased rapeseed production within Canada. In 1942, Dr. Stevenson, the Head of the Crop Division of Canada Department of Agriculture, was mandated to begin increasing Canadian production. Dr. Stevenson planted the 1942 harvest of *B. napus*, 2,600 lbs., for the 1943 planting along with 41,000 pounds of *B. napus* of Argentine origin (Argentine type) purchased from the U.S. (Khachatourians et al. 2001). The Polish and Argentine rapeseed types were well suited for the prairies of Canada and provided much needed marine lubricant for the U.S. Navy during WWII.

After WWII, Canada had significant production and processing capacity, but faced an uncertain market. Rapeseed contained approximately 40-42% oil on a dry weight basis, and the resultant meal contained approximately 38-42% protein (Appelqvist and Ohlson 1972, Khachatourians et al. 2001). However, the high erucic acid content of rapeseed oil, about 55%, was determined to have heart damaging effects (Appelqvist and Ohlson 1972, Bell 1982, Khachatourians et al. 2001), and the resultant meal cake contained levels of glucosinolates that were harmful to livestock. To address these issues, Canada pursued three lines of research: utilization of meal cake for livestock feed, development of edible oil, and plant breeding (Khachatourians et al. 2001).

Traditional breeding programs were initiated to address the challenges of high erucic acid and high glucosinolate levels in rapeseed. The initial programs took place in Saskatoon, and the Universities of Alberta and Manitoba. Key researchers, such as Drs. Keith Downey and Baldur Steffanson, used new technologies and techniques (e.g., gas-liquid chromatography combined with half-seed breeding), to develop low erucic acid and high nutrition (low glucosinolates) meal cake varieties (Bell 1982, Khachatourians et al. 2001).

The first Canadian variety with low erucic acid, 'ORO' (*B.napus*), was released in 1968 (Bell 1982, Khachatourians et al. 2001). 'Tower' (*B. napus*), released by Dr. B. R. Steffanson in 1974, was the first Canadian variety to contain both low erucic acid and low glucosinolate content (Bell 1982, Khachatourians et al. 2001). Interestingly, the low glucosinolate character found in Tower came from a low erucic acid, low glucosinolate content Polish variety 'Bronowski' (*B. napus*), released in Poland in 1955 (Bell 1982, Khachatourians et al. 2001). 'Candle' released by Dr. R. K. Downey in 1977 was the first *B. rapa* variety to contain both low erucic acid and low glucosinolates (Bell 1982, Khachatourians et al. 2001). The release of Tower and Candle mark the beginnings of the "double low" or 00 designations for rapeseed oil, and thus, a new generation of rapeseed cultivars. These events signaled the genesis of a new global commodity crop. In 1978, the name "Canola" was trademarked to represent these new low erucic, low glucosinolate varieties (Canola Council of Canada, 2014b). "Canola is a contraction of Canada and ola, meaning oil" (Canola council of Canada 2014a).

Canola quality rapeseed oil is derived from three *Brassica* species, *B. rapa*, *B. napus*, and *B. juncea*, but oil can be extracted from all six cultivated *Brassica* crop species. Canola must meet the following internationally regulated standards: "the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Canola council of Canada 2014a).

CANOLA OIL QUALITIES OF INTEREST

Canola grade oil, processed rapeseed oil from *B.rapa*, *B.napus*, or *B. juncea*, must fall below two important anti-nutritional thresholds. This was accomplished by using traditional breeding methods, but producers needed to highlight the beneficial properties that made canola attractive to a wide variety of end users.

The compositional components of canola provided the beneficial properties when compared to other vegetable oils on the market. Dietary oils contain fatty acids that are used by the body as fuel in the form of adenosine triphosphate (ATP). Fatty acids are composed of a carboxylic acid on one end (termed the alpha end) and a long aliphatic chain (no ring structure) with an even number of carbon atoms (i.e., 12-28) terminating in an alkyl group (termed the omega end). A saturated fatty acid (SFA) occurs when only single bonds exist between the carbon atoms in the chain. Additionally, there are two types of unsaturated fatty acids. Monounsaturated fatty acids (MUFAs) contain one double bond between carbon atoms in the aliphatic chain with the remainder having single bonds. By contrast, polyunsaturated fatty acids (PUFAs) contain two or more double bonds between carbon atoms in the chain.

PUFAs are named according to the first carbon to carbon double bond from the omega (ω) end. To clarify, omega-3 (ω -3) and omega-6 (ω -6) fatty acids are PUFAs with the first carbon to carbon double bond at the third and sixth carbon counting from the omega end, respectively. Omega-3 and omega-6 fatty acids are essential fatty acids, but they cannot be synthesized by the human body. Thus, they must be obtained from food sources. Three types of omega-3 fatty acids are available: α -linolenic acid (ALA) found

in plant oils, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in marine oils i.e., algae and fish. Sources for omega-6 or linoleic acid (LA) include food oils and certain fruits and nuts.

It is well known that a diet high in SFAs contribute to cardio vascular diseases that can lead to stroke or death (Asiimwe et al. 2007, Mishra and Manchanda 2012, Lin et al. 2013). Diets rich in the amounts of MUFAs and PUFAS as found in canola have proven heart and health benefits (Connor 2000, Gillingham et al. 2011, Mishra and Manchanda 2012, Lin et al. 2013, Fleming and Kris-Etherton 2014). Interestingly, canola contains only 7% SFAs, the lowest among common cooking and salad vegetable oils. Canola contains 18.64% linoleic acid (ω -6), 9.14% α -linolenic acid (ω -3), and 63.23% mono-unsaturated fatty acid (Table 1.6). Canola oil also is a beneficial source of tocopherols (vitamin E) an antioxidant (Lin et al. 2013), and phytosterols that help reduce cholesterol (Mishra and Manchanda 2012). Lastly, like all vegetable oils canola is cholesterol free. The health benefits of canola oil have enhanced the adoption of canola as a healthy dietary source across the globe. This has resulted in an increased number of countries planting acres to canola production.

GLOBAL PRODUCTION OF RAPESEED/CANOLA

Canola (oilseed rape) has achieved worldwide acceptance and is cultivated on six of the seven continents. Between 1961 and 1991, global consumption of canola rose 1175% (Phillips and Grant 2001). Globally, rapeseed/canola is now the third most important source for vegetable oil for human consumption after palm and soybean oils (Table 1.1), ranked first and second, respectively. In addition, worldwide rapeseed/canola protein meal ranks second to soybean (Table 1.2). Globally rapeseed/canola oilseed production is second only to soybean oilseed production (Table 1.3). The total global productions of meal, oil, and oil seed rapeseed in 2015-2016 are expected to exceed 38.4, 25.9, and 64.6 million metric tons, respectively. The top three in world production of meal, oil, and oilseed rapeseed include the European Union, China and Canada ranked from first to third, respectively (Table 1.4). Canada makes a clear distinction between canola and rapeseed, but other markets and countries use other terms for canola grade oils (Gunstone 2004) i.e., oil rapeseed or low erucic acid rapeseed (LEAR). Further, total global rapeseed/canola production numbers, outside of Canada, may include rapeseed production that is not canola grade and used for other purposes, such as industrial products e.g high erucic acid rapeseed (HEAR).

In the United States (U.S.), canola was granted GRAS (generally recognized as safe) status in 1985 by the FDA (Raymer 2002), thus paving the way for canola to be used in foods for the U.S. market. For example, canola oil could be used for salad oils, cooking oils, and baby formula (see fig. 1.2 for further examples of end uses). In turn,

U.S. farmers began to plant canola to meet expected demand. In 1991, the U.S. acreage planted to canola was 155,000 acres (62,726 hectares) that produced 191 million pounds (86.636 metric tons) of seed valued at approximately \$18.5 million. In 2015, 1.7 million acres (687,965 hectares) were projected to be planted that will produce 2.5 billion pounds (11.3 million metric tons) of seed with an approximate value of \$426 million (USDA 2015a).

Most U.S. canola production is in the Northern Great Plains. In 2014, the economic value in the top four states in production was \$334.14 million (North Dakota), \$14.42 million (Oklahoma), \$14.1 million (Montana) and \$9.8 million (Minnesota) (Table 1.5). North Dakota's production represented 86% of all U.S. canola production. In the U.S. and across the globe, canola production and use will continue to rise in order to meet global market needs. Whether it is through increasing acreage for production, increased yield potential, or specialty cultivars to meet end user needs, researchers and breeders will be required to meet the demands of the future

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Table 1.1 WORLD VEGETABLE OIL PRODUCTIONS

The table demonstrates the global oil production levels derived from oilseed crops.

Table 1.1 World vegetable oil production, 2010/11-2014/15. Source: Foreign Agricultural Service, USDA					
Vegetable oil	2010/11	2011/12	2012/13	1/ Preliminary 2013/14 1/	2/ Forecast 2014/15 2/
<i>Million metric tons</i>					
Coconut	3.71	3.41	3.66	3.46	3.43
Cottonseed	4.96	5.22	5.21	5.13	5.13
Olive	3.27	3.45	2.44	3.15	2.34
Palm	49.14	52.44	56.49	59.42	62.44
Palm Kernel	5.75	6.16	6.57	6.99	7.29
Peanut	5.31	5.29	5.49	5.58	5.52
Rapeseed/Canola	23.03	24.10	24.92	26.43	26.98
Soybean	41.40	42.73	43.10	44.96	47.37
Sunflowerseed	12.21	14.73	13.27	15.75	15.16
Total	148.76	157.53	161.13	170.87	175.65

Source: Economic Research Service, United States Department of Agriculture.
<http://www.ers.usda.gov/data-products/oil-crops-yearbook.aspx>

Table 1.2 WORLD PROTEIN MEAL PRODUCTION

The table demonstrates the main sources and production levels for meal protein between 2010- 2015.

Table 1.2 World protein meal production, 2010/11-2014/15. Source: Foreign Agricultural Service, USDA						
Protein meal					1/ Preliminary	2/ Forecast
	2010/11	2011/12	2012/13	2013/14	1/	2014/15 2/
Million metric tons						
Production						
Copra	1.97	1.82	1.95	1.84		1.82
Cottonseed	14.82	15.65	15.65	15.51		15.45
Fish	5.02	4.18	4.37	4.12		4.30
Palm Kernel	6.78	7.26	7.82	8.38		8.70
Peanut	6.46	6.46	6.73	6.83		6.76
Rapeseed/Canola	34.04	35.69	36.90	39.21		39.95
Soybeans	174.43	180.45	181.29	189.34		200.82
Sunflowerseed	12.99	15.66	14.02	16.60		16.01
Total	256.50	267.16	268.71	281.84		293.80

Source: Economic Research Service, United States Department of Agriculture.
<http://www.ers.usda.gov/data-products/oil-crops-yearbook.aspx>

Table 1.3 WORLD OILSEED PRODUCTION

The table demonstrates the main sources and production levels for oilseed between 2010-2015.

Table 1.3 World oilseed production, 2010/11-2014/15. Source: Foreign Agricultural Service, USDA								
Oilseed			1/ Preliminary		2/ Forecast			
	2010/11	2011/12	2012/13	1/	2013/14	1/	2014/15	2/
Million metric tons								
Production								
Copra	5.89	5.56	5.80		5.58		5.53	
Cottonseed	44.42	48.24	46.52		45.35		45.04	
Palm kernel	12.97	13.83	14.89		15.73		16.49	
Peanuts	39.82	38.33	40.12		39.84		38.98	
Rapeseed/canola	60.56	61.57	63.76		71.18		71.33	
Soybeans	264.25	240.49	268.77		283.74		315.06	
Sunflowerseed	33.07	39.68	35.97		42.91		39.78	
Total	460.97	447.70	475.82		504.31		532.20	

Source: Economic Research Service, United States Department of Agriculture.
<http://www.ers.usda.gov/data-products/oil-crops-yearbook.aspx>

Table 1.4 WORLD RAPESEED PRODUCTION BY COUNTRY

The table demonstrates the worldwide production of meal, oil, and oilseed by country.

Table 1.4 World Rapeseed production by country, 2013/14-2015/16. Source: Foreign Agricultural Service, USDA									
Country	Meal,Rapeseed			Oil,Rapeseed			Oilseed,Rapeseed		
	2013/14	2014/15	2015/16	2013/14	2014/15	2015/16	2013/14	2014/15	2015/16
	Thousand metric tons								
China	11631	11505	10975	6579	6500	6195	14458	14600	14100
India	3720	3250	3600	2400	2100	2320	7300	6310	7150
Canada	3925	4150	3945	3050	3230	3070	18551	16410	13300
Japan	1353	1360	1360	1054	1075	1075	2	2	2
European Union	13780	14370	13398	9946	10371	9670	21304	24394	21300
Other	5026	5586	5198	3479	3837	3641	10473	10193	8840
World Total	39435	40221	38476	26508	27113	25971	72088	71909	64692

Source: Economic Research Service, United States Department of Agriculture.

Source: <http://www.ers.usda.gov/data-products/oil-crops-yearbook.aspx>

Table 1.5 U.S. STATES PRODUCING CANOLA

The table demonstrates the economic value of canola production in the U.S.

Canola Price per Cwt and Value of Production – States and United States: 2012-2014						
State	Price per cwt			Value of production		
	2012	2013	2014	2012	2013	2014
	(dollars)	(dollars)	(dollars)	(1,000 dollars)	(1,000 dollars)	(1,000 dollars)
Idaho	25.80	22.00	16.00	16,951	17,501	9,792
Minnesota	26.40	26.40	20.00	11,448	8,494	4,455
Montana	26.20	19.00	16.70	15,563	20,189	14,058
North Dakota	26.50	20.60	17.00	532,094	343,052	364,140
Oklahoma	25.20	20.30	15.00	35,192	42,346	14,415
Oregon	24.00	22.00	18.70	3,395	4,259	2,805
Washington	26.10	21.50	18.40	6,812	13,158	10,378
Other States ¹	24.90	19.60	17.00	8,798	7,449	5,753
United States	26.50	20.60	17.00	630,253	456,448	425,796

¹ Other States include Colorado and Kansas and data withheld above.

Source: National Agricultural Statistic Service (NASS), USDA. Crop values 2014 summary (February 2015).

<http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1050>

Table 1.6 FATTY ACID COMPOSITIONS OF VEGETABLE OILS

The table demonstrates the fatty acid composition of various vegetable oils.

Vegetable oil per 100 g	Calories (k/cal)	SFA (g)	MUFA (g)	PUFA (g)	ω -6 PUFA (g)	ω -3 PUFA (g)	Vitamin E (mg)	Vitamin K (μ g)
Canola	884	7.37	63.23	28.14	18.64	9.14	17.46	71.3
Coconut	862	86.5	5.8	1.8	1.8	0	0.09	0.5
Cottonseed	884	25.9	17.8	51.9	51.5	0.2	35.3	24.7
High oleic canola	884	6.51	71.99	17.1	14.34	2.07	21.8	0
Olive	884	13.81	72.96	10.52	9.76	0.76	14.35	60.2
Palm	884	49.3	37	9.3	9.1	0.2	15.94	8
Palm kernel	862	81.5	11.4	1.6	1.6	0	3.81	24.7
Soybean	884	15.65	22.78	57.74	50.42	6.79	8.18	183.9
Sunflower	884	10.3	19.5	65.7	65.7	0	41.08	5.4

Source: National Nutrient Database for Standard Reference Release 27, USDA
<http://ndb.nal.usda.gov/ndb/search>

Figure 1.1 THE TRIANGLE OF U

The triangle of U represents the genomic relationship between the six cultivated crop species of *Brassica*.

www.hort.purdue.edu taken from (Raymer 2002)

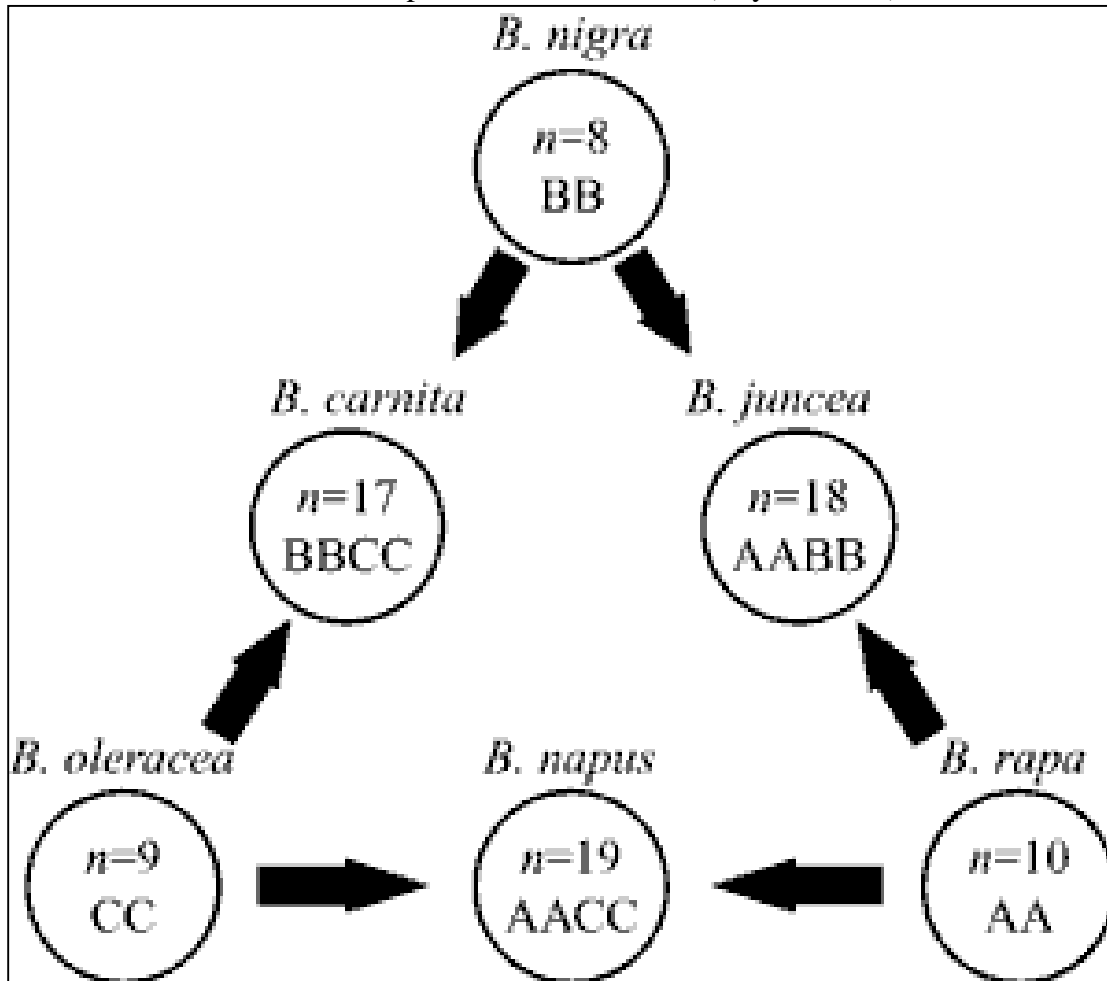
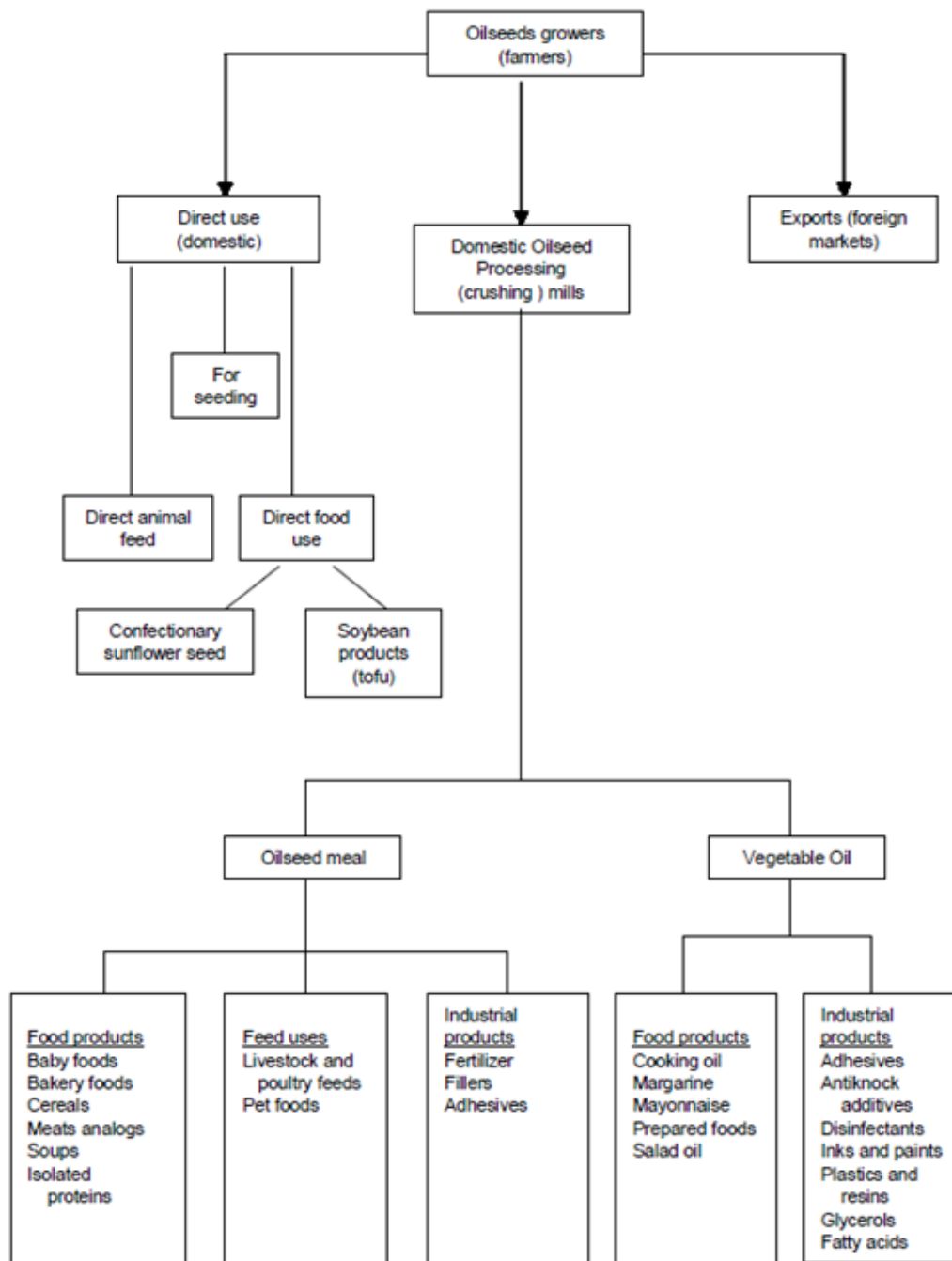


Figure 1.2 OILSEEDS: STRUCTURE OF U.S. INDUSTRY

This illustration demonstrates the possible end uses for canola in the U.S.



Courtesy of the United States International Trade Commission

Source: <http://www.usitc.gov/publications/332/pub3576.pdf>

CHAPTER 2

BREEDING CANOLA

Mankind has practiced domestication and cultivation of plants for approximately ten millennia (Riehl et al. 2013). Though they may not have been aware of the processes involved, farmers from that early era of domestication depended on spontaneous mutation, open pollination, and hybridization. Farmers selected for desired qualities by saving the seeds from their best plants to use in subsequent plantings, i.e., mass selection. In addition, the expansion of civilizations through time introduced and spread new variation from which to select superior types. George Acquaah (2009) defines domestication as "the process by which genetic changes (or shifts) in wild plants are brought about through a selection process imposed by humans." Modern plant breeding is an extension of the domestication of plants. This chapter is an overview of selection and breeding techniques of traditional plant breeding prior to the late-1990s and technological advances in breeding since the late 20th century. Particular emphasis will be given to the selection and breeding techniques that have contributed to the development and improvement of canola.

TRADITIONAL PLANT BREEDING

Key discoveries used in traditional plant breeding can be traced through the 17th, 18th and 19th centuries. The German botanist, Rudolf Jakob Camerarius described sexual differentiation of male and female plant parts. Joseph Gottlieb Kölreuter researched the role of pollen in hybridization of tobacco plants. The seminal work of Gregor Johann Mendel with peas in the 19th established genetic inheritance. However, prior to these

discoveries, mass selection, pedigree selection and bulk breeding were already employed to some extent and are still widely used today (Acquaah 2009). In breeding canola from rapeseed, mass selection, pedigree selection, bulk breeding, and haploidy have been used singularly or in combination with other techniques (Patel et al. 2014). The basis for mass selection was developed by Danish biologist Wilhem Ludvig Johannsen in 1903 (Acquaah 2009). Mass selection was used to improve the average performance of a population as a whole, but this improvement is limited to the genetic variability within the base population. Selection is based on phenotype. Hence, mass selection can be used on large populations to produce one generation for the next cycle, adaptation to a new region, and to maintain a cultivar's purity. Mass selection can be used in self- and cross-pollinating plants.

Pedigree selection was first described by H.H. Lowe in 1927, and it differs from mass selection in that hybridization is used to introduce variability (Acquaah 2009). It is primarily used in self-pollinated crops, but it can be adapted for use with cross-pollinated crops that are hybrids. A base population is established by crossing selected superior parents, e.g., hybrids or elite inbreds. Extensive record keeping is used to manage the increasingly segregating populations to allow the breeder to trace a promising candidate's parent-progeny lineage back to the F_2 plant. The goal of pedigree breeding is to obtain a cultivar with the desired traits by selecting and selfing superior plants through successive generations. The desired traits must be highly heritable in order to reach a desired level of homozygosity (Shahidi 1990, Burton et al. 2004, Udall et al. 2004, Acquaah 2009, Rahman et al. 2011, Rahman 2013)

Bulk breeding was initially developed by Swedish botanist Herman Nilsson-Ehle and expanded upon by Harry V. Harlan and colleagues at the USDA in the 1940s (Acquaah 2009). Characteristics of bulk breeding include delaying artificial selection for later generations after natural selection has eliminated unfit crosses, i.e., from abiotic stresses such as drought, cold and heat. Bulk breeding was primarily used for self-pollinated crops that are also closely spaced in production, e.g., small grains, but it can be adapted to inbred cross-pollinated crops. Single plant selections are made in later generations, and these plants are more homozygous and adapted to the local environment.

The Canadian Agricultural Research Station in Saskatoon, Canada was the starting point for the development of canola, which began with the rapeseed breeding program in 1944 that continues to this day (Khachatourians et al. 2001). The initial breeding program centered around agronomic traits, such as yield, height, uniformity, maturity, shattering, and disease resistance (Bell 1982). *Brassica* species that are used to generate canola in breeding programs have two different methods of sexual reproduction. *B. napus* and *B. juncea* are autogamous or self-pollinating and *B. rapa* and *B. oleracea* are allogamous or cross-pollinating. Consequently, the breeding techniques used to achieve canola characteristics are dictated by these differences.

There are no known wild relatives of canola or *B. napus*, and there are no *Brassica* species native to North America (Rollins 1993, Khachatourians et al. 2001, Prakash et al. 2011). Therefore, breeding the desired oil and meal qualities into canola from rapeseed required the use of many established techniques and development of new methods, e.g., half seed breeding. A partial list of the techniques include recurrent selection, reciprocal crosses, half seed breeding, backcross breeding, and hybrid

breeding. The production of hybrid canola required the development of additional techniques including cytoplasmic male sterility (CMS), generation of haploidy through microspore culture and embryo rescue, protoplast fusion, resynthesis, and advanced biotechnology methodologies, such as marker assisted selection (MAS) and genetic engineering. These breeding techniques used for canola will be discussed and expanded upon further in the following section.

BREEDING TECHNIQUES

Recurrent selection

Acquaah (2009) and Patel et al. (2014) described recurrent selection as a cyclical and systematic technique used to improve the populations of allogamous or autogamous *Brassica* species. Ideally, several different non-related parents are used for the initial crossing, and these parents should exhibit high performance for the desired traits. In canola breeding, recurrent selection was used to select for low erucic acid content, low glucosinolates, disease and insect resistance, less lodging, and less pod shattering. Intermating of the parental material will produce a genetically heterozygous population, and the recombination of genes through crossing can also increase the genetic diversity of the population. After the initial crossing, superior individuals are selected and advanced to the next generation where they are crossed in all possible combinations. This cycle is repeated until the breeder feels all the genes of interest are assembled into a population.

Reciprocal cross

A reciprocal cross is a breeding technique used to determine the inheritance pattern of a desired trait, e.g., determine if the trait of interest is passed on paternally or

maternally (sex-linked) or cytoplasmic inheritance. For instance, a male plant expressing a desired trait is crossed to a female plant not expressing the desired trait and vice versa. After crossing progeny segregation is examined. If both crosses produce similar progeny then the trait is autosomal, i.e., not sex-linked. On the other hand, if the progeny segregate for the trait of interest then the trait is sex linked. The selected parents used in reciprocal crosses must be true breeding, and the trait(s) of interest must be observable phenotypically or quantifiable by chemical or genetic analysis.

Reciprocal crosses have been used in canola breeding to select for important agronomic traits. High levels of resistance to blackleg have been found in European winter varieties of *B. napus*. Transfer of blackleg resistance to spring varieties of *B. napus* cultivated in Australia is desirable but difficult. This indicates that blackleg resistance may be linked to vernalization. Light et al. (2004) made reciprocal crosses in order to determine the inheritance patterns of vernalization. Their results indicated that vernalization were not maternally inherited. Reciprocal crosses are used to identify cytoplasmic male sterility genes, which are maternally inherited (Yamagishi and Bhat 2014). Reciprocal crosses were useful in recovering several male sterile plants with high female fertility from intergeneric hybrids between *B.napus* and *Orychophragmus violaceus* (Hu et al. 2002). Reciprocal single-cross canola hybrids were used to evaluate the performance of triazine-tolerant varieties (Beverdors and Kott 1987). In the initial breeding of rapeseed for low erucic acid content, reciprocal crosses indicated that low erucic acid concentration in *B.napus* were not sex-linked (Downey and Harvey 1963).

Half-seed breeding

The half-seed breeding technique was developed in the early 1960s by Drs. R. K. Downey and B. L. Harvey. This technique was instrumental to breeding low erucic acid and low glucosinolates rapeseed cultivars. Canola seeds are dicotyledonous, permitting the use of one half of the seed, the cotyledon without the embryo, to be analyzed for fatty acid content by gas-liquid chromatography. The remaining cotyledon with the embryo would be saved. If the analysis revealed the desired oil qualities, the remaining cotyledon could be planted for further breeding needs (Downey and Harvey 1963). Prior to the discovery of the half-seed technique, fatty acid analysis was a destructive technique that required about 200,000 whole seeds (1 kilogram) and about two weeks to perform one fatty acid analysis (Murphy 2006). With half-seed breeding, the genotype could be determined one generation earlier and provide more accurate classification of oil characteristics. The increased efficiency of classification and reduced seed and space requirements provide considerable advantages.

Backcross breeding

Backcross breeding is a method to replace an undesirable gene in an otherwise well adapted cultivar or breeding line with a gene that expresses a desirable trait, e.g., disease resistance (Khachatourians et al. 2001, Acquaah 2009, Patel et al. 2014). For successful backcross breeding, the trait of interest must be highly heritable, dominant (though backcrossing for recessive genes is possible), and produce an easily observed phenotype. Backcross breeding involves a recurrent parent and a donor parent. The recurrent female parent is well adapted except for the one undesirable trait of interest,

e.g., disease susceptibility. This parent is crossed to the donor male parent that possesses the desired trait of interest, i.e., disease resistance. After the initial cross, F_1 plants possessing the desired trait are selected and then backcrossed (BC_1) to the recurrent female parent. This is repeated for several cycles until the BC_nF_1 which possesses the desired proportion of the recurrent parent genome, plus the desired trait from the donor parent. After the last backcross, the plants are selfed in order to fix the desired trait in the homozygous state (Acquaah 2009, Patel et al. 2014).

Backcross breeding is an effective technique for autogamous and allogamous reproduction systems. In canola production, backcross breeding has been used to achieve a variety of breeder objectives. Researchers used backcross breeding to introgress agronomic improvements to their patented canola inbred varieties (Kebede et al. 2012, Patel et al. 2014). Backcrossing was used to develop triazine tolerant canola (Beverdors and Kott 1987). Backcross breeding has been used for the creation of interspecific hybrids (Waara and Glimelius 1995, Rakow and Raney 2003, Rahman 2005, 2013, Murphy 2006) and intergeneric hybrids (Pelletier et al. 1983, Hu et al. 2002) to increase genetic diversity. In addition, backcross breeding can be combined with other techniques such as embryo rescue (Kott et al. 1990), resynthesis (Seyis et al. 2010), and to overcome linkage drag, i.e., when introgression of a trait brings with it other undesirable genes that are linked to the desired gene (Hou et al. 2014).

Hybrid breeding

Hybrids are defined by (Acquaah 2009) as "the progeny of a cross between two different species, races, cultivars, or breeding lines." Canola is the product of sexual and somatic (parasexual) hybridization of *B.rapa*, *B. napus*, and *B. juncea*. *Brassica* species can express strong heterosis, i.e., the increased vigor of the hybrid over the parents. In general, the greater the genetic distance between the parents (inbred or hybrid) of the F₁ hybrid, the greater the expression of heterosis in the F₁ hybrid (Lefort-Buson et al. 1987, Brandle and McVetty 1990, Udall et al. 2004, Rai and Rai 2006, Yamagishi and Bhat 2014). The techniques used in canola hybrid breeding include cytoplasmic male sterility, haploidy through microspore culture and embryo rescue, protoplast fusion (somatic hybridization), and resynthesis.

Cytoplasmic male sterility

Cytoplasmic male sterility (CMS) has been instrumental in the production of canola F₁ hybrid cultivars. CMS describes a plant that is female fertile, but it cannot produce functional anthers, pollen, or male gametes (Gustafson et al. 2009, Patel et al. 2014, Yamagishi and Bhat 2014). In CMS breeding, hand-emasculation of the female parent is eliminated, saving considerable time and labor resources. CMS systems are an effective pollination control system used in the formation of hybrid seed because it allows pollination only from one parent (male fertile) to the male sterile female plant. A number of CMS systems have been described, but *Ogu* CMS is widely used in canola breeding. *Ogu* CMS, was originally described in Japanese radish (*Raphanus sativa* L.) (Ogura 1968). *Ogu* CMS was transferred into *Brassica* species through protoplast fusion (e.g., intergeneric hybridization) and backcrossing (introgression) (Patel et al. 2014,

Yamagishi and Bhat 2014). CMS traits are encoded by a gene located in the mitochondria, and thus, it is maternally inherited (Acquaah 2009, Yamagishi and Bhat 2014). In canola, CMS can occur spontaneously or be produced by mutagenesis, artificial hybridization through sexual interspecific or intergeneric crosses, or parasexually through protoplast fusion (Acquaah 2009, Gustafson et al. 2009, Patel et al. 2014).

Components of the CMS system include an A-line (female parent) that is male sterile, a B-line (male parent) that is male fertile (maintainer line) and the R-line with the fertility restorer i.e., the fertility restoring (*Rf*) gene is located within the nucleus. The A-line is crossed with the B-line to increase the A-line for commercial hybrid production. The A-line x B-line progeny is then crossed with the fertile R-line to produce the fertile F₁ hybrid. In addition, both the B-line and R-line are self-pollinated to produce the seed quantities required for commercial hybrid production (Acquaah 2009, Patel et al. 2014, Yamagishi and Bhat 2014). CMS has been used to introduce traits into canola such as clubroot (*Plasmodiophora brassicae* Woronin) resistance (Rahman et al. 2011), create inbred lines in cross pollinating species (Acquaah 2009), and to introduce agronomic traits, i.e., a change in fatty acid composition (Downey and Bell 1990), from intergeneric species (Hu et al. 2002).

Microspore culture

The generation of haploid plants ($n = 1$) through microspore culture or in concert with embryo rescue has contributed greatly to canola breeding. Microspore culture is an *in-vitro* process that involves the timely extraction of immature pollen grains (microspores) from the anthers of unopened flower buds. After extraction, the microspores are cultured on specific media where they are induced to develop into

embryos. Following embryogenesis, haploid plantlets are grown out. These haploid plants are then treated with colchicine, a mitotic inhibitor that doubles the chromosomes of the meristematic tissues and all the subsequent growth. This chromosome doubling produces a double haploid (DH) plant. The DH plant is homozygous, and thus the genetically desired character traits are now fixed. The DH plants can be used as parental material for various breeding techniques, e.g., backcross, recurrent selection, and resynthesis (the artificial production of allopolyploids of naturally occurring allopolyploid plants by utilization of the presumed parental species) (Kott et al. 1990, Henderson and Pauls 1992, Udall et al. 2004, Murphy 2006, Acquaah 2009, Qiong et al. 2009, Ferrie and Caswell 2011).

Embryo rescue

Embryo rescue is one of the oldest *in-vitro* techniques dating back to 1925 (Reed 2005). Embryo rescue involves timely extraction of an immature embryo, ovule, or ovary to a defined media for culture to develop and mature (Reed 2005). Embryo rescue can be used in concert with microspore culture as described above or as a standalone technique. It is particularly useful for hybridization of interspecific or intergeneric crosses (wide hybridization) where sexual incompatibilities exist from either pre or postzygotic barriers (Kott et al. 1990, Reed 2005, Murphy 2006, Acquaah 2009, Rahman 2013, Sosnowska and Cegielska-Taras 2014). Embryo rescue has been employed to transfer traits from wild relatives to canola/rapeseed such as disease resistance to blackleg (*Leptosphaeria maculans*), clubroot (*Plasmodiophora brassicae*) and leaf blight (*Alternaria brassicae*), resistance to cabbage aphid (*Brevicoryne brassicae*), and triazine herbicide resistance (Kott et al. 1990, Murphy 2006). In addition, embryo rescue can be

used in the development of resynthesised rapeseed/canola (Kott et al. 1990, Seyis et al. 2010, Sosnowska and Cegielska-Taras 2014). Lastly, both microspore culture and embryo rescue can accelerate canola breeding programs 30-40% (Kott et al. 1990).

Protoplast fusion

Somatic hybrids can increase the genetic diversity of rapeseed/canola cultivars through interspecific and intergeneric fusion of protoplast (Waara and Glimelius 1995). Protoplasts from the cross between *B.oleracea* x *B.rapa* can be used to resynthesize *B.napus* for different fatty acid compositions (Kott et al. 1990, Heath and Earle 1995, 1997). Protoplast fusion between *Orychophragnus violaceus* and *B. napus* was used to transfer CMS and fatty acids traits (Hu et al. 2002). Pelletier et al. (1983) used protoplast fusion to combine the triazine resistant *B. rapa* x *R. sativa* (CMS trait) and *B.napus* x *R. sativa* (CMS trait) to create a hybrid with two desired traits, i.e., triazine herbicide resistance and CMS capability.

Protoplast fusion (somatic hybridization), i.e., fusion of the genetic information within isolated protoplasts from two distinct species to create a somatic hybrid, is a useful technique for the transfer of genes of interest between sexually incompatible species (interspecific hybridization) or genera (intergeneric hybridization) (Constabel 1976, Kott et al. 1990, Tomar and Dantu 2010). "A protoplast is all the cellular components of a cell excluding the cell wall" (Acquaah 2009). In *Brassica* crops, cells from protoplast fusion can be sourced from leaf mesophyll cells, hypocotyls, roots, stem peels, zygotic embryos or haploid plants (Kott et al. 1990). Protoplast culture and fusion is an *in-vitro* process that involves four steps: protoplast isolation, protoplast fusion,

somatic hybrid selection, and regenerating complete somatic hybrid plants (Kott et al. 1990, Tomar and Dantu 2010).

Protoplast isolation from plants using enzymes was first pioneered by E.C. Cocking (Cocking 1960). To isolate protoplasts, pectinase, cellulase, and hemicellulase enzymes are used to degrade the cell wall, thus releasing the naked cell. These naked cells are purified to remove burst cells, enzymes, and other debris. Protoplast fusion is induced by one of three methods: high Ca^{2+} and high pH, polyethylene glycol (PEG), or an electric field (Tomar and Dantu 2010). Different methods can be used to select for somatic hybrids such as selection media, complementary selection, i.e., selection based on some character expressed by the hybrid that is not present in either parent, mechanical selection by visual means, and the morphology of the regenerated plant. After selection, the somatic hybrids are grown out. (Kott et al. 1990, Tomar and Dantu 2010).

In conclusion, protoplast fusion (somatic hybridization) has incorporated valuable agronomic traits into canola such as desired fatty acid compositions and nutritional qualities, increased seed size, color, yield, and resistance to drought, heat, lodging, pest and disease from two otherwise sexually incompatible species.

Resynthesis

Resynthesis refers to the artificial production of allopolyploids of naturally occurring allopolyploid plants by utilization of the presumed parental species. The cytogenetical relationships among the six *Brassica* crops were described by (Morinaga 1934), which were later verified and diagrammatically represented with The Triangle of U (Nagaharu 1935). As discussed earlier, three species of *Brassica* are used for

rapeseed/canola production, *B. juncea*, *B. napus*, and *B. rapa*. *B. juncea* and *B. napus* are allopolyploids while *B. rapa* is diploid. It is believed that the allopolyploid species, *B. juncea* (AABB, n=18), *B. napus* (AACC, n= 19), and *B. carinata* (BBCC, n = 17) were the results of natural hybridization that evolved from the sympatric areas of their progenitors *B. rapa* (AA, n= 10), *B. nigra* (BB, n = 8), and *B. oleracea* (CC, n = 9) (Morinaga 1934, Nagaharu 1935, Prakash et al. 2011).

The allopolyploid *Brassica* species contain only a subset of the genetic material from their progenitors. In addition, due to intensive breeding for low erucic acid and low glucosinolates, the genetic base of rapeseed/canola has been narrowed. Resynthesized allopolyploids can introduce novel genotypes to breeding programs. Hence, the extant un-adapted diploid progenitors of *B. rapa*, *B. juncea*, and *B. carinata* represent a potential reservoir of genetic variability for breeders (Kott et al. 1990, Waara and Glimelius 1995, Udall et al. 2004, Seyis et al. 2010, Karam et al. 2014). Microspore culture, embryo rescue, and protoplast fusion techniques have been used separately or in combination to resynthesize allopolyploid species. In turn, these hybrids have been incorporated into breeding programs to meet the breeder's objectives. Resynthesized allopolyploid species have been used to introduce valuable character traits, such as heterosis, resistance to diseases, pests, salt, drought, seed yield, seed oil and protein composition. Furthermore, resynthesis of an allopolyploid is not limited to hybridization of their diploid progenitors. Karam et al. (2014) have resynthesized *B. juncea* using non-parental diploids. Their group has presented an alternate method whereby two allopolyploids, i.e., *B. carinata* (BBCC) x *B. napus* (AACC), were crossed to produce an F₁ hybrid. The F₁ hybrid was then treated to double its chromosomes, selfed, and selected for *B. juncea* (AABB) (the C

genome was lost during chromosome doubling) thus, producing a novel genotype distinct from the conventional resynthesized method. Incorporation of resynthesized allopolyploids from rapeseed/canola into breeding programs should increase with continued research and technological advances.

Marker assisted selection

Marker assisted selection (MAS) is a method for discriminating among variability to advance the breeding population. The assumption by breeders is that molecular markers are either closely linked to alleles that have a quantitative effect on a trait or can be used for selection of qualitative traits (Acquaah 2009, Patel et al. 2014). Markers can be classified into two categories, morphological and molecular markers. Morphological markers are generally selected at maturity. For example, yellow seed coat in canola can indicate lower fiber and higher oil content (Rakow and Raney 2003). Growth habits can be used for selection, e.g., leaf clasping on the stem (Khachatourians et al. 2001) or the presence or lack of reproductive organs. Molecular markers can be used to discriminate genetic differences that may or may not have phenotypic expression. The ability to characterize and segregate genetic relationships between desirable and undesirable traits allows breeders to accelerate breeding programs, e.g., in backcrossing schemes, parent selection, and the ability to segregate prospective candidates to advance from a large population.

There are a number of molecular markers techniques available to the breeder today. A partial list of these would include isozyme electrophoresis, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), sequence characterized amplified regions (SCAR),

amplified fragment length polymorphism (AFLP), and single nucleotide polymorphisms (SNPs). Molecular markers have been applied to many aspects involved in canola breeding. Gustafson et al. (2009) used simple sequence repeats (SSR) molecular markers to determine fertility restoration in CMS lines. Pelletier et al. (1983) used known nuclear and cytoplasmic markers that were expressed morphologically to characterize and select their somatic hybrids. In the review on somatic hybridization, Waara and Glimelius (1995) note that isozyme and RFLP analysis were used to characterize somatic hybrids in Brassicaceae. To characterize the intergeneric hybrids between *O. violaceus* x *B. napus* HU et al. (2002) used RAPD. Burton et al. (2004) used AFLP to assess the genetic diversity of 77 breeding lines of canola quality *B. juncea* from three major breeding programs.

The reviews written by Murphy (2006) and Rahman (2013) outline the increasing use of several molecular markers techniques involved with breeding canola. Yellow seed color in canola is associated with 55% reduced fiber content in the meal, greatly increasing the value for livestock feed. Kebede et al. (2012) constructed a genetic linkage map using SSR markers for yellow seed color in *B. rapa* that was used to determine the qualitative trait loci (QTL). This knowledge helped breeders identify the causative gene for yellow seed color. Banga and Kaur (2009) resynthesized a novel allopolyploid *B. juncea* (AABB) from two non-parental allopolyploids, *B. napus* (AACC) x *B. carinata* (BBCC). SSR markers were used to select, characterize, and demonstrate the distinctiveness of this novel allopolyploid compared to a conventionally resynthesized allopolyploid, thus accelerating backcross breeding (Gepts 2002). Furthermore, molecular markers helped elucidate the entire genomic sequence of *B. rapa* accession

Chiifu-401-42 (Wang et al. 2011). Also, other *Brassica* species are being sequenced with the help of molecular markers. The information gathered will be invaluable to canola breeders moving forward, and this will have particular relevance to those working with transgenic traits in canola.

Transgenics

Transgenes in canola breeding are used to express various phenotypes and genotypes of agronomic interest, e.g., resistance to disease and pests, herbicide resistance, and altered seed characteristics (Patel et al. 2014). Transgenesis is a genetic engineering process whereby an exogenous gene is inserted into the genome of a well-adapted cultivar using transformation. Transformations in canola are mediated by *Agrobacterium tumefaciens* Smith & Townsend. Cardoza and Stewart Jr. (2006) have demonstrated high-frequency *A. tumefaciens* mediated transformation in canola.

The first transgenic canolas were commercially released in 1995, and they expressed glufosinate (Liberty Link) and glyphosate (Roundup) herbicide resistance (Devine and Buth 2001, Stringam et al. 2003). In 2000, a third transgenic canola resistant to the herbicide bromoxynil was released (Devine and Buth 2001). A number of other transgenes have been inserted into the canola genome to improve cultivar performance. Wang and Fristensky (2001) identified a pea defense gene that expresses resistance to aggressive blackleg isolates and to *Rhizoctonia solani* Krühn that was successfully transferred to *B napus*. Also a gene from *Arabidopsis thaliana* L. that increases trichome density has been introgressed that deterred feeding by flea beetles (*Phyllotreta* spp.) (Soroka et al. 2011). Other examples of transgenes used in canola include tolerance to

flooding stress at a metal-contaminated site (Farwell et al. 2007), expression of a Bt (Cry1Ac) toxin against resistant ecotypes of diamondback moth (*Plutella xylostella* L.), introgression of desired traits with hybrids that possessed transgenes for male sterility and fertility restoration (Udall et al. 2004), and modification of lipid composition (Murphy 2006).

It should be expected that as more canola species are sequenced researchers would be better able to elucidate the function and the locations of genes for traits of interest. Hence, breeders will have greater access to information to further their breeding objectives. Transgenic technologies offer exciting opportunities for introducing valuable traits, but it is not a standalone technique in plant breeding. Traditional breeding methods, e.g., those mentioned in this chapter, will always be necessary because of the unforeseen and unpredictable gene by environmental interactions that influence cultivar performance and market success.

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CHAPTER THREE

CANOLA PRODUCTION

The *Brassica* species, *B.napus*, *B.rapa*, and *B. juncea*, are grown for canola grade oil and meal. Canola production occurs primarily in Europe, Asia, Canada, and Australia and to a limited extent here in the United States. Canola is produced using both spring and winter varieties. Winter varieties are fall sown, require a vernalization period in order to flower the following year, and generally yield better than spring varieties (CCC 2014a). Winter varieties of *B.napus* are grown in Europe, and parts of China. Further, *B. napus* varieties matures on average 105 days from seeding to harvest requiring more frost free days than *B.rapa* varieties that require on average 85 days from seeding to harvest (CCC 2014a, Khachatourians et al. 2001). Spring varieties of *B. napus* are grown in Canada, northern Europe, United States, and China. In the United States and Australia spring varieties of *B.napus* can be grown as a fall-sown winter crop. Spring varieties of *B.rapa* are grown in Canada, northern Europe, China, and India. Spring varieties of *B.juncea*, which are more drought and heat tolerant than *B.napus* and *B.rapa*, are the dominant *Brassica* species grown in India (Raymer 2002, Gunstone 2004). There are many factors that can influence the agronomic practices for producing canola. These vary by continent and country and include latitude, species, variety, and regional pest and disease pressures as well as market forces. This chapter will give particular emphasis to production in North America, i.e., Canada and the United States of America.

PLANTING

Cultivation of canola is suited to temperate regions and can tolerate a wide range of soil pH values (5.5 - 8.0). In Canada, canola is grown in the western provinces of Alberta, Saskatchewan, and Manitoba and to a limited extent in Ontario and Quebec (CCC 2014c). In the United States, canola is grown in the Great Plains states of North Dakota, Montana, Oklahoma, Colorado, and Kansas, as well as, Washington, Minnesota, Idaho, and Oregon (NASS 2014). *Brassica* species used in canola production are cool season crops that are best suited to temperatures between 12 - 30°C (53.6-86°F) with 21-25°C (69.8-77°F) considered optimum (CCC 2014b, Khachatourians et al. 2001, Gunstone 2004).

Winter temperatures in the regions of Canada and the northern U.S. states where canola is grown are too severe for winter varieties of canola to be grown reliably. Thus, spring varieties are sown according to predicted last spring frost in order to mature before freezing temperatures occur in the fall, i.e., April - mid May. Delayed or late plantings risk exposure to summer heat and drought that adversely affect flowering and pod set or the crop may not reach maturity prior to the first frost of winter, thus reducing yields significantly (Johnston et al. 2002). Soil temperatures should exceed 2 - 4°C (~36 - 40°F) for successful seed germination (CCC 2014d, 2104e). Lower soil temperatures will delay germination and may lead to seed rot and lower emergence rates. Some factors that affect seed germination can be monitored by the grower such as, soil temperature, and soil moisture. While other factors that affect seed germination such as, seed bed preparation, seed planting depth, and seed to soil contact are directly under the control of the grower.

The higher cost of hybrid seed versus conventional seed can impact profit margins. Hence, overplanting hybrid seed can reduce profits. Research conducted by Shirtliffe and Hartman (2009) demonstrated that increasing seeding rates do not always equate to increased yields. Therefore, planting strictly according to pounds acre⁻¹ or kg hectare⁻¹ should be avoided as there exist great variation between 1000 seed weight (TSW) counts between seed lots due to size of seed and species. Instead, it is suggested that a grower plant to a desired plant populations based on seed size to achieve the optimum yield potential specific to their area. In part, optimum yield potential is based on final plant stands as determined by in-field germination rates (assuming germination rates are equal to seedling survival and 50% being the average) (CCC 2014d). In the northern United States, such as North Dakota target optimum plant populations are 10 - 16 plants ft⁻² (100 - 160 plants m⁻²) (Kandel and Knodel 2015) while in Canada target plant populations are 7-10 plants ft⁻² (70 -100 plants m⁻²) (Shirtliffe and Hartman 2009). To effectively manage the seed purchase, the following formula has been developed: seeding rate (lb./ac.) = $[9.6 \times \text{desired plant density (plants/ft}^2) \times \text{TSW (grams)}] \div \text{estimated seedling survival (\%, expressed as a whole \#)}$ (CCC 2014d). For example, in North Dakota if the TSW was 3.5 grams then; $[9.6 \times 10 \text{ (plants/ft}^2) \times 3.5 \text{ (grams)}] \div 50\%$ (estimated seedling survival) = 6.7 lb./ac or 7.5 kg/ha. The grower should follow up with stand counts throughout the season to determine if desired plant populations were achieved.

Canola should be planted into a fine, firm, moist, and well-structured seedbed at a depth of 1/2" - 1" (12 to 25 mm) (CCC 2014f, Gunstone 2004, Kandel and Knodel 2015). While a variety of seeding equipment can be used to plant canola, typically a grain drill

or precision drill is used. This ensures good seed to soil contact, and uniform depth, plant, and row spacing. To prevent soil erosion and preserve soil moisture, canola growers' use no-till or modified-till systems, i.e., only the seed row is tilled, in the semiarid regions of North America. In China, growers use transplant seedlings from seedbeds that are transplanted by hand into the fields because the availability of affordable labor (Gunstone 2004).

FERTILITY MANAGEMENT

Optimum fertility management before, during, and after the growing season encourages the development of vigorous and healthy plants. Vigorous plants are better able to defend against disease, pest, and abiotic stressors, thus maximizing yield potential. In addition, fertility management protects the soil resource ensuring long-term sustainable productivity. Factors that influence soil fertility include soil type, organic matter content, cropping history, fertilizer regimes, irrigation, residue management, and other management practices.

Soil sample testing can provide adequate estimates of soil fertility. Soil fertility should be determined prior to seeding, either in late fall when soil temperatures 41-45°F (5-7°C) have reduced microbial activity, or early spring as soon as the ground has thawed. Soil tests will quantify the amount of important nutrients within the soil profile analyzed, such as nitrogen (N), phosphorous (P), potassium (K) and sulfur (S). Further, soil tests measure soil properties that can affect nutrient availability. Soil properties measured by a soil test include pH, soil organic matter (SOM), soil texture, i.e., the proportion of sand, silt and clay that play an important role in nutrient retention or

leaching, and cation exchange capacity (CEC) i.e., the capacity of roots and soil aggregates to either attract cations (e.g., NH^{+4}) or repel anions (e.g., NO^{-3}).

In order to collect a representative soil sample, proper sampling methods should be followed. *Brassica* crops develop deeper roots systems than cereal crops, thus the sampling depth should be to 60 cm (24"). Ideally, two sample depths should be collected 0-15cm (0-6") and 15-60cm (6-24"), but if only one sample depth can be collected then collect samples from 0-15cm (0-6") for analysis (CCC 2014g). In a uniform field, 20 random soil samples (cores) are collected into one composite sample, and that sample is sent to a lab for analysis. Collect soil cores from representative areas and take care to avoid known problems or unusual areas, e.g., hilltops, low depressions, and saline areas. If there were differences noted within a field, e.g., from historical yield data, then a separate sample should be collected for site specific areas to allow for precise analysis and site specific fertilizer application. However, soil test results from the same samples collected can differ between labs due to different techniques, calibration standards and equipment used. For this reason, a grower should stick with one lab over time to maintain relevant and consistent records on which to base the appropriate fertility management decisions into the future.

Nitrogen

Other than water, nitrogen is the most limiting nutrient for canola production. It has been estimated that every bushel of canola requires 2.9-3.5 lbsN (1.3-1.6 kg) (CCC 2014h). In North Dakota, which produced 85% of the canola in the U.S., the average yield from 2005-2014 was 31.12 bushels acre⁻¹ or 1556 lbs acre⁻¹ (1744 kg ha⁻¹) (NASS 2015), thus requiring 90-109 lbs-N acre⁻¹ (101- 122 kg ha⁻¹). However, this does not take

into account N loss due to leaching, mineralization, residue or microbial tie-up, or volatilization. Work at North Dakota State University suggests the nitrogen rates for optimum canola growth in cooler, moist regions should have an upper limit of 150 lbs acre⁻¹ (168 kg ha⁻¹) and 120 lbs acre⁻¹ (135 kg ha⁻¹) for warmer drier regions (Franzen 2011). Mineralization is the process where microorganisms decompose soil organic matter into a more plant accessible form, thus increasing root available N throughout the growing season. The rate of mineralization is dependent on soil characteristics, available moisture and temperature, thus mineralization estimates range from 6-20 lbs acre⁻¹ (7-22 kg ha⁻¹) for every percentage point of soil organic matter (SOM). For example, for soils with 2% SOM (typical for agricultural soils), 12-40 lbs acre⁻¹ (13-45 kg ha⁻¹) will be available during the growing season (CCC 2014h).

A grower can estimate how much N to apply by incorporating the results from the soil tests and the previous crop credit found in Table 3.1 into the following formula from Franzen (2010):

$$NR = (YP \times 0.065) - STN - PPC$$

Where NR = supplemental nitrogen recommended

YP = yield potential in lb/acre

STN = soil test nitrate-N (0-24" depth) (to convert ppm to lb acre⁻¹ multiply ppm x 2 to get lb acre⁻¹).

PPC = previous crop credit (for leguminous crops e.g., alfalfa or peas). General estimates from this formula can be found in Table 3.2.

Phosphorous and Potassium

Phosphorous and potassium are essential nutrients for the vegetative and flowering growth stages in canola. Canola is a good scavenger of mineral phosphorous and phosphate fertilizers. On a per bushel basis, canola will take up 1.3 - 1.6 lbs (.6-.7 kg) of phosphate fertilizer (P₂O₅) with 0.9 - 1.1lbs (.4-.5 kg) being removed with the seed

(CCC 2013). Phosphorous has limited mobility in the soil, particularly in cool wet soils. Thus, phosphorous should be placed within the seed row for early uptake, i.e., as a starter rate of approximately 20 - 30 lbs acre⁻¹ (22-34 kg ha⁻¹) P₂O₅. The rate of phosphorous fertilizer can be determined from the results of the soil test referenced against the phosphorous fertilizer table (Table 3.2). A yield of 35 bu acre⁻¹ will require 56 lbs acre⁻¹ (63 kg ha⁻¹) of phosphorous (P₂O₅), and about 38 lbs acre⁻¹ (43 kg ha⁻¹) phosphorous (P₂O₅) will be removed from the field in the seed. This phosphorous requirement exceeds the amount that can safely be applied with the seed, thus soil phosphorous reserves will be depleted with time. Consequently, growers will have to apply higher rates of phosphorous to other rotational crops in order to maintain soil fertility and productivity.

Potassium is usually present in adequate quantities in most soil types where canola is grown. Most of these soil types have adequate clay content and eroding clay particles replenish soil potassium. Furthermore, most of the potassium taken up by the plant remains within the plant biomass and returns to the soil in the residue after seed harvest. However, the grower should review their soil test results and refer to the potassium recommendations table (Table 3.2).

Sulfur and Micronutrients

Canola has a high requirement for sulfur (S) (Grant and Bailey 1993), and sulfur deficiencies can severely impact yield. Sulfur (SO₄) is soluble within the soil profile and this result in highly variable sulfur content within a field. Hence, soil test results for sulfur can also be highly variable. Consequently, recommendations for sulfur when test results are low to medium are 20 - 30 lbs acre⁻¹ (22-34 kg ha⁻¹) and 10 - 15 lbs acre⁻¹ (11-17 kg ha⁻¹) with medium to high results (Frazen 2011). Sulfur can be applied as

ammonium sulfate, ammonium thiosulfate, and potassium thiosulfate. Elemental sulfur should be avoided, as it will not be available for plant uptake within the same season of application.

Micronutrient deficiencies in canola production in North America are not common. But micronutrient deficiencies should not be discounted should symptomology appear or soil test results revealed deficiencies. Suspect micronutrient deficiencies may be diagnosed with plant tissue analysis.

DEVELOPMENT AND GROWTH STAGES

Global demand and market prices have encouraged farmers to commit more acres to canola production. To maximize yield and profits, timely applications of management strategies and inputs are critical for success. Many management practices and control measures (e.g., fertilizer and pesticide applications) are dependent on specific growth stages of the crop in order to be most effective. Therefore, a universal system is needed to describe the developmental stages of canola. A uniform decimal code for growth stages of crops was described by Lancashire et al. (1991) and developed by BASF, Bayer, Ciba-Geigy and Hoechst called the BBCH decimal system used to describe canola growth stages (CCC 2014i). The BBCH system is a two-digit code whereby the first digit designates the principal growth stage. The principal stages include: 0) germination, 1) leaf development, 2) formation of side shoots, 3) stem elongation, 5) inflorescence emergence, 6) flowering, 7) development of fruit, 8) ripening, and 9) senescence. The second digit in the code describes the incremental developmental stages within the principal stage. Fertility and herbicide management are usually completed before the formation of side shoots (BBCH 20) and pest and disease management occurs through to

development of fruit (BBCH 70). In addition, it is important to understand which growth stages are most influenced by environmental factors, e.g., water, hail, heat stress. The complete BBCH scale can be viewed in Table 3.3

WATER MANAGEMENT

Water availability can be one of the most limiting factors in canola production. Dry or wet extremes during any of the developmental growth stages can impact yield potential. Water use in canola production can vary by year, season, and location because it is influenced by humidity, temperature, wind and available light. In general, about 5-6" (127-158mm) of moisture is required before any yield is attained, and for every inch (25mm) of additional water use about 175 lbs acre⁻¹ (196 kg hectare⁻¹) of seed is produced (Nielsen 1997, Johnston et al. 2002, Hergert et al. 2011, CCC 2014j). A grower cannot control the weather, but a grower can use strategies that enhance and manage the stored and available moisture within a field. Factors that affect the soil's capacity to hold moisture include reducing tillage that uses crop residues to increase soil fertility, capture snow moisture, and increase SOM.

Moisture retention in soils is related to soil texture and structure. Soil moisture retention is greater in finer textured soils (soils with greater proportions of silt and clay). These soils have increased surface areas for water and minerals to bind to than coarser textured soils. Water moves through more quickly and is retained less in coarser soil textures (greater proportion of sand particles) (Fig 3.1) (OMAFRA 2011). A soil test can help to characterize the soil texture in a particular field.

Adequate fertilization increases the plants water use efficiency (Krogman and Hobbs 1975, CCC 2014j). Canola plants that have adequate soil fertility early in the vegetative growth stages produce extensive root systems that are better able to exploit soil moisture deeper into the soil profile. Canola roots can extend 65" (1.65m) down into the soil profile (Nielsen 1997). Consequently, above ground growth is enhanced and the crop canopy can cover the soil faster, reducing evaporation from the soil. Crop canopy closure is also influenced by temperature, row spacing and population density.

Moisture from snowfall can contribute approximately 25-35% of annual precipitation (CCC 2014j). Capturing available snow moisture will enhance available moisture in the spring. Cardillo et al. (2015) have demonstrated that the stubble left from the wheat crop conferred a significant yield advantage to the following canola crop. Further, Cardillo et al. (2015) posit that tall stubble (50cm) was more efficient than short stubble (25cm) at capturing winter snow and helped slow evaporation and soil drying. Moisture retention through stubble management will reduce water stress later in the season.

The adoption of no-till and modified-till practices have prevented soil erosion, reduced moisture evaporation due to tillage, and increased moisture retention. No-till management systems leave crop residues, which cover the ground and reduce impact from rain, increase infiltration, and reduce run-off. In addition, crop residues reduce surface wind speed that in turn, reduces water vapor loss, and residues provide cover from the sun's rays, further reducing water loss due to evaporation. Decomposition of the extensive root systems of grass and legume rotations add to the SOM, improving soil aggregation, which increases plant available nutrients, and soil moisture. Management

practices that conserves even small amounts of moisture could make a difference later in the season should heat and or water stress occurs.

HARVEST AND STORAGE

Harvest

Implementing good agronomic practices (GAP) to maximize yields throughout the growing season can only be realized if the crop can be brought to market with minimal seed losses and superior seed quality. Canola seed harvest and storage must be timely, i.e., low percentage of green seed, and meet certain criteria in order to secure maximum seed yield, and quality. In a profitable canola operation, pre-harvest and harvest related seed losses must be kept to a minimum. Pod drop and pod shatter are the primary contributors to pre-harvest losses (Cavaliere et al. 2014). Untimely harvest and inappropriate harvesting techniques contribute to harvest losses (Vera et al. 2007, Haile et al. 2014). In North America, the equipment used to harvest cereal crops can be used to harvest canola with minimal adjustment. Canola is either swathed into windrows to dry and mature or direct combined.

Swathing has many advantages over direct combining including: 8-10 day earlier harvest, quicker dry down, more even seed maturity (important in a field that has uneven maturity), reduced pod shattering, can be done around the clock, and prevent further seed set in weeds (CCC 2014k). Timing of canola swathing is dependent on optimum average seed moisture of 30-35%, which represents physical maturity. Premature swathing that can result in a higher percentage of green seed that will lower the canola grade level. Seed moisture can be estimated by percentage of seed color change, i.e., from green to

brown or black, on the main stem. A grower should start looking for seed color change about 10 days after the end of flowering, i.e., BBCH 69. The Canola Council of Canada has increased their past recommendation from 30-40% seed color change on the main-stem up to 60% as the optimum time to swath (CCC 2014k). Researchers in North Dakota and Minnesota recommend that swathing of Argentine type canola (*B. napus*), begin at 15% color change, and in Polish type canola (*B. rapa*) swathing can begin at 20-25% color change (Nowatzki et al. 2014). A grower should consult their local extension personnel for the appropriate seed color change recommended for swathing in their area. Dry down of the windrow to a desired seed moisture level of 8-10%, required for storage and market, is dependent on the temperature and humidity levels after swathing. Dry down may take 5-10 days or longer in cooler wet weather. Green seeds can taint the canola oil because of the presence of chlorophyll and makes processing the oil more expensive and lowers the canola grade quality. The dry down period allows green seeds to mature and cure.

Direct combining reduced operating costs, because only one pass is made on the field versus two for swathing and combining. Also, direct combining must be done when seeds have matured. Consequently, there is less green seed content. The canola species can influence the decision to swath or direct combine. Varieties of *B. rapa* are less prone to shattering with direct combining than *B. napus*. Regardless of variety, direct combining should be done during the cooler part of the day to reduce shattering, e.g., when damp with dew or rain or at night during weather periods of hot day time temperatures (CCC 2014k).

Storage

Several factors can affect seed quality while in storage such as, seed maturity and condition (green seed and damage free), moisture level (8-9%), temperature (20°C), molds, insects, and storage climate and handling methods. Green and damaged seeds can lower the grade rating. Temperature and moisture influence biological activities. High temperatures combined with high moisture levels promote the growth of molds causing spoilage. In addition, molds affect fatty acid composition and meal quality imparting a tobacco like odor that is difficult for oilseed processors to remove. Generally whole seeds are less vulnerable than crushed seed to insects. Also, cool and dry conditions in storage bins generally do not favor insect growth. Molds, insect, and mites interact together in canola seed storage bins. Insects and mites damage the seed when they feed upon them. Damaged seeds provide an infection court for molds and pathogens (CCC 2014l).

Sweating is the term used to describe the respiration rate of freshly harvested seed. After harvesting, sweating can occur for six weeks while in storage before the seed enters dormancy. Seed respiration in storage must be monitored because seed respiration can add heat and moisture to a storage bin. These conditions favor heat damage and the growth of molds, two processes that will result in downgrading the marketability of the seed. Therefore, seed should be stored in cool, dry conditions, i.e., maximum moisture of 8% for long term storage (Appelqvist and Ohlson 1972, CCC 2014l). The use of conditioning and aeration systems can maintain optimum long-term storage conditions.

The practices used to harvest and store canola seed have direct impact on the final marketability of the product. There are specific determinants used to grade canola, which determine the final value of a harvest (see Table 3.4). Appropriate and timely harvest and

storage procedures can help the grower receive the best grade possible for their harvest, thus securing a profitable return on investment.

WEED MANAGEMENT

Farmers have battled against weed species since the beginning of plant domestication 10,000 years ago (Vaughan et al. 2007). In agronomic crop production, weed species can reduce growth and productivity. Weed species compete with crops for light, nutrients, and water. In addition, weed species can serve as an alternate host for plant pathogens or provide habitat for pestiferous insects, e.g., *Lygus* species in cruciferous weeds (Butts and Lamb 1991). Also, sexually compatible weed species from the mustard family can contaminate canola seed and meal and reduce the grade and quality.

Integrated weed management (IWM) is a long term management strategy that will maximize returns. For the grower to obtain sustainable yields and profits, an IWM strategy should be implemented. IWM uses two or more different agronomic practices to reduce the reliance on any one weed management technique, e.g., herbicide tolerant crops. Therefore, it is important to understand the agronomic practices that could be used in IWM.

Timely weed removal

To manage weeds species in agronomic crops, the grower must know when the critical weed free period occurs. The critical weed free period is the specific period in the life cycle of a crop that must be weed free in order to prevent yield loss (Nieto et al. 1968, Van Acker et al. 1993). In canola, Martin et al. (2009) determined the critical weed

free period, i.e., to prevent >10% yield loss, to be up to the fourth leaf stage (BBCH 14). Currently, most of the canola being grown in North America is herbicide tolerant, e.g., Round-up Ready (glyphosate), Liberty-Link (glufosinate) or Clearfield (imidazolinone). Timely herbicide applications are critical in managing weed species and reducing unnecessary applications. Reducing unnecessary herbicide applications lowers input costs, selection pressure for resistance, and environmental impact. In a study using small-scale plots, Harker et al. (1999) demonstrated a delay in the application of glyphosate herbicide of three weeks after emergence on glyphosate resistant canola would reduce yields by 25%. In addition, large scale field studies showed a 20% reduction in yield when herbicide application in Clearfield canola was delayed until the six- seven leaf stage (Harker et al. 2008).

Uniform seeding and spacing

Canola seedlings are poor competitors against weeds. Field studies in canola have determined that only 50% of the canola seed planted actually emerges (Harker et al. 2003). Poor emergence and non-uniform stands are less competitive with weeds and require more applications of herbicides to control weeds. Canadian researchers Yang et al. (2014) compared the yields of non-uniform stands versus uniformed stands at low yielding and high yielding sites. Their results indicated that uniform stands had a 32% increase at the low yielding sites and a 20% increase at the high yielding sites. Seeding depth can influence canola emergence. When moisture is available, shallow seed planting depths allow for quicker emergence and could decrease the time to canopy closure, flowering, and maturity. Harker et al. (2012) planted seed in depths ranging from 1cm (.39 in) to 4 cm (1.6 in). Under moist soil conditions their results indicated an average

emergence of 37% at 4 cm versus 67% at 1 cm. The results also indicated a reduction in days to emergence, days to flowering, days to maturity, and green seed levels at harvest, as well as an increase in canola ground cover.

Fertilizer timing and placement can be an effective weed management strategy. For example, during seeding a band of fertilizer should be placed close to and below the seed rather than broadcast to reduce weed populations. The results from O'Donovan et al. (1997) indicated that banding nitrogen fertilizer had the potential to control green foxtail (*Setaria viridis* L.) and field pennycress (*Thalpsi arvensis* L) in barley. The study by Blackshaw et al. (2004) indicated a reduction in the seedbank of certain weed species (25 to 63%) with point injection when compared to broadcast nitrogen in spring wheat. Blackshaw et al. (2004) also noted that spring applied fertilizer reduced weed biomass when compared to fall applied fertilizer. To create a uniform stand, careful attention must be given to planting density, speed, depth, row spacing, and fertilizer placement.

Competitive ability

A crop's competitiveness is defined by the ability to outcompete a weed species for resources, such as light, water and nutrients, while maintaining grain yield and quality. Planting highly competitive canola genotypes could be used as a low cost tactic for weed management. In Australia, Asaduzzaman et al. (2014a, 2014b) identified genotypes of *B. napus*, from a world-wide collection of 70 genotypes, that were competitive against the weed species annual ryegrass (*Lolium rigidum* Guad.), shepherd's purse (*Capsella bursa-pastoris* L.), Indian hedgemustard (*Sisymbrium orientale* L.), and false barley. (*Hordeum leporinum* L.). Another study in Australia testing sixteen genotypes of canola indicated that at flowering, strongly competitive canola genotypes

had successfully reduced the weed biomass of annual ryegrass and volunteer wheat (*Triticum aestivum* L.) by 50%, thus reducing the weed seedbank (Lemerle et al. 2014).

Crop diversity and rotations

"Diverse crop rotations are the cornerstone of all sustainable pest management and crop production systems" (Blackshaw et al. 2008). Crop diversity and rotations use the principle of varied selection pressure to keep weed communities off balance and reduce the long term build up of weed species (Derksen et al. 2002). Canola is generally grown in a 4-year rotation with cereals, pulses, forages, and/or other oilseeds. The different management practices for each crop in rotation, along with the different life cycles of the crops, present different challenges to weeds species that prevent unrestricted growth and reproduction (Blackshaw et al. 2007). In contrast, short rotations or continual canola on canola increases selection pressure for those individuals that can persist and eventually build resistance. For effective weed management growers should strive to balance crops types in the rotation, e.g., broadleaf versus grasses and spring, or summer, versus fall planted (Derksen et al. 2002).

Combining optimal agronomic practices

In order to have an effective IWM strategy, the grower should combine all available practices at their disposal to maximize weed management over the long-term. These include the practices outlined above, but also include tillage practices and chemical controls, i.e., herbicides. For example, in a multisite study in Canada, Blackshaw et al. (2008) indicated superior weed management was achieved by combining the use of a competitive canola cultivar, higher seed rates, and early weed removal. Further when

compared to standard agronomic and weed control practices, they witnessed a 41% yield increase. In a barley-field pea rotational study combining an early seeding date, higher seed rates, spring applied fertilizer, and timely application of herbicide, Blackshaw et al. (2005) maintained high yields. A barley study by Harker et al. (2009) looked at the effectiveness of combining single, double, and triple optimal agronomic practices to control wild oats (*Avena fatua* L.). Their results indicated that the triple treatment of double seeding rate, crop rotations (barley-pea and barley-canola), and tall cultivars, along with a quarter rate of herbicide (a high management tactic) reduced wild oat biomass 19-fold and wild oat seed production more than 90% when compared to the low management full rate herbicide regime. Judicious and timely use of herbicides combined with a diverse mixture of effective agronomic practices should enable more effective weed management, lower costs, and optimize yields.

INSECT MANAGEMENT

Economically important insect pests in canola production can be found in the orders Coleoptera, Hemiptera, Homoptera, Leiodoptera, Diptera, and Hymenoptera (Lamb 1989). Insect pests present in canola production vary between Europe, Asia, Australia, and North America. In India, the cause serious losses in oilseed rapeseed (Kular and Kumar 2011). In Australia, the redlegged earth mite (*Halotydeus destructor* Tucker) and three species of blue oat mite, i.e., *Penthaleus major* (Duges), *P. falcatus* (Qin and Halliday), and *P. tectus* (Qin and Halliday) are the most regular and damaging pests in canola (Gu et al. 2007). In Europe, the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) is a serious pest on oilseed rapeseed, particularly since the ban on neonicotinoid insecticide seed treatments (AHDB 2015). In North America, crucifer flea

beetle (*Phyllotreta cruciferae* Goeze) and the striped flea beetle (*P. striolata* Fabricius) are the most serious insect pests in canola (Knodel and Olson 2002). Some other canola pests of world-wide importance include the bertha armyworm (*Mamestra configurata* Walker), the diamondback moth (*Plutella xylostella* L.) and the root maggot (*Delia radicum* L.). The following section provides an overview of the crucifer flea beetle and current management tactics.

Crucifer flea beetle

The crucifer flea beetle was introduced from Eurasia to the west coast of North America in the early 1920s (Milliron 1953). Distribution of the crucifer flea beetle extends across northwestern and southern Canada and the Great Plains of the United States (Knodel and Olson 2002, NDSU 2015). In 2013, crucifer flea beetle damage to seedlings in many parts of Montana was in excess of 80% (Reddy et al. 2014). Damage losses in North America caused by the crucifer flea beetle are in excess of \$300 million annually (Knodel and Olson 2002, Soroka 2013). Overwintering adults emerge in the spring and feed on the cotyledons and first true leaves of emerging seedlings. Signs of feeding appear as pitting and "shot-gun" holes in the leaves reducing photosynthetic area and weakening the seedling. Excessive feeding can result in seedling death and substantial stand loss. Examples of flea beetle feeding on leaves and pods can be found in Figures 3.2 and 3.3.

Life cycle

The small, oval-shaped, black-colored adult crucifer flea beetle measures about 1/32 - 1/8 inch (2-3mm) with a bluish sheen on the elytra. Crucifer flea beetles have a univoltine life style. The life cycle begins in the spring when temperatures warm to 14° C

(58°F). The emerging adults that have overwintered in the leaf litter of shelterbelts and grassy areas begin feeding on volunteer canola and related weed species e.g., wild mustards. Crucifer flea beetles move, i.e., walk, fly or hop, into newly planted canola fields as the seedlings emerge. Warmer temperature increase their activity, i.e., feeding and movement throughout the field. The adults are active through late June and then begin to die. The females oviposit eggs into the soil where they incubate and hatch in about 12 days. The larvae feed on the roots, and after three instars, lasting 25 - 34 days; they enter a pupal stage for another 7 - 9 days. The next generation of adult crucifer flea beetles emerge in late July through September, feed on the foliage, stems, and pods of the maturing canola, and related *Brassica* species. In the fall, adults return to the ground litter of overwintering sites (Knodel and Olson 2002).

Cultural control

Integrated pest management (IPM) is broad-based approach that integrates practices for the economic control of pests while minimizing risks to people and the environment. Cultural practices found to be effective in managing the feeding damage by the crucifer flea beetle include, no-till, row spacing, seeding density, seeding date, and seed size.

Tillage affects the amount of seedling feeding damage caused by the crucifer flea beetle. The results in Canada indicated that zero-till (no-till) systems had a greater reduction in seedling feeding damage from crucifer flea beetle when compared to conventional tillage (Dosdall et al. 1999). This may be explained in part by the microclimate created by the presence of crop residues, i.e., high moisture levels, and

cooler temperatures affects the crucifer flea beetles behavior, because it is more active in hot and dry conditions.

Row spacing may also be used to manage feeding by crucifer flea beetles. Research indicates that optimum row spacing for reducing feeding damage should be about 14 cm (5.5 in) and 30 cm (12 in) for *B.napus* and *B. rapa*, respectively (Doddall et al. 1999). In addition, increasing seeding rates to 7 kg ha⁻¹ (6.2 lbs acre⁻¹) and 10 kg ha⁻¹ (9 lbs acre⁻¹) for *B.napus* and *B. rapa*, respectively, resulted in less crop damage (Doddall et al. 1999, Doddall and Stevenson 2005).

Seeding date can be used as a crucifer flea beetle management tactic. Fall dormant seeding, i.e., planting seed late in the fall prior to frost to prevent germination and induce dormancy, and early spring planting, allows canola to grow beyond the seedling stage prior to the emergence of overwintering adults (Doddall et al. 1999, Knodel and Olson 2002, Doddall and Stevenson 2005, Knodel et al. 2008). Growth beyond the seedling stage will have more available leaf area and the larger plants can tolerate more feeding damage.

Interestingly, seed size and seed weight affects seedling establishment, seedling vigor and tolerance to *Phyllotreta* species. Studies by (Elliott et al. 2007, 2008) indicated that the seedlings produced from large seeds of *B. napus* and *B. rapa* were more vigorous, had greater shoot biomass, and were better able to tolerate feeding damage from flea beetles than seedlings produced from small and medium sized seeds. A grower could purchase seed with greater 1000 seed weights to take advantage of this tactic.

Chemical control

Undoubtedly, the use of synthetic insecticides for controlling crucifer flea beetles in canola production is still the number one management tactic. In canola production, insecticides are applied as either seed treatments or post emergence foliar sprays. Seed treatments are systemic insecticides that are designed to protect a seedling against sucking and chewing pests. In general, seed treatments provide protection for 1-2 weeks after emergence (Knodel and Olson 2002). Neonicotinoids are a class of insecticidal compounds now commonly used as seed treatments. Insecticidal compound groups registered for use as foliar sprays in managing crucifer flea beetles include organophosphates, pyrethroids, and carbamates (Knodel and Olson 2002, CCC 2014m).

Crucifer flea beetles can do serious damage quickly; therefore, daily field scouting should be practiced during critical growth stages, e.g., emergence and flowering. Field scouting throughout the growing season is crucial for timely, economically, and environmentally sound management of crucifer flea beetle. Scouting of the fields should begin in early spring as soon as the temperature rises to 14° C (58°F) for extend periods. The economic threshold to trigger foliar application of insecticide is 25% defoliation (Knodel et al. 2008). Research with spring planted canola conducted in North Dakota indicated that a high dose seed treatment combined with a foliar treatment 21 days after planting was the optimum strategy to reduce feeding injury and protect yield (Knodel et al. 2008).

Biopesticides

"Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals" (EPA 2015). Biopesticides offer a new

and exciting alternative to synthetic insecticides that would fit well into IPM strategies. A study in Montana by Reddy et al. (2014) compared the effectiveness of an entomopathogenic nematode (*Steinernema carpocapsae*), two entomopathogenic fungi (*Beauveria bassiana* and *Metarrhizium brunneum*), neem (azadirachtin), fatty acids (M-pede), petroleum spray oil (PSO), two pyrethroid foliar sprays (deltamethrin and bifenthrin), and one neonicotinoid (imidacloprid) seed treatment at managing crucifer flea beetles in canola. In part, their results indicated that the treatments with the entomopathogenic nematode and the two entomopathogenic fungi gave considerable control of *P. cruciferae*. More research will be required to bring such technologies to market, but it does present possible options for the future.

An IPM strategy for managing *Phyllotreta cruciferae* would combine optimal cultural practices, such as no-till, planting date, row spacing, seeding rate, seed size, and judicious use of selective insecticides. Adoption of an IPM strategy would provide a number of benefits over conventional methods. For example, IPM strategies work synergistically to reduce the amount of chemical compounds released into the environment and conserve beneficial insects. Reduced insecticide use will reduce selection pressure and input costs, thus maximizing yields and profits. IPM is not just good for the environment it is good for business.

DISEASE MANAGEMENT

Globally, the incidence and severity of diseases that attack canola vary between countries, agronomic practices, and climatic conditions. World-wide there are many plant pathogens that attack canola. Plant pathogens of canola can be bacterial, fungal, viral, or phytoplasmal and may affect below and above ground plant structures throughout the

growing season. Economically important canola diseases with a cosmopolitan distribution include blackleg (*Leptosphaeria maculans*-(Desm.) CEs. & deNot , anamorph *Phoma lingam*-(Tode) Desmaz.), Sclerotinia stem rot or white mold (*Sclerotinia sclerotiorum*-(Lib.) de Bary), and clubfoot (*Plasmodiophora brassicae*-Woronin). The following section provides an overview of blackleg (*Leptosphaeria maculans*) a major disease worldwide and its current management tactics.

Blackleg

Blackleg is caused by the Ascomycota fungus (*Leptosphaeria maculans* (Tul. & C. Tul) Ces. & Not.(anamorph = *Phoma lingam*-(Tode) Desm.) and is also known as phoma stem canker. The disease blackleg is now reported to be comprised of two species, a weakly virulent species *Leptosphaeria biglobosa* (Shoemaker & Brun (current name: *Plenodomus biglobosus* (Shoemaker & Brun) Gruyter, Aveskamp & Verkley) and a highly virulent species *L.maculans*. *L.biglobosa* or *P.biglobosus* (the weakly virulent species) infects canola later in the growing season and rarely forms stem cankers that cause lodging and yield loss, thus it is not considered to be economically important.

The highly virulent species of blackleg (*L. maculans*) is capable of causing serious yield losses in North America, Europe, and Australia (Fitt et al. 2006a). The first case of blackleg in the United States was reported in Kentucky in 1989 with a 100% yield loss (Ash 2000). In 1991, a blackleg outbreak in North Dakota resulted in yield losses up to 68% in some fields (Nepal et al. 2014). Recorded yield losses due to blackleg outbreaks in Canada have been around 50% (Kutcher et al. 2011). Both France and Australia have reported up to 90 % yield losses due to blackleg disease outbreaks (Sprague et al. 2006). Understanding the biology of *L. maculans* and the factors that

favor or suppress outbreaks in canola production will allow producers to effectively manage blackleg disease.

Biology

L. maculans is a hemibiotrophic pathogen, i.e., it is parasitic for some time and then continues to live in dead tissue. *L. maculans* has a heterothallic life cycle: two different compatible mating types must be present for sexual reproduction. Sexual reproduction of *L. maculans* takes place on crop residues (Rouxel et al. 2003). Sexual reproduction produces pseudothecia, a sexual fruiting body, on decaying leaves and basal stems (Fig. 3.3). The pseudothecia contains bitunicate asci, i.e., asci with a clearly differentiated inner and outer cell wall. Within the asci are eight biserial ascospores that are the primary source of inoculum. Ascospores are dispersed by the wind over long distances. Asexual reproduction is via pycnidia occurring in diseased tissues. The pycnidia produce pycnidiospores. The pycnidiospores are unicellular and colorless and serve as a secondary source of inoculum. Under favorable conditions pycnidia will ooze a pink/amethyst colored tendril (cirrhi) rich with pycnidiospores. Pycnidiospores are dispersed by rain splash over short distances, i.e., to neighboring plants (Ash 2000).

Disease cycle

After harvest, *L. maculans* can persist as a saprophyte on the remaining stubble (Salam et al. 2003). However, blackleg inoculum overwinters primarily as mycelium, pseudothecium, and pycnidium in the crop residue and stubble, such as basal stems and non-harvested seeds. Primary infection occurs when pseudothecia release their ascospores under favorable conditions. This occurs after a rainfall, heavy dew and or high humidity coinciding with temperatures ranging from 8-12°C (46-54°F) (Ash 2000, Salam

et al. 2003, Toscano-Underwood et al. 2003). The released ascospores are then wind dispersed over distances as far as 5 kilometers. Under favorable conditions, the release of ascospores can continue for 3-4 months (Salam et al. 2003). Ascospores that alight on canola plants require the presence of free water and temperatures between 4-28°C (40-82°F) to germinate and penetrate stomates (Ash 2000). Hyphal structures penetrate the leaf tissues intercellularly and acquire nutrients in a biotrophic manner. At some point the fungus becomes necrotrophic invading mesophyll tissues causing cell death and the appearance of gray-green or grey-ash lesions (Fig. 3.4) (Ash 2000). Eventually, the fungus colonizes xylem and stem cortex tissues and moves through the vascular system, causing stem cankers that form quickly at temperatures of 20-24°C (68-75°F). Because the fungus can travel through the vascular system, it can also colonize the seed pods and the forming seeds. If the infection occurs early in seed formation the seeds may be rendered unviable. Additionally, biotic and abiotic stressors, i.e., mechanical, insect, or herbicide injury, will increase disease severity. Lodging occurs when basal stem cankers are severe enough to pinch-off water and nutrient flow in the vascular tissues.

Stem and leaf lesions initiated by ascospore infection lead to the formation of the asexual fruiting structures (pycnidia) (Fig. 3.4). The pycnidium releases pycnidiospores under moist conditions. Pycnidiospores require up to 16 hours of wetness and a temperature range of 20-25°C (68-77°F) in order to germinate and another 13 days to produce new inoculum after infection (Ash 2000).

Cultural control

Cultural practices to reduce blackleg in canola have centered on avoiding or limiting exposure to inoculum sources, such as crop rotation, residue management and

certified pathogen free-seed. *L.maculans* can survive saprophytically on stubble for years, thus it is important to avoid planting canola into fields that have canola stubble. A four-year rotation of non-host crops has been a proven standard for reducing pathogen carry-over in crop residue and soil (Krupinsky et al. 2002, Kutcher et al. 2011). Removal of volunteer canola or related *Brassica* species ensures that alternate hosts are not available. Tillage can also be used to incorporate stubble into the soil, hastening microbial decomposition and reducing the discharge of ascospores into the air (Ash 2000). Keep in mind that in dryer climates crop residues take longer to break down.

Although windborne ascospores can travel long distances, research in Australia indicated that planting no closer than 500 meters to a field that had canola stubble from the previous year would reduce blackleg severity significantly (Marcroft et al. 2004). Lastly, seed can also be infected with *L.maculans*, and initiate a new disease cycle upon germination. Thus, growers are encouraged to purchase only certified pathogen free seed.

Chemical control

Fungicide use in management of blackleg in North America is centered on the use of seed treatments and foliar sprays. Canola seedlings are vulnerable to blackleg. In areas with a history of blackleg, the grower would be well advised to apply a fungicide seed treatment. Early recognition and control of blackleg may reduce the severity of the stem canker phase. Because foliar infection can infect canola from emergence to flowering given favorable conditions, decision support systems, such as predictive disease forecasting models, could be helpful in timing foliar applications (Salam et al. 2003, Kutcher et al. 2011). Growers should consult with their extension or coop personnel for further information.

Genetic resistance

A major strategy for controlling blackleg is the use of cultivars with resistance to *L. maculans* (Fitt et al. 2006). Because *L. maculans* reproduces sexually and asexually it has a high potential for gene flow and can evolve quickly. For example, French growers and researchers witnessed the breakdown of resistance in three years due to large scale cropping of the same resistant cultivar (Rouxel et al. 2003). A similar experience of resistance breakdown occurred in Australia (Sprague et al. 2006). Rotating resistant cultivars with different resistant genes prolongs the use and effectiveness of resistant genes against blackleg by reducing selection pressure (Marcroft et al. 2012).

Combining agronomic practices, such as fungicide applications, crop rotations, growing and rotating resistant hybrids, planting pure seed, removing volunteer canola and related weed species, and separation from canola stubble both temporally and spatially have reduced the incidence of blackleg (Krupinsky et al. 2002, Sprague et al. 2006, Kutcher et al. 2011, 2013, Marcroft et al. 2012). But market forces and reliance on resistance cultivars has moved some growers away from a more integrated management approach. Incredibly, continuous and two-year rotations are being used. The risk of shorter rotations increases the risk for greater disease incidence and severity by inoculum carry-over, resistance breakdown, and potential yield loss.

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Table 3.1 PREVIOUS CROP NITROGEN CREDIT

Use this table to determine the nitrogen credit from the previous crop.

Previous crop	Credit
Soybean	40 lb N/acre
Edible bean	40 lb N/acre
Pea and lentil	40 lb N/acre
Chickpea	40 lb N/acre
Sweet clover that was harvested	40 lb N/acre
Alfalfa that was harvested and unharvested sweet clover:	
> 5 plants / sq ft	150 lb N/acre
3-4 plants / sq ft	100 lb N/acre
1-2 plants / sq ft	50 lb N/acre
<1 plant / sq ft	0 lb N/acre
Sugar beet	
Yellow leaves	0 lb N/acre
Yellow / green leaves	30 lb N/acre
Dark green leaves	80 lb N/acre

Source: Previous crop credit table taken from: North Dakota Fertilizer Recommendations Tables and Equations. Frazen (2010).

Table 3.2 N, P, K FERTILIZER RECOMMENDATIONS

Use this table in conjunction with soil test results to determine the amount of fertilizer to apply to achieve expected yield potential.

Yield potential	Soil N plus fertilizer N required	Bray-1 Olsen	Soil Test Phosphorus, ppm					Soil Test Potassium, ppm				
			VL 0-5 0-3	L 6-10 4-7	M 11-15 8-11	H 16-20 12-15	VH 21+ 16+	VL 0-40	L 41-80	M 81-120	H 121-160	VH 161+
lb/a	lb/acre-2'		lb P ₂ O ₅ /acre					lb K ₂ O/acre				
1000	65		33	24	15	6	0	47	34	20	6	0
1500	100		49	36	23	9	0	71	50	30	10	0
2000	130*		65	48	30	13	0	94	67	40	13	0
2300	150		75	55	35	18	0	108	77	46	15	0
2500	150		82	60	38	16	0	118	84	50	16	0
3000	150		98	72	46	18	0	142	100	60	20	0
Nitrogen recommendation			= 0.065 YP-STN-PCC with a 150 lb max limit					Note: Canola has a high requirement for sulfur				
Bray-I P recommendation			= (0.036-0.0017 STP)YP					Application of 20 to 30 lb/a S is recommended				
Olsen P recommendation			= (0.036-0.0022 STP)YP					regardless of soil test results for this crop.				
Potassium recommendation			= (0.054-0.00034 STK)YP					Apply S as sulfate or thiosulfate form.				

Source: N, P, K, recommendation table taken from: North Dakota Fertilizer Recommendations Tables and Equations. Frazen (2010).

Table 3.3 BBCH-IDENTIFICATION KEYS OF CANOLA

Describes the morphological characters used to determine the growth stage.

Source: adapted from Lancashire et al. (1991), and (CCC 2014i)

Growth stage	Code	Description	
0: Germination	00	Dry seed	
	01	Beginning of seed imbibition	
	03	Seed imbibition complete	
	05	Radicle emerged from seed	
	07	Hypocotyl with cotyledons emerged from seed	
	08	Hypocotyl with cotyledons growing towards soil surface	
	09	Emergence: cotyledons emerge through soil surface	
	1: Leaf development ¹	10	Cotyledons completely unfolded
		11	First leaf unfolded
12		2 leaves unfolded	
13		3 leaves unfolded	
1 .		Stages continuous till . . .	
19		9 or more leaves unfolded	
2: Formation of side shoots		20	No side shoots
	21	Beginning of side shoot development: first side shoot detectable	
	22	2 side shoots detectable	
	23	3 side shoots detectable	
	2 .	Stages continuous till . . .	
	29	End of side shoot development: 9 or more side shoots detectable	
3: Stem elongation ²	30	Beginning of stem elongation: no internodes (“rosette”)	
	31	1 visibly extended internode	
	32	2 visibly extended internodes	
	33	3 visibly extended internodes	
	3 .	Stages continuous till . . .	
	39	9 or more visibly extended internodes	
5: Inflorescence emergence	50	Flower buds present, still enclosed by leaves	
	51	Flower buds visible from above (“green bud”)	
	52	Flower buds free, level with the youngest leaves	
	53	Flower buds raised above the youngest leaves	

	55	Individual flower buds (main inflorescence) visible but still closed
	57	Individual flower buds (secondary inflorescences) visible but still closed
	59	First petals visible, flower buds still closed (“yellow bud”)
6: Flowering	60	First flowers open
	61	10% of flowers on main raceme open, main raceme elongating
	62	20% of flowers on main raceme open
	63	30% of flowers on main raceme open
	64	40% of flowers on main raceme open
	65	Full flowering: 50% flowers on main raceme open, older petals falling
	67	Flowering declining: majority of petals fallen
	69	End of flowering: 10% of plants have flowers
7: Development of fruit	71	10% of pods have reached final size
	72	20% of pods have reached final size
	73	30% of pods have reached final size
	74	40% of pods have reached final size
	75	50% of pods have reached final size
	76	60% of pods have reached final size
	77	70% of pods have reached final size
	78	80% of pods have reached final size
	79	Nearly all pods have reached final size
	8: Ripening	80
81		10% of pods ripe, seeds dark and hard
82		20% of pods ripe, seeds dark and hard
83		30% of pods ripe, seeds dark and hard
84		40% of pods ripe, seeds dark and hard
85		50% of pods ripe, seeds dark and hard
86		60% of pods ripe, seeds dark and hard
87		70% of pods ripe, seeds dark and hard
88		80% of pods ripe, seeds dark and hard
89		Fully ripe: nearly all pods ripe, seeds dark and hard
9: Senescence	97	Plant dead and dry
	99	Harvested product

Table 3.4 CANOLA PRIMARY GRADING DETERMINANTS

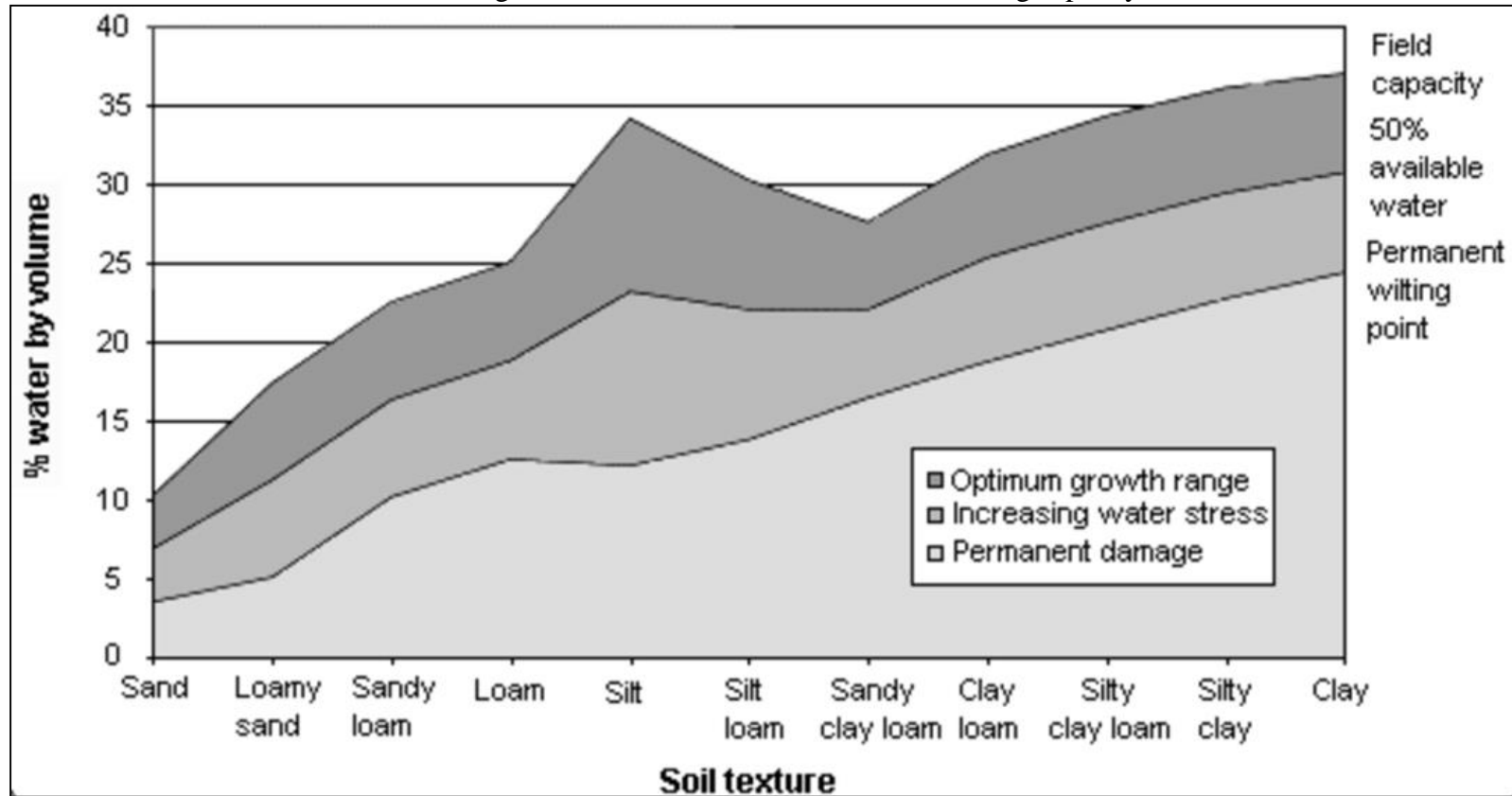
Illustrates the factors that affect the canola seed grade quality received.

Grade name	Standard of quality		Standard of cleanliness				
	Degree of soundness		Commercially pure seed				
No. 1 Canada	Reasonably well matured, sweet, good natural colour		Not more than 1.0% of other seeds that are conspicuous and that are not readily separable from canola, to be assessed as dockage				
No. 2 Canada	Fairly well matured, sweet, reasonably good natural colour		Not more than 1.5% of other seeds that are conspicuous and that are not readily separable from canola, to be assessed as dockage				
No. 3 Canada	May have the natural odour associated with low-quality seed, not distinctly sour, musty, rancid, or any odour that would indicate serious deterioration		Not more than 2% of other seeds that are conspicuous and that are not readily separable from canola, to be assessed as dockage				
Grade, if No. 3 specs not met			<i>Canola, Sample Canada Account Admixture</i>				
Grade name	Damage						
	Distinctly green %	Heated %	Total %				
No. 1 Canada	2	0.1	5				
No. 2 Canada	6	0.5	12				
No. 3 Canada	20	2	25				
Grade, if No. 3 specs not met	<i>Canola, Sample Canada Account Damaged</i>	<i>Canola, Sample Canada Account Heated</i>	<i>Canola, Sample Canada Account Damaged</i>				
Grade name	Foreign material included in dockage						Inconspicuous admixture %
	Ergot %	Excreta %	Insect excreta %	Sclerotinia %	Stones %	Total Conspicuous admixture %	
No. 1 Canada	0.05	0.02	0.10	0.05	0.05	1.0	5
No. 2 Canada	0.05	0.02	0.20	0.10	0.05	1.5	5
No. 3 Canada	0.05	0.02	0.3	0.15	0.05	2	5
Grade, if No. 3 specs not met	<i>Canola, Sample Canada Account Ergot</i>	<i>Canola, Sample Canada Account Excreta</i>	<i>Canola, Sample Canada Account Excreta</i>	<i>Canola, Sample Canada Account Admixture</i>	<i>2.5% or less—Canola Rejected (grade) Account Stones, or Canola, Sample Canada Account Stones. Over 2.5%—Canola, Sample Salvage</i>	<i>Canola, Sample Canada Account Admixture</i>	<i>50% or less—Canola, Sample Canada Account Admixture. Over 50%—Refuse screenings</i>

Source: Canadian Grain Commission (CGC 2015)

Figure 3.1 AVAIALBLE SOIL MOISTURE BY SOIL TEXTURE

Illustrating the effects of soil texture on water holding capacity.



Source: Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA 2011)

Figure 3.2 FLEA BEETLE DAMAGE IN CANOLA-BBCH 51

Demonstrating the "shotgun hole" damage from flea beetle feeding. Flea Beetles are circled in red.



Source: Photograph taken by Kenneth Roche' in Montana 2015

Figure 3.3 FLEA BEETLES FEEDING ON PODS

This image depicts flea beetles feeding and damage to pods that result in lower photosynthetic area and seed abortion.



Photograph taken by J. Knodel, North Dakota State University.

Source: <https://www.ag.ndsu.edu/.../crucifer-flea-beetle-biology-and-integrated-pest-management-in-canola>

Figure 3.3 BLACKLEG CANKER ON THE STEM OF A CANOLA PLANT

Stem canker with pseudothecia (blackdots) that are the primary inoculum for the following canola crop.



Photograph courtesy of North Dakota State University.

Source:

www.ag.ndsu.edu/archive/entomology/ndsucpr/Years/2005/may/26/ppath_26may05.htm

Figure 3.4 BLACKLEG LESIONS ON A CANOLA LEAF

Blackleg lesions with pycnidia (blackdots) on a diseased canola leaf.



Photograph courtesy of North Dakota State University.

Source:

www.ag.ndsu.edu/archive/entomology/ndsucpr/Years/2005/may/26/ppath_26may05.htm

CHAPTER FOUR

DOCTORAL INTERNSHIP

INTERNSHIP

The University of Nebraska-Lincoln Doctor of Plant Health program is a professional doctorate program with a comprehensive approach to plants and agriculture. The program emphasizes a broad interdisciplinary education across all plant-related disciplines, practical learning, research, and experience through internships. For my final required internship, I worked as a senior agricultural research intern with Research Designed for Agriculture (RD4AG) in Montana. RD4AG is a contract research organization (CRO) based in Yuma, AZ with over thirty-years of experience.

RD4AG offers agronomic research testing services in field, forage, grape, vegetable, and citrus. RD4AG conducts a variety of field trials, such as the effect of novel technologies on plant health, drought and heat tolerance, variety trials, and United States Department of Agriculture (USDA) regulated articles testing (RD4AG 2015). In addition, RD4AG contracts crop protection research services that are used by a sponsor for product development in order to meet Good Laboratory Practices (GLP) registration requirements in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which is regulated by the Environmental Protection Agency (EPA) of the United States of America. The Organization for Economic Co-operation and Development (OECD 1997) defines GLP as "...a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived, and reported".

During my three month internship at RD4AG in Montana, I was the project lead on fifteen trials and assisted with another twenty-six projects that included winter and spring wheat, field pea, and canola cropping systems. The wheat and field pea trials included agronomic evaluations by variety and testing substances for either crop enhancement or crop protection. Examples of data collected include the response of varieties to the dry-land farming conditions experienced in Montana, i.e., available moisture, heat tolerance and pests' pressures, pesticide regimes, or seed treatments for disease protection.

However, a large portion of my responsibilities centered on managing the GLP (40 CFR Part 160 FIFRA Good Laboratory Practice Standards) specific to the regulated canola trials that were undertaken in Montana for industry sponsors. The canola trial sites, about 20 acres in total, were planted in three separate locations across Montana ranging one hundred fifty to two hundred and thirty miles between each location. This was to ensure the data gathered would be representative of canola production in Montana. The canola trials included evaluations with regulated, i.e., genetically engineered (GE), and non-regulated materials. The data gathered included varietal agronomic trait responses (e.g., days to emergence, days to flowering, and days to maturity), plant parts sample collections, i.e., leaves, roots and seeds, used to determine protein expression and polyunsaturated fatty acid content (PUFA) analysis, herbicide tolerance response, and bulk seed increase for later evaluation by the sponsor.

I assisted with the planting at all three canola trial sites and participated in all plot maintenance for the duration of my internship. But more importantly, I was responsible for gathering, monitoring, and collating, all the raw data as per the standard operating

procedures (SOPs) outlined by the study director and sponsors and in accordance with GLP standards. A complete review of GLP standard, i.e., 40 CFR Part 160 FIFRA Good Laboratory Practice Standards, can be found at the U.S. Government Publications Office (CFR 2011). This responsibility allowed me to interact with the study director, the sponsor stewardship and development personnel, as well as the third party quality assurance unit (QAU) personnel during sampling events and inspections.

SYNTHESIS

The goal of the Doctor of Plant Health (DPH) Program at the University of Nebraska–Lincoln is to produce plant practitioners with broad expertise and experience across the various disciplines, i.e., plant pathology, plant science, weed science, soil science, and entomology, that impact plant health and plant management. DPH internships require the student to apply knowledge gained through the DPH program in a practical real-world setting. This internship allowed me to experience the intricacies of private sector research and product development prior to commercial release of a novel technology or hybrid cultivar.

The doctoral document is intended for the student to take their internship experiences, synthesize those experiences, then dive into the available literature to further explore and expound upon what they have learned. Through this document I have synthesized my experiences with canola production in Montana to explore canola further. I have examined the historical context regarding the domestication of *Brassica* crops and the transition to canola, the breeding techniques used to develop canola from traditional rapeseed species, i.e., *B.napus*, *B.rapa*, and *B.juncea*, and touched upon some the

production requirements for canola, such as planting, fertility, water, weed, insect and disease management.

Canola has a multitude of end uses. In North America, canola is used primarily as an edible vegetable oil for frying, salad oils, margarine, and processed foods. In the European Union, canola is also consumed as an edible vegetable oil, but most of their canola oil production is used for biofuel production, i.e., biodiesel. Globally, canola consumption is on the rise so that nations are committing more land to canola production, but market forces are also tempting growers to shorten rotations. The consequences of shorter rotations to canola could be deleterious. Shorter rotations increase selection pressure for herbicide resistant weed species. Additionally, shorter rotations allow disease inoculum loads to build-up, increasing the incidence and severity of disease and hastening the breaking down of resistant cultivars. In effect, shorter rotations reduce the durability of the two most important tools for integrated crop management in canola: herbicide and disease resistant cultivars. To ensure an adequate supply of canola to meet market demands, growers would be wise to heed academic and extension recommendations regarding integrated management practices. Adoption of an integrated approach to sustainable production is necessary if canola growers want to be productive in the long-term.

Through out the process of researching and writing this paper, I have observed that there exist two potential opportunities for improvement in canola production. The first opportunity was briefly discussed in chapter two, 'Breeding Canola', resynthesis of *B.juncea* and *B.napus* to expand the genetic diversity and variability available to breeders. Advancements in high-throughput technologies will increase the

characterization of the genomic sequences of *Brassica* and related species. As the genomic sequences become available, breeders will have the knowledge necessary to expand the research and development of resynthesized *Brassica* species. Introgression of desired traits from resynthesized *Brassica* species using traditional breeding techniques, such as recurrent selection, back-crossing and protoplast fusion along with modern techniques (transgenic) would greatly enhance a breeder's ability to meet future challenges.

Challenges such as those presented by market forces that continue to encourage growers to choose agronomic practices that can quickly breakdown resistance to diseases such as blackleg, white mold, and clubroot. In addition, identification and introgression of genes with desired traits, such as host plant resistance to insect feeding, drought, heat, and cold tolerance could expand the temporal and spatial areas that canola could be cultivated. These are important considerations in light of the developing global climatic conditions, such as the expected extremes in weather variability and limited resources and areas in which to grow canola.

A second opportunity I see is with the current average emergence percentages compared to germination percentages. Currently, Grade 1 canola seed must have a germination rate of about 90% and grade 2 canola seed 80-89% (CCC 2013). Yet, a 50% average emergence/seedling survival is common. There is an apparent disconnect between these two occurrences. While this paper has outlined some cultural practices (adequate fertility, timely weed control, large seed size, uniform planting depth, higher seeding rate, row and seed spacing) that can improve emergence rates and uniform stands, more research is needed. Research that seeks answers concerning the

discrepancies between germination, emergence, or seedling survival percentages and how to minimize them could prove to be helpful in increasing and maximizing yields.

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