

Volunteer Canola (*Brassica napus* L.) Interference with Soybean (*Glycine max* L.  
[Merr.]

BY

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## Abstract

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Soybean (*Glycine max* L. [Merr.]) has become a popular crop choice for producers in Manitoba as the number of seeded hectares has grown from 40 500 ha in 2005 to 665 320 ha in 2016. Lack of diversity in crop rotations is part of the reason why increasing populations of glyphosate-resistant volunteer canola (*Brassica napus* L.) in glyphosate-resistant soybeans are now a concern. Currently, farmers tend to ignore population density of volunteer canola in the field or control volunteer canola based on aesthetics. In 2012 and 2013, 6 paired field experiments were established across southern Manitoba as a randomized complete block design. In each year, three experiments were seeded with a narrow row-spacing of 25 cm, and three experiments were seeded with a wide row-spacing of 75 cm. Increasing densities of volunteer canola were broadcast onto soybean plots to determine the action threshold in soybean as well as evaluate soybean growth and development parameters and their relative importance to yield loss. Height and branching were determined to be important parameters in describing the effects of interference between volunteer canola and soybean in both narrow and wide row-spaced soybean. Other growth and development parameters impacted by the interference of volunteer canola were soybean leaf area, biomass, light interception, seed weight and seed size. Row-spacing did not appear to have a significant impact on the action threshold. The action threshold (calculated at 5 % soybean yield loss) in narrow row-spaced soybean was

3.0 plants m<sup>-2</sup> while the wide row-spaced soybean action threshold was 2.7 plants m<sup>-2</sup>.

Digital images were captured between V3 and V4 to determine if early-season volunteer canola and soybean ground cover can be related to yield loss in soybean. There were no apparent differences between row types for image analysis and the method shows promise relating early-season ground cover to soybean yield loss that could serve as a decision making tool to manage volunteer canola in soybean.

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Derek Lewis

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Jenny Pieniuta

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## **Dedication**

To my amazing wife Kaitlin! There were some tough days and some really tough days, my dear, you supported me the whole way and I can't thank you enough! Cold falls, and hot summers, muddy days, slow days, busy days, and bad days, you understood it all, my wife, I love you so much. You pushed me, helped get me this far, thank you so much. And of course, to my little 7 lb 6 oz baby girl Natasha, dad loves you lots. You are adorable, cute, funny, love cats, and especially love your mom. I will cherish moments, little moments, but meaningful to me. Some nights were long, but I never thought our baby would be so amazing. I loved having you sleep in my office while I worked.

Kaitlin you're an amazing wife and mother, I dedicate this thesis to you and can't wait to see where life leads us that, for the first time since we've been married, neither of us are in university ; )

Thank you to my parents Michel and Michelle, and Brian and Jane for asking me how things are going and supporting me along the way. I know it seemed like it would never end, but you've helped so much with allowing me the chance to close out this chapter in my life.

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## 1.0 Introduction

In Manitoba, 2 of the 3 most frequently seeded crops are genetically-engineered for resistance to herbicides. In 2016, canola (*Brassica napus* L.) was seeded to 1 284 200 hectares and soybean (*Glycine max* L. [Merr.]) was seeded to 666 300 hectares. For soybean this marks an increase of 1 645 % over a span of 11 years from 2005 when only 40 500 hectares were seeded to soybean (Lange 2016). Soybean, accounting for nearly 18 % of all seeded hectares in Manitoba in 2016 is used to produce edible products such as tofu, soy milk and margarine as well as industrial products such as inks, waxes and resins for spray foam insulation (Statistics Canada 2009).

Of the soybeans grown in Manitoba, 93-95 % are glyphosate-resistant (MAFRD 2012). The benefits from low costs and ease of weed management in-season and for future weed populations have led to the popularity of using herbicide-resistant canola and soybean varieties (Gulden et al. 2009a; Ivany 2004; Scursoni et al. 2006). The widespread production and the ability for canola to disperse, in combination with canola seedlot contamination, have resulted in volunteer canola becoming the fifth most abundant weed in Manitoba (Friesen et al. 2003; Knispel and McLachlan 2010; Leeson et al. 2016). Furthermore, herbicide-resistant soybeans are seeded to several different row-widths and populations in Manitoba, there is a group of farmers seeding soybeans with the same seeder they would use to seed their cereal crops; while other farmers use precision planters. Consequently, the increased popularity of herbicide-resistant crops and the array of farm management practices in Manitoba are some of the reasons why there are wide ranges of densities of herbicide-resistant volunteer canola in soybean contribution to yield loss.

The purpose for this project was to assess the impact of volunteer canola interference on soybean yield, growth and development and to determine action thresholds for volunteer canola in soybean in Manitoba.

The goals for the following research are to assess the following three objectives:

- 1 – To determine the impact of increasing densities of volunteer canola on soybean growth, development and yield.
- 2 – To develop an action thresholds for managing volunteer canola in narrow and wide row-spaced soybean.
- 3 – To link early-season ground cover from increasing densities of volunteer canola to yield loss in soybean.

## **2.0 Literature Review**

### **2.1 Soybean**

#### **2.1.1 Soybean History**

The earliest records that refer to soybean are from China in 2823 B.C. These records refer to soybeans as plants with medical properties, and soybean is thought to be 1 of the 5 essential crops to early Chinese civilisation. In 1765, the first soybean seeds were shipped from China and seeded in the United States, and in 1890 the first soybean research trials were conducted at the Kansas Agricultural Experiment Station (Hymowitz and Harlan 1983; Morse 1949). Commercial production in Canada was restricted by climate to southern Ontario until 1976. Since then, varieties requiring fewer days to maturity have been produced in eastern Canada which were then introduced to western Canada (Statistics Canada 2009). The scientific name for soybean, *Glycine max*, is derived from the Greek word for “glykys” meaning sweet, alluding to the sweetness of the roots and leaves (Smith 1997).

#### **2.1.2 Soybean Production**

Global soybean production in 2016 was 313.1 million tonnes with 89.4 % of that production produced by the United States of America, Brazil and Argentina at 36.9, 34.2 and 18.2 % of total global production, respectively (Global Soybean Production 2017). In 2013, a total of 1 828 800 ha that produced 6 094 000 tonnes of soybean were planted in Canada, and Manitoba contributed 23 and 18 %, respectively, to that total Canadian production (Canadian Soybean Canada 2014; MAFRD 2014). Soybean has become a popular crop choice for producers in Manitoba as the number of seeded hectares has grown from 40 500 ha in 2005 to 666 300 ha in 2016, making soybean the crop with the

third largest acreage in Manitoba following wheat and canola (Lange 2016). The increase in value of soybeans, from \$242 per tonne in 2005 to \$427 per tonne in 2011 (MAFRD 2014) is one reason that has led producers in Manitoba and Western Canada to include soybean in their crop rotations.

### **2.1.3 Soybean Uses**

Soybean seed is used for edible and industrial products as oil and protein for a wide range of consumers. Edible uses for human consumption include tofu, soy milk, margarine and edamame, and simulated meats such as bacon bits among others, while soybean meal can be used for the livestock industry to produce feed (Statistics Canada 2009). McNiven et al. (2004) determined that roasting soybean seed for livestock feed can produce a beef product that is healthier for human consumption by promoting a superior fatty acid composition. Industrial products derived from soybean include inks, waxes for crayons and candles, solvents, plastics and adhesives. Soybeans have been bred for high seed oil and protein content, but also for seed coat color depending on the final use of the seed. Light color seeds and a light color hilum are generally preferred and used for human consumption (Statistics Canada 2009). The prominent challenges to future development of the industrial use market are the technologies and costs associated with the production and development of new soybean oil traits such as the fatty acid composition of soybean oil (Cahoon 2003).

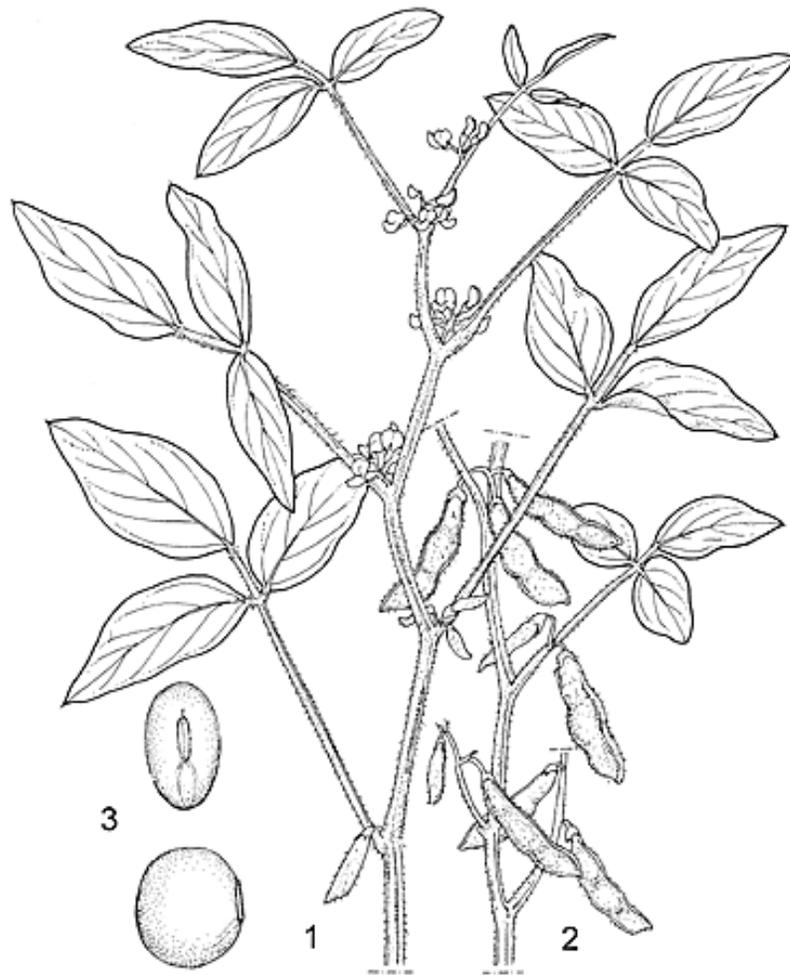
### **2.1.4 Soybean Morphology**

The morphology of soybean is described by Lersten and Carlson (2004), detailing vegetative and reproductive morphology. Soybean growth stage is commonly described

by vegetative phases (V1-Vn), and 8 reproductive phases (R1-R8) (OMAFRA 2014) (Figure 2.1). Soybean is an annual plant that typically grows between 75 to 125 cm in height. Depending on the cultivar and growing conditions (eg. fertility, row spacing and daylength) branching can be dense or sparse. Stems are cylindrical and soybean develops three different types of leaves during a growth cycle: the cotyledons, a pair of simple primary leaves and trifoliates. The simple primary leaves are ovate occurring oppositely at the first node above the cotyledons. Following the simple primary leaves, the remaining leaves that develop are trifoliates arranged alternately with an oblong, ovate to lanceolate shape. Trifoliolate leaflets range from 4 to 20 cm in length and 3 to 10 cm in width.

Below ground, the root system is fibrous and soybean develop several secondary roots that, when grown alone, are capable of reaching depths of 200 cm in a diameter of 250 cm around the crown, however, when grown in competition with other plants the root system is more compact. In ideal conditions, the radicle can emerge from the seed in only 1 to 2 days after planting and seedlings can emerge from the soil in as few as 4 to 5 days. Soybean is a leguminous plant capable entering a nitrogen fixing symbiosis with *Bradyrhizobia japonicum* (Kirchner). Nodules develop on the root when *B. japonicum* penetrate the root causing the root cortical cells to dedifferentiate, become meristematic and develop into special organs housing these bacteria to begin a nitrogen-fixing relationship as early as 10 days after planting. The bacteria in the nodules reduce atmospheric dinitrogen to ammonium which is used by the soybean plant to produce proteins and other nitrogen containing compounds. The centers of nodules that are actively fixing nitrogen have a reddish pink coloration.

The duration of anthesis in soybean is dependent on when soybean is seeded, flowering may last 3 to 5 weeks. Flowers begin development between the third and sixth node then continue to produce flowers on lower and upper parts of the plant. Pods range from 2 to 7 cm in length and produce on average 2 to 3 seeds per pod; the number of pods per plant and where the pods are located on the plant varies among cultivars. The achene is round to oval, tan in color; the hilum can be 1 of 7 different colors based on genetics and the soybean size will vary greatly by variety and growing conditions but on average is about 1 cm long, 0.6 cm wide and 0.8 cm tall.



**Figure 2.1.** *G. max*; 1. Flowering branch, 2. Branch with developed pods, 3. Soybean seed (Prota 2015).

### **2.1.5 Soybean Production Practices in Manitoba**

As seeded hectares continue to increase in Manitoba by producers who have and have not previously grown soybean, different field production practices are being employed as producers work towards determining the most productive and economic practices for this region and their farms. In Manitoba, soybean is typically seeded between May 15 and May 25, when the soil temperature has reached at least 10°C (MAFRD 2014). Soybean crops require approximately 135 days to maturity requiring on average 1 500 to 1 700 growing degree days (base temperature, 5°C) (Agriculture and Agri-Food Canada 2014). Seeds can be planted up to 4 cm deep in sandy soils and are generally recommended to be seeded 2 cm into the soil in order to have sufficient contact with soil and moisture (MAFRD 2015a). Seeding rates for soybeans can vary between 440 000 to 520 000 seeds ha<sup>-1</sup> depending on the producer's desired plant stand. Producers seed using row-widths ranging on average between 25 cm to 75 cm. Inoculating soybean crops is recommended as they are capable of nitrogen fixation through nodules on their root system, and as a result no additional nitrogen is required when seeding soybean. Soybean crops typically require 10 to 40 kg ha<sup>-1</sup> of phosphorus, 35 to 65 kg ha<sup>-1</sup> of potassium and 20 kg ha<sup>-1</sup> of sulphur. The fertilizer requirements are influenced by soil texture, fertilizer placement, row-width and crop history.

Following seeding, fields are typically rolled to push rocks into the soil and create a level field surface to facilitate harvest. Mature soybeans need to be cut close to the ground with a swather or combine in order to catch all of the low hanging pods. Soybeans should be harvested at about 14 % seed moisture content to prevent seed damage and seed losses due to shattering (Manitoba Pulse & Soybean Growers 2014). The seed moisture content

for soybean should be below 13 % for long-term storage to avoid any hot spots from biological activity leading to seed spoilage.

## **2.1.6 Weed Management**

### **2.1.6.1 Glyphosate-Resistant Production Systems**

Growing glyphosate-resistant soybean varieties in Manitoba is very popular as 93 to 95 % of all the soybean hectares are planted to glyphosate-resistant varieties (MAFRD 2014). Gulden et al. (2009a) compared weed control and weed communities over 6 years in conventional herbicide systems to glyphosate-resistant herbicide cropping systems in corn (*Zea Mays* L.) and soybean cropping systems in Ontario and assessed the impact of these herbicide programs on weed ground cover, weed density and yield. In 4 of 5 of the treatments total weed ground cover was lower in the glyphosate-resistant system than in the conventional system. Glyphosate-resistant systems also had lower mid-season weed densities than conventional systems. In 3 of 5 of the glyphosate-resistant treatments soybean yield increased from 7 to 14 %.

A benefit of using glyphosate-resistant soybean varieties includes relatively inexpensive and effective weed management from a single application of glyphosate (Ivany 2004). A single application of glyphosate can provide optimum weed control and crop yield with lower herbicide costs as compared to other conventional herbicide options available. Scursoni et al. (2006) determined that midseason weed species diversity in glyphosate-resistant systems were similar or greater than in conventional systems with a single glyphosate application, while a second application of glyphosate decreased weed species diversity as a result of fewer weed escapes. Another experiment conducted in the Maryland, USA (Kratovichil et al. 2004) determined yield loss for 4 glyphosate-resistant

soybean varieties; seeding rates were reduced by 20 and 40 % across 3 different row spacings. Significant yield loss was observed in 1 of 4 varieties when seeding rates were reduced to 20 % in 19 and 38 cm row spacing while the other 3 varieties showed no significant yield loss. When seeding rates were reduced by 40 %, significant yield loss was observed in all 4 glyphosate-resistant soybean varieties.

## **2.1.6.2 Weed Interference with Soybean**

### **2.1.6.2.1 Weed Competition**

Numerous studies have assessed the impact of weed interference between soybean and different weed species to develop an understanding of their interactions and the impact on soybean yield and development. Dekker and Meggitt (1983) evaluated the effect of velvetleaf (*Abutilon theophrasti* Medik.) interference on soybean dry matter, branching and seed production near Lansing, Michigan. Experiments were setup as an additive design, average weed populations for increasing weed densities were 0, 3.1, 6.0, 9.1 and 17.1 plants m<sup>-2</sup>. Soybean data was collected mid-season and at harvest. Dry matter, branching nodes and yield decreased on average by 33, 26 and 42 %, respectively, at the two lower weed densities and decreased on average by 46, 45 and 64 %, respectively, at the two higher weed densities. Greater losses were observed at higher weed densities, but the rate of loss was less as weed densities became higher.

Shurtleff and Coble (1985) compared the interference of 5 species of weeds with soybean and the impact on soybean height, leaf area and yield in North Carolina. The numbers of weed seeds planted per 10 meters of soybean row were 0, 2, 4, 8 and 16 seeds. Weed seeds were also planted at 3 different distances from the soybean row (15, 30 and 60 cm), soybean leaf area decreased as the distance between soybean plants and

weeds decreased, this trend coincided with yield loss as the soybean plants were in closer proximity to weeds. At an average distance of 30 cm from the soybean row, soybeans were shorter when grown in competition with common cocklebur (*Xanthium strumarium* L.), common lambsquarters (*Chenopodium album* L.), sicklepod (*Senna obtusifolia* L.) and common ragweed (*Ambrosia artemisifolia* L.). In the case of red root pigweed (*Amaranthus retroflexus* L.), soybean height increased, they suggested that soybean may be displaying an ability to compete for space with taller weeds.

A study by Umarani and Selvaraj (1996) at the Tamil Nadu Agricultural University in India compared the germinability of harvested soybean seed under different levels of weed interference. Weed densities were maintained throughout the season at 0, 25, 50, 75 and 100 percent of the weedy control treatment. At maturity, soybean plants were threshed and the collected seed was subjected to a germination test. As the density of weeds increased, germinable soybean seed was reduced by 5.1 % when grown with the highest weed densities. In a similar study by Millar et al. (2007), where different levels of weed competition with soybean were evaluated, a decrease in seed oil content was observed. The effects of volunteer canola interference on the soybean have not been studied before.

#### **2.1.6.2.2 Effect of Row Spacing and Soybean Population Density on Weed Interference**

Many studies have examined the effect of row-spacing on soybean growth and development to the optimal spatial arrangement for productivity. Row-spacing has been shown to be a key contributor to the crop-weed interference and the resulting crop yield loss. Harder et al. (2007) conducted 3 field studies in 2004 and 2005 to determine the

effect of row width and soybean population density on weed populations, crop canopy, crop yield and profit margins. Soybeans were planted at three different row widths (19, 38 and 76 cm) and at 3 different population densities (averages between both years were 191 500, 302 500 and 445 000 plants ha<sup>-1</sup>). Naturally occurring weed populations included annual grasses and broadleaf weeds such as common lambsquarters, common ragweed and wild mustard (*Sinapis arvensis* L.). Leaf area index (LAI) was measured to determine light penetration through the soybean canopy and found to be similar between narrow and medium row-spacing, but LAI in these row-spacing's were greater than in soybeans planted to wide rows indicating more rapid canopy closure and more effective light capture at more narrow row-spacing's. Different soybean populations for wide row-spaced soybeans did not have an impact on LAI. Canopy closure in the medium population of wide row-spaced soybean was delayed by 2 weeks compared to the high population of narrow-spaced soybeans. Following a herbicide application, fewer weeds emerged and weed biomass was reduced in narrow row-spaced soybean as compared to wide row-spaced soybeans.

Similar research (Legere and Schreiber 1989) demonstrated the relationship between canopy closure in narrow row-spaced soybean to suppress additional flushes of weeds and consequently resulted in increased yields in narrow row-spaced soybean. Harder et al. (2007) also suggested that increased weed emergence in wide row-spaced soybean may not reduce yield loss, but low-density weed escapes can set large amounts of seed contributing to the seed bank. In the weedy controls, soybeans planted to narrow and medium row-spacing's had higher yields at all population densities compared to soybeans planted to 76 cm row-spacing signifying the competitive ability of soybeans planted in more narrow row-spacing. Gross profit margins were higher for narrow row-spaced

soybeans compared to wide row-spaced soybean for all soybean populations. The importance of soybean row spacing on the outcome of soybean-volunteer canola interference is not known.

#### **2.1.6.2.3 Critical Period for Weed Removal**

In 1990 and 1991, three field experiments were established in southern Ontario to determine the critical period for weed control in soybean (van Acker et al. 1993). Field experiments were planted at 60 cm row-spacing and naturally occurring weed populations were allowed to grow. All weeds were removed and the plots were kept weed-free for the remainder of the growing season when the following developmental stages of soybean were reached: the first trifoliolate, second trifoliolate, third trifoliolate, fourth trifoliolate, beginning bloom, beginning pod set, beginning of seed formation and one treatment remained unweeded throughout the growing season. In addition, analogous treatments were established where plots would remain weed-free until each of these developmental stages in soybean and weeds emerging after these stages would be allowed to grow for the remainder of the growing season. Researchers observed that a weed-free period between 21 to 30 days after emergence would limit yield loss to 2.5 %, while a shorter weed free period of 0 to 15 days after emergence resulted in 10 % yield loss in soybean which was likely a result of more weed competition during the early stages of soybean development.

Field experiments were conducted in 1999, 2000, and 2001 (Knezevic et al. 2003) to determine the effect of row-spacing on the critical period for weed removal in soybean. Soybeans were planted to three different row-spacings of 19, 38 and 76 cm, all at the same population density. Naturally occurring weed populations were allowed to grow

until the average soybean had reached the one trifoliolate, two trifoliolate, three trifoliolate, beginning of pod formation and beginning of seed formation developmental stages. Yield data showed that wide row-spaced soybean (76 cm) exhibited reduced early-season crop tolerance to weeds (first trifoliolate), thus requiring earlier weed management practices than when soybean were grown in moderate row-spaced soybean (38 cm, second trifoliolate), and the narrow row-spaced soybean (19 cm) had the latest critical period for weed removal (third trifoliolate).

## **2.2 Canola**

### **2.2.1 Canola History**

Plants from the *Brassica* genus, such as mustard, kale and cabbage, have been important parts of a human diet in Europe and India around 2000 to 1500 B.C. (Bell 1981; Downey 1965). Oilseed rape was first introduced in 1936 in Saskatchewan by a Polish immigrant farmer, these seeds then became a source of seed for the Canada Department of Agriculture to test across Canada. Originally grown as an industrial oilseed, canola was developed to have the desirable edible qualities of low glucosinolate and low erucic acid, in 1974 the first canola variety, Tower, was released (Canola Council of Canada 2015a; Gulden 2008). The name canola is a combination of Canada ‘can’ and ‘ola’, meaning oil.

### **2.2.2 Canola Production Statistics**

Manitoba produced 2 313 300 tonnes of canola on 1 185 700 ha in 2014 while Canada produced 15 555 100 tonnes of canola on 8 074 700 ha in 2014, Manitoba produced 15 % and seeded 15 % of the entire Canadian market (Canola Council of Canada 2014). Canola

production in Manitoba has been fairly consistent, in 2004, 1 011 700 ha of canola was seeded, that value has increased slightly to 1 185 700 ha of seeded canola in 2014, making canola the second largest seeded crop in Manitoba behind wheat. The value of canola per tonne of crude oil has increased from \$785.07 in 2004 to \$947.44 in 2014.

### **2.2.3 Canola Uses**

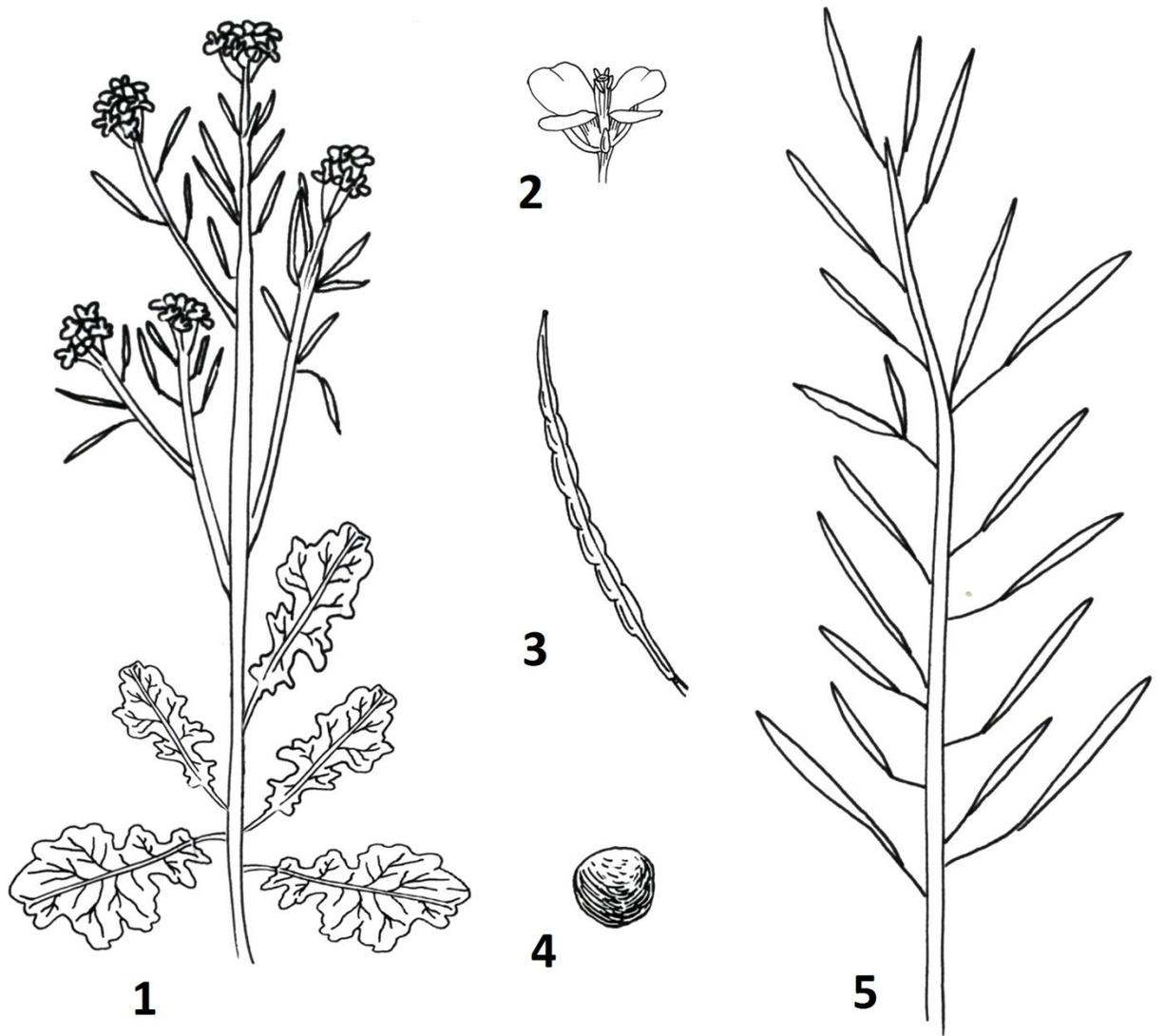
Canola is used for the edible and industrial oil market by crushing the oil from the seed (Canola Council of Canada 2015b). Canola oil contains high levels of monounsaturated and polyunsaturated fats, vitamin E and is cholesterol free. The flavor and light-clear colored oil have made canola oil a popular choice for chefs, food processors and general consumers. The by-product of crushing the canola for oil is meal which is a widely used protein source in animal feed. Canola seed is also being crushed to produce biodiesel, the meal by-product from crushing is once again sent to be used for animal feed

### **2.2.4 Canola Morphology**

Canola morphology is described in *The Biology of Canadian weeds – Brassica napus* L. and on the Canola council of Canada website (Canola Council of Canada 2015b; Gulden et al. 2008) (Figure 2.2). This summer annual crop has a small dark round seed 1.8 to 2.7 mm in diameter. The seed germinates between 4 to 10 days after planting depending on soil and environmental conditions. Cotyledons have a distinct kidney shaped, as the plant develops rosette shape leaves are 5 to 20 cm long, hairless and are generally ovate, elongated and lobed. Canola develops a taproot and will develop many secondary roots; the length of the root system will vary, but on average reach 140 cm

below the surface. Stem elongation or 'bolting' coincides with the start of flowering and branch initiation, nutrient translocation from the leaf to the fruit begins as the fruit begins to develop. Branches develop alternately and the plant grows straight up and can be 0.5 to 1.3 meters high.

The classic yellow canola field in July is a result of the pale to bright yellow petals arranged in a cross during flowering; canola will flower between 14 to 21 days under average conditions. Nearly cylindrical pods are made up of two separate segments about 4.5 to 7.5 cm long and 2 to 5 cm thick. Pods typically hold between 1 to 40 small seeds and on average weigh 3.5 to 5.5 grams per 1000 seeds.



**Figure 2.2.** *B. Napus*; 1. Flowering branch, 2. Flower, 3. Pod, 4. Seed, 5. Mature branch (Loewen 2016).

### **2.2.5 Canola Production Practices in Manitoba**

The seeded hectares for canola in Manitoba have been steady for the past 20 years suggesting that producers are comfortable with the management and predictability of the crop. Canola is a cool season crop and prefers moderate heat and moisture, extreme climate conditions can lead to yield loss and disease outbreak. Canola is planted 1 to 4 cm into the soil in May and into the first few weeks on June. The range for canola to mature is broad from 74 to 124 days to maturity in Manitoba and on average canola varieties in Manitoba require 82 days to complete a life cycle (Canola Council of Canada 2015b). Ideally, canola is seeded to densities of 70 to 110 plants m<sup>-2</sup>. Canola seeds are coated with insecticide and a fungicide to protect the seed and the early stages of plant development, in recent years new products have included micro-nutrients and bacteria to promote phosphorus uptake in addition to pesticides. Following a wheat crop, canola typically requires 75 to 95 kg ha<sup>-1</sup> of nitrogen, 35 to 45 kg ha<sup>-1</sup> of phosphorus, 35 to 70 kg ha<sup>-1</sup> of potassium and 25 kg ha<sup>-1</sup> of sulphur, based on soil texture, fertilizer placement and cropping history (MAFRD 2015b).

Canola has been genetically modified to support three weed control options other than conventional herbicides including resistance to imidazolinone chemistries, glyphosate and glufosinate ammonium (Canola Council of Canada 2015b). Canola may be straight-cut or swathed, timing is critical to reduce harvest losses and may be desiccated to accelerate crop dry-down and improve crop moisture uniformity (MAFRD 2015c). Combining can commence when seed moisture is at or below 10 % and can be stored for long-term storage between 8 to 9 % moisture at temperatures below 20°C.

## **2.2.6 Volunteer Canola as a Weed**

### **2.1.6.1 Harvest Losses and the Seedbank**

Canola seed loss during harvest has driven volunteer canola populations in fields and innate characteristics have allowed canola to be able to develop persistent seedbanks. To determine seedbank additions at harvest, 35 producer fields were sampled within 150 km of Saskatoon, Saskatchewan in 1999 and 2000 (Gulden et al. 2003b). Soil was collected with a wet-dry vacuum at three random locations within the field; soil and residue were separated from the seed and the seed was counted and tested for germinability.

Germination test in 2000 concluded that an average of 82 % of the seeds collected were viable. Canola seed harvest losses were on average 3 000 seeds m<sup>-2</sup> with counts ranging from 1 530 to 7 130 seeds m<sup>-2</sup> and one field with losses of 13 900 seeds m<sup>-2</sup>. Considering between 70 to 100 seeds m<sup>-2</sup> is a typical seeding rate for canola, harvest losses in terms of seeding rate means that 18 to 22 times the seeding rate is being added to the soil. The small seed size for canola is certainly a contributing factor to harvest losses, but primarily harvest losses measured to be as high as 4 447 to 6 429 seeds m<sup>-2</sup> (Cavalieri et al. 2016) and as high as 13 900 seeds m<sup>-2</sup> (Gulden et al. 2003b) are likely the result of the maturity of the crop while windrowing and the combine settings and speed during harvest

Persistence in the seedbank from secondary seed dormancy allowed volunteer canola to be a weed in 17 % of fields in 2002 in Manitoba and the 5<sup>th</sup> most abundant weed in Manitoba (Leeson et al. 2016; Van Acker et al. 2002). In 1999, Gulden et al. (2003) established two field trials near Saskatoon, Saskatchewan where 2000 seeds m<sup>-2</sup> of canola from 8 different varieties were applied to the soil surface in the fall then followed by three years of continuous wheat (*Triticum aestivum* L.). Each canola variety was exposed to

either conventionally-tilled or zero-till treatments to compare treatments with and without soil disturbance by tillage equipment.

Concurrently, a study with the same varieties was performed to categorize the six canola varieties into either high or medium potential to develop secondary seed dormancy (Gulden et al. 2003a). In the three years following establishment the high potential canola varieties exhibited 6 to 12 times greater persistence in the soil than the medium potential canola varieties. The canola genotype groups separated by potential for secondary dormancy affected the persistence of the seedbank and indicated that growing varieties with a lower potential for secondary dormancy would significantly reduce persistence of seeds in the soil. Varietal differences for developing secondary dormancy ranged from 0.7 % dormant seed to 80 % dormant seed indicating a clear genetic link to this trait (Gulden et al. 2004a; Pekrun et al. 1997). The genetic link for seed size classes within a variety contributed 21 % of the variation to the potential for canola seed to develop secondary seed dormancy (Gulden et al. 2004a). When comparing the soil disturbance treatments, seedbank persistence was similar (not significantly different) in the zero-till system compared to the conventional-till system.

This is supported by work by Legere et al. (2001) explaining how volunteer canola can exhibit secondary dormancy under zero-till conditions and has been shown to persist for 4 to 5 years after production (Simard et al. 2002) where large quantities of residue are present (Gulden et al. 2003a; Legere et al. 2001). Contrasting research has detailed that canola seed burial from increasing levels of soil disturbance largely contributes to seedbank persistence (Harker et al. 2006; Pekrun et al. 1998). The depth of the seed has been shown to be a significant contributing factor as shallow seedbanks are more susceptible to influences such as temperature, precipitation, predation and winter

survivorship (Gulden et al. 2004b). Abiotic factors also appeared to play a role in seedbank persistence as lower levels of precipitation in the fall resulted in fewer canola seedbank losses to lethal germination (Gulden et al. 2003a). Other contributing factors include the pre-harvest environment that appears to have an influence on the potential for development of secondary dormancy in canola (Gulden et al. 2004b) and that greater persistence in the soil was observed when canola was grown on fine-textured soil (Gulden et al. 2003a).

#### **2.2.6.2 Cross Contamination of Herbicide-Resistant Traits**

The presence of herbicide-resistant volunteer canola is found in fields which have and have not had a history of herbicide-resistant canola production. Friesen et al. (2003) investigated the complaint that farmers were observing glyphosate-resistant volunteer canola plants in their fields after harvesting a herbicide-resistant canola crops resistant to herbicide modes of action other than glyphosate. Commercially available certified canola seedlots from a number of different companies were sampled by collecting several cups of seed from 33 different seedlots to assess the level of cross contamination of herbicide resistance traits. Each seed source was planted and sprayed with 1 of 6 different treatments of glyphosate, glufosinate and thifensulfuron to test for the presence of plants resistant to additional herbicides different from that specified, referred to as herbicide resistance trait contamination (HRTC) from here on. Of the 33 seed sources, 1 seedlot had no detectable HRTC, 18 sources had less than 0.25 % of the population had HRTC and 14 had HRTC above 0.25 % and 3 seed sources had HRTC in excess of 2 %. Of the 14 seed sources above 0.25 % with HRTC, 9 were a result of adventitious presence of resistance to glyphosate and 5 sources with HRTC were a result of adventitious presence of

resistance to glufosinate. Although this research was not replicated, the research suggests that some certified seed is not meeting industry guidelines to be considered ‘certified’ seed. Seedlot contamination appears to be a source of unwanted and unexpected HRTC in volunteer canola present in the seedbank which is related to the frequency that farmers plant canola.

Widespread cultivation and large-scale dispersal of canola seed allow genetically modified herbicide-resistant volunteer canola to become permanently entrenched as a weed in western Canada (Knispel and Mclachlan 2010). Volunteer canola populations being actively managed depend on being dispersed by agricultural transport and processes to maintain and establish new populations. Theoretically, any transgenic organism may be next to impossible to remove from a population once released if that organism has inherent advantages and will likely be cost-prohibitive to eradicate (Marvier and Van Acker 2005). Knispel and Mclachlan (2010) suggest that a coexistence strategy will only exacerbate the impact of volunteer canola as a weed and that perhaps distinct regions should be divided to produce genetically modified material and non-genetically modified material to ensure cropping system choice is maintained.

### **2.2.6.3 Breeding Objectives and Their Relationship to Weediness in Canola**

The relative abundance of volunteer canola in fields in Manitoba has increased from ninth most abundant weed in Manitoba in 2002 (van Acker 2002) to fifth most abundant weed in Manitoba in 2016 (Leeson et al. 2016) and has the ability to be a very competitive weed to most crops (Hall et al. 2005). These authors also present a list of weedy characteristics still present in canola. Factors contributing to canola to be a competitive volunteer are that canola is bred for disease, lodging and herbicide resistance

and is therefore adapted to stresses such as disease pressure, windy environments or control by certain herbicides.

#### **2.2.6.3.1 Canola Plasticity**

Canola is bred to be successfully grown across a wide range of environments; plasticity is the ability to balance growth and development and compensate for yield losses amid poor growing conditions. High phenotypic plasticity was shown in a study by Angadi et al. (2003) where plant density and stand uniformity were correlated to canola yield and biomass. They determined that yield was lower at lower plant populations, but yields were also unaffected across a wide range of plant population densities indicating a propensity to capture available space and resources. Shoot biomass produced from lower plant densities was not statistically different from that at higher plant densities, and there was no effect on biomass accumulation comparing uniform and non-uniform plant stands. Although stand uniformity does have an impact on canola yield, a recent study (Yang et al. 2014) measured that there was a 32 % yield increase when canola plant stands were in a spatially uniform arrangement at low-yield sites and a 20 % yield increase was observed when canola plant stands were spatially uniform at high-yielding sites compared to plant stands that were not in a spatially uniform arrangement. Plasticity is also bred into canola to have high rates of early-season crop biomass accumulation and increased seedling vigour has been shown to accumulate more resources that contribute to suppressing weeds or crops across varying environments (Beckie et al. 2008; Hall et al. 2005).

### 2.2.6.3.2 Seed Dispersal

Canola weediness is also represented by the ability to disperse over time and space, often a key characteristic for weeds to survive and persist (Hall et al. 2005). Canola is capable of forming a moderately persistent seedbank (Gulden et al. 2003a) providing dispersal in time and in addition canola seed is very small and can easily be dispersed among locations by human actions when moving farm equipment or transporting canola seed from the field to storage facilities and beyond (Knispel and McLachlan 2010). A survey conducted in 2005 (Yoshimura et al. 2006) along popular shipping railways and roads within Saskatchewan destined for the port of Vancouver to detect the occurrence of herbicide-resistant canola. They found that the mean plant densities along railways were greater than along roads and that two-thirds of the plants sampled were herbicide-resistant varieties. Furthermore, a single hybrid cross between *B. rapa* and *B. napus* was detected suggesting that populations could persist and spread under current railway and roadside control measures.

Another study to determine the persistence of volunteer canola populations in natural habitats along the roadways surrounding London, UK was established over a 10 year period between 1993 and 2002; 3658 quadrats were placed along roadways to observe the populations dynamics of feral populations of canola (Crawley and Brown 2004). Key observations were that the majority of populations survived between 0 and 4 years and few of the quadrants recorded populations over the entire survey period. Researchers noted that feral populations along roadsides did not appear to self-replace but that these populations were dependent on seed additions from seed spilled from trucks to persist. Similar results were observed in Western Australia where populations were found to

persist for three years before the population would become extinct (Busi and Powles 2016).

#### **2.2.6.3.3 Managing Seedbank Persistence**

Volunteer canola seeds tend to exhibit dormancy to germinate and grow under the most favorable field conditions (reviewed in Gulden et al. 2008). Another key weedy characteristic bred into canola is a high capacity to reproduce, meaning high yields made up of large volumes of comparatively small seeds relative to other crops. As a result, preventing seed bank replenishment may be the best solution to reduce herbicide-tolerant volunteer canola (Hall et al. 2005; Harker et al. 2006; Johnson et al. 2004; Kumur and Jha 2015). Following a canola crop producers are more likely to cultivate a field shortly after harvest when conditions are favorable for fieldwork but by doing so they may be encouraging secondary seed dormancy in future years by seed burial (Gulden et al. 2004b).

Soil disturbance often occurs again in some fields when fall-applied nitrogen is applied, potentially increasing seedbank persistence from increased soil disturbance (Gulden et al. 2004b; Harker et al. 2006). Studies in Europe have demonstrated that limiting soil disturbance to late fall or the following spring significantly reduces canola volunteers by promoting canola seed predation, exposure to the environment and winter kill (Gruber et al. 2004). In North America opposite effects have been observed, where a light harrow or limiting the depth and aggressiveness of cultivation (eg. smaller shovels or strip tillage) immediately after harvesting canola can promote germination rather than promoting burial and reduce volunteer canola recruitment in the following spring due to winter kill of germinated seeds and emerged seedlings (Charles Geddes personal

communication). Limiting the depth of cultivation and seed burial appears to be critical to limit persistence that occurs in deeply buried seeds.

An example of weed seed persistence in the soil is a study established in Colorado (Zorner et al. 1984) to evaluate weed seed longevity of kochia (*Kochia scoparia L.*). Mesh cloth bags were used to hold 400 kochia seeds and buried at different depths and replicated across time such that bags would be removed from the soil after 1, 2, 4, 6, 9, 12, 18, 24, 30 and 36 months. Kochia seed buried 5 cm into the soil had 10 % germinable seed after just 12 months; after 18 months, 3 % of the seed was germinable. This rapid reduction in germinable kochia seed is supported by a study linking seed coat thickness and weed seed mortality in the soil. Kochia has a seed coat thickness of 27.7  $\mu\text{m}$  (Davis et al. 2008), while canola has on average a seed coat thickness of 50  $\mu\text{m}$  (Hu et al. 2013), these two species have reduced longevity in the soil and seed coat thickness appears to influence the susceptibility by external attacks and increasing seed decay and longevity in the soil (Gardarin et al. 2010).

### **2.3 Yield Loss Model**

A two-parameter rectangular hyperbola yield loss model was adopted by Dr. Roger Cousens and others (1985) who compared this model against seventeen other linear and non-linear 2- and 3-parameter equations using 22 independent data sets. Results determined that the two-parameter rectangular hyperbolic yield loss model which is similar to the Michaelis–Menten function used for enzyme kinetics best described these data sets and has been adapted as a predictive tool. In addition, the equation uses biologically meaningful parameters. A strong argument was made for the rectangular hyperbola over the popular sigmoidal model because the rectangular hyperbola was better

at describing the effect of yield loss from interference at low weed densities. Yield loss models continue to be based on weed density as the input variable because weed density can be determined at the earliest developmental stages, allowing this and other density-based yield loss model to be a predictive and decision-support tool as there is still time to manage weeds in-crop if predicted yield losses are above tolerable levels. To determine weed-crop competition, experiments need to be established as an additive design study where the crop species is seeded at the same population density throughout all treatments while the weed species is seeded at increasing densities which is expected to cause different levels of yield loss. The rectangular yield loss model is as follows:

$$\text{Yield loss} = I * d / ( 1 + I * d / A ) \text{ [eq.1]}$$

where, yield loss is the percent yield loss due to weed competition, I is the percent yield loss per weed as the weed density approaches zero, d is the weed density and A is the maximum predicted yield loss as weed density approaches infinity.

Several studies have used this equation to quantify the link between yield losses and weed density. This yield loss model was used in two experiments (Lewis and Gulden 2014; Lewis et al. 2015) to quantify the effects of increasing weed density on yield in sunflower (*Helianthus annus* L.). In 2009-2011, kochia seed was broadcasted targeting 6 different population densities into sunflower plots. Several crop and weed measurements were recorded including yield. Using the yield data and weed density, the parameters ‘I’ and ‘A’ were determined and showed a yield loss of up to 76 % in sunflower from kochia interference [eq.1]. Yield loss data were used to determine an action threshold at 5 % yield loss in sunflower and this threshold was 4 kochia plants m<sup>-2</sup> [eq.2]. The action threshold for a given percentage of yield loss is determined as follows:

$$d = ( A * Y_L ) / ( I * A - I * Y_L ) \text{ [eq.2]}$$

where, the  $d$  is the weed density,  $A$  is the percent yield loss as weed density approaches infinity,  $Y_L$  is the yield loss is the percent yield loss due to weed competition,  $I$  is the percent yield loss per weed as the weed density approaches zero. The 2015 study (Lewis et al. 2015) determined the yield loss associated with biennial wormwood (*Artemisia biennis* [Willd.]) interference in sunflower which reached a maximum of about 46 % [eq.1]. Again, an action threshold at 5 % yield loss was determined and found to be variable. At 2 site years, the action threshold was 40 biennial wormwood plants  $m^{-2}$ , while at other sites more biennial wormwood plants  $m^{-2}$  were required to reach this threshold. Another experiment compared the effect of weed density and row-spacing on chickpea (*Cicer arietinum* L.) yield loss (Whish et al. 2002). Experiments were established with increasing densities of wild oats (*Avena fatua* L.) in chickpea plots in 1996 and 1997. After calculating parameters 'A' and 'I', there was on average 18 % less yield in wide row-spaced (64 cm) weed-free plots compared to narrow row-spaced (32 cm) weed-free plots. There were no significant differences among weedy plots comparing row-spacing and they suggested that characteristics of the crop (leaf size and shape, plant height, etc.) could contribute to differences in the relationship between yield loss and row-spacing for more and less competitive crops.

Yield loss models are often disregarded because competition from multiple species is rarely studied and analysis and interpretation of the results are difficult and complicated (e.g., Ali et al. 2013; van Acker 1996). The rectangular hyperbola models are best suited to situations where a single weed species competes with the crop. Glyphosate-resistant volunteer canola in glyphosate-resistant soybean is a good example of this as at the moment, this is the only weed that remains in most situations after glyphosate has been applied to soybean. In this instance, a decision-support tool that can help decide whether

an additional control measure (herbicide or other) is required to manage this weed in soybean to prevent economic losses is valuable. Predictive yield loss models to manage weeds have been developed for a producer to reduce the number of unnecessary herbicide application. The Guide to Field Crop Protection published annually in Manitoba offers similar decision-support tools; for example, based on one of three crop densities, and the relative emergence of wild oats before, at the same time, or after the crop, a producer can count the density of wild oats in their field and determine the percent yield loss to be expected from wild oat interference in barley (*Hordeum vulgare* L.) and decide if the value of the yield loss is greater than the cost of application and control which is called the economic threshold (MAFRD 2015d) which for simplicity has been replaced by the action threshold. The action threshold is the point where the producer commences weed control at weed populations above or below the economic threshold substantiated by other drivers such as aesthetics, sociological pressure or aversion to risk (Coble and Mortensen 1992).

## **2.4 Experiment Objectives**

The goals for the following research are to assess the following three objectives:

- 1 – To determine the impact of increasing densities of volunteer canola on soybean growth, development and yield.
- 2 – To develop an action thresholds for managing volunteer canola in narrow and wide row-spaced soybean.
- 3 – To link early-season ground cover from increasing densities of volunteer canola to yield loss in soybean.

### **3.0 Volunteer Canola (*Brassica napus* L.) Interference with Soybean (*Glycine max* L. [Merr.]**

*Abstract.* Volunteer canola is a common weed in glyphosate-resistant soybean in Manitoba as canola and soybean are 2 of the 3 most seeded crops in the province. The seeded canola hectares have been fairly constant over the past decade while the number of seeded soybean hectares has risen from 40 500 ha in 2005 to 666 300 ha in 2016. Yet, no studies have evaluated the effects of interference of volunteer canola on soybean to determine at what threshold weed management should occur or what the impacts of such interference are on soybean growth and development. In 2012 and 2013, six paired field experiments were established at three southern Manitoba locations as randomized complete block designs with four replicates. Experiments were planted with Dekalb 23-10RY (2325 CHU) to a narrow row-spacing of 25 cm, and a wide row-spacing of 75 cm. Canola seed was broadcast at soybean planting at increasing densities of 0, 10, 20, 40, 80, 160, 320 and 640 seeds m<sup>-2</sup> (640 seeds m<sup>-2</sup> in 2013 only). Measurements collected include: heights, crop densities, leaf area, light interception, biomass, branch counts, seed return and analysis of harvested seed. Soybean height and the number of branches were found to be the most significant growth and development parameters affected by interference and differences in these measurements at the early pod stage were the most significant at describing soybean yield. Action thresholds between row-spacing treatments were significantly different at two of the six locations; however, in a combined analysis the effect of row-spacing had no apparent impact on the action threshold for volunteer canola in soybean. On average, the action threshold (5% soybean yield loss) in

narrow row-spaced soybean was 3.0 plants m<sup>-2</sup> while the wide row-spaced soybean action threshold was 2.7 plants m<sup>-2</sup>.

### **3.1 Introduction**

Large amounts of canola shattering in the field and particularly losses during harvest have been recorded at an average of 4 447 to 6 429 seeds m<sup>-2</sup> (Cavaliere et al. 2016) and as high as 13 900 seeds m<sup>-2</sup> in western Canada (Gulden et al. 2003b). Large harvest losses in canola contribute to volunteer canola interference in subsequent crops. Part of the reason why volunteer canola has become the fifth most abundant weed in Manitoba (Leeson et al. 2016) is that canola has high phenotypic plasticity and the ability to capture available space and resources (Angadi et al. 2003; Beckie et al. 2008). Moreover, seed characteristics allow volunteer canola to disperse through time by forming a moderately persistent seedbank (Gulden et al. 2003a) as well as throughout the environment by being transported on field equipment in the field and while being hauled between storage facilities (Knispel and McLachlan 2010; Yoshimura et al. 2006). Ultimately, in glyphosate-resistant soybean, glyphosate-resistant volunteer canola has the ability to evade herbicide control, resulting in farmers to either paying for more expensive herbicides to control volunteer canola in soybean or accepting the in-season soybean yield loss and volunteer canola seedbank additions.

As soybean hectares grew 1 645 % since 2005 to 666 300 ha in Manitoba in 2016, there is a greater need for farmers to have more and better information going forward as seeded soybean hectares are expected to reach 1 214 000 ha by 2022 (Lange 2016). Yet, a search of the literature revealed that no studies have assessed volunteer canola

competition in soybean and determine at what threshold the farmer should consider other more expensive herbicide options or what the impacts could be on soybean growth and development. In addition, farmers continue to experiment on farm with different row-spacings as some farmers are just starting to grow soybean or have only grown soybean for a few years. Other soybean competition studies have shown that under competition, soybean yield, height, branching, biomass and leaf area, among other growth and development parameters, will be impacted negatively as weed densities increase (Dekker and Meggitt 1983; Shurtleff and Coble 1985).

The objective for this study were to determine the effects of increasing densities of volunteer canola in soybean planted to narrow or wide row-spacing on soybean yield, and growth and development. The resulting yield loss was used to determine action thresholds at 5 % (which is about equal to the cost of herbicide and application) soybean yield loss to assist farmers when planning to apply an additional herbicide to manage volunteer canola in soybean.

The specific hypotheses tested in this study were i) maximum yield loss in soybean caused by canola interference differs among locations, ii) more narrow row spacing leads to reduced yield losses in soybean in response to volunteer canola interference, and iii) soybean growth and development will be reduced by increasing densities of volunteer canola.

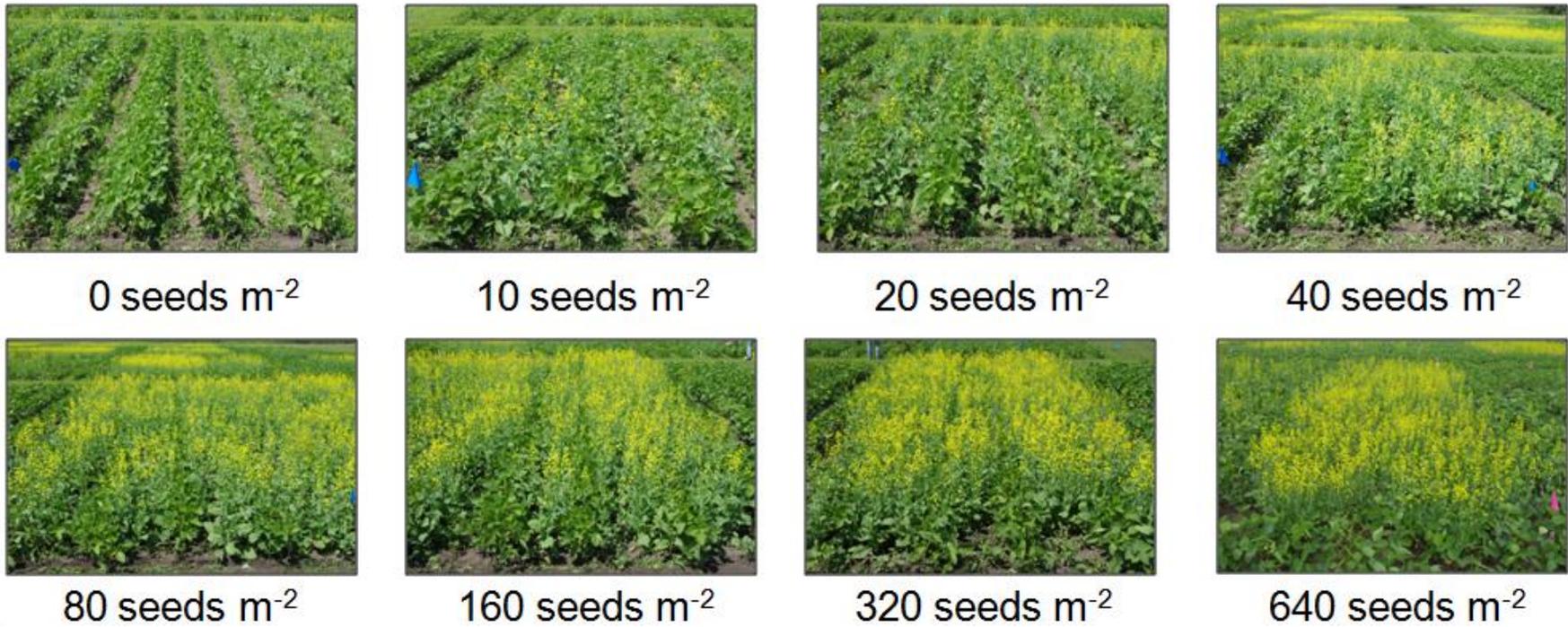
## **3.2 Materials and Methods**

### **3.2.1 Experiment Locations**

Three field experiments were established in 2012 and in 2013 near Kelburn Farms, MB (49°41'15.782", 97°7'22.334"), at the Ian N. Morrison Research Farm near Carman, MB (49°30'3.471", 98°1'49.992") and near Melita, MB (49°15'47.099", 100°59'39.631"). Detailed environmental conditions, soil characteristics and soil analysis are found in the results (sections 3.3.3, 3.3.4 and 3.3.5).

### **3.2.2 Experimental Design, Establishment**

The three paired field experiments were laid out as a randomized complete block design with four replicates in 2012 and again in 2013. At each of the three sites, one of the paired experiments was seeded to a narrow row-spacing of 25 cm and the second set of three experiments was seeded to a wide row-spacing of 75 cm. Both narrow and wide row-spaced experiments were seeded to 445 000 plants ha<sup>-1</sup>. The soybean variety was 23-10 RY. Individual experimental units (plots) were 8 m long by 3 m wide. Bare canola seed (variety 73-45 RR) was hand broadcast evenly over designated plots at rates of 0, 10, 20, 40, 80, 160 and 320 seeds m<sup>-2</sup> in 2012, and in 2013 a higher density treatment of 640 seeds m<sup>-2</sup> was added (Figure 3.1) to better define the yield loss model. In each replicate there were three control treatment plots (0 seeds m<sup>-2</sup>) to account for spatial differences in volunteer canola seedling recruitment. None of the experiments received any fertilizer. All experiments were seeded with granular inoculants at 4.5 kg ha<sup>-1</sup> (Cell-Tech West granular, *Bradyrhizobium japonicum*). All locations were cultivated at least once in the fall or spring and were seeded into wheat stubble. All experiments received two in-crop applications of glyphosate at 1.66 L ha<sup>-1</sup> (commercial rate for weed control), a carrier volume of 100 L ha<sup>-1</sup> at 275 kPa using flat-fan nozzles.



**Figure 3.1** Images of representative plots demonstrating the eight densities (0, 10, 20, 40, 80, 160, 320 and 640 seeds  $\text{m}^{-2}$ ) of canola in wide row-spaced soybean in 2013 at Kelburn.

### 3.2.3 Data Collection

Data were collected at four key time points during the growing season. The first data collection time point was when soybean reached the V1 developmental stage (one fully developed trifoliolate). The heights of three soybean plants were measured in each plot and averaged per plot; volunteer canola heights were also collected the same way by standing the canola plant upright and measuring to the uppermost part of each plant. A 25 cm by 25 cm quadrat was used to determine the density of soybean and canola in each plot, the quadrat was centered on a soybean row in a representative area of the plot.

The second data collection occurred when soybean reached the V3 to V4 developmental stage (three to four fully developed trifoliolates). Once again, height was captured the same way as described for the first data collection time point. Total leaf area for soybeans was measured by removing all leaves from 5 soybean plants per plot and determining the total leaf area for each plant using a leaf area meter (LI-30 Area Meter, LI-COR, Lincoln, Nebraska); leaf area values were then averaged for each plot. Light interception data also were collected; data were collected between 1 and 3 pm on days where there were no clouds in the sky to affect incoming solar radiation. A light sensor (Line-Quantum Sensor, Li-Cor, Lincoln, Nebraska) was held above the crop canopy to capture a baseline reading of solar radiation before collecting the in-plot data. A second light sensor reading was collected by placing the light sensor at ground level into the crop canopy parallel to a soybean row (about 3 cm from the row). A third reading was collected by placing the light sensor perpendicular to the soybean row; all three measurements were collected within 1 min for each plot.

The third data collection point was when the canola had reached physiological maturity. Heights and densities for both soybean and canola were collected in the same

fashion as earlier in the growing season. Five soybean plants were collected per plot and the number of branches per soybean plant were counted on the main stem and averaged per plot. Biomass samples for both soybean and canola were collected by removing a 50 cm by 50 cm area of the plot and separating the soybean from the canola, fresh weights were recorded for both groups of plants then the biomass for each crop was placed in a 60°C oven for 72 hours and re-weighed. The canola biomass samples were hand-threshed then passed through a sieve and the total seed weight was collected.

The fourth data collection time point was at soybean maturity. Each plot was harvested and the soybean seed was measured for final weight and thousand kernel weight; after harvest, seed size and area of the harvested soybean seed were determined using APS Assess 2.0 (APS Press, St. Paul, MN, USA)

### **3.2.4 Statistical Analysis**

Statistical analysis was performed using SAS 9.4 (SAS Institute, Carey, NC, USA). To model soybean yield loss in response to increasing densities of volunteer canola using the rectangular hyperbola (Cousens et al. 1985) and examine differences among the curve parameters, a non-linear Mixed model (Proc NLmixed) approach as described by Knezevic et al. (2002) was used. Briefly, a mixed model approach was used to examine the data and determine initial error term estimates required for the non-linear mixed model. Prior and during mixed model ANOVA, data were examined for the assumptions of ANOVA (i.e., whether the residuals conformed to the normal distribution and heterogeneity of residuals). For this analysis, the fixed variables were site-year, treatment (target density) and row-spacing, while random effects included replicate nested within site-year and the main plot error replicate by row-spacing. As site-year was expected to

be an important factor contributing to differences in soybean yield loss in response to volunteer canola, it was included as a fixed effect.

Following this analysis, data were subjected to non-linear mixed model analysis to determine whether they conformed to the rectangular hyperbola model. In addition to modelling the data to the rectangular hyperbola yield loss model, a series of single-degree-of-freedom estimates were constructed to determine differences among the *I*-values and a similar series of estimates was used to determine differences among the *A*-value estimates (Equation below). Separate NLMixed model analyses were conducted to determine differences between row-spacing treatments within each site-year and in a combined analysis among all site-years.

Mean action thresholds over all experiments were determined using a combined analysis examining the differences between *I*- and *A*-values in narrow and wide row-spaced soybean. This analysis was conducted twice. The first analysis included all site years, while the second and likely more accurate analysis for a meaningful average action threshold excluded Carman 2012 (did not conform) and Melita in 2013 (significantly different from all other sites) (Table 3.2)

A separate, but similar analysis was conducted to determine differences in the *I*- and *A*-values among site-years. Prior to utilizing this method, an extra sums of squares method, described in Lindquist et al. (1996) was used to determine if there were differences between *I*- and *A*-values among site-years (Appendix). The extra sums of squares method is an older method where manual removal of up to 8 or 9 site-years was required for site-years to be considered the same. The analysis used in this study is a newer, better suited analysis for this type of experiment because this extra sums of squares method is an older, less powerful type of method. The following equation was used to calculate the action

threshold, which is based on the parameter estimates from the hyperbolic yield loss model:

$$\frac{\text{Action}}{\text{Threshold}} = \frac{Y_L * A}{(I * A) - (I * Y_L)}$$

$Y_L$  is the determined percentage of yield loss

$A$  is the maximum yield loss as weed density reaches infinity

$I$  represents the percent yield loss per weed at low weed densities

The effect of increasing volunteer canola densities on soybean or volunteer canola growth and development parameters was determined using linear regression analysis. To do this, regressions within site-year and row-spacing were conducted in PROC GLM and linear and/or quadratic effects were determined for each response variable.

Finally, to determine the relative contribution among growth and development in soybean to soybean yield in response to increasing densities of volunteer canola, a multiple regression approach was used within each row-spacing treatment. Prior to regression analysis, all growth and development parameters were normalized to a mean of zero and unit variance and standardized by location and by row-spacing. Model selection criteria included concomitant maximization of the adjusted  $R^2$  and minimization of the AIC. In the multiple regression analysis, multicollinearity among variables was determined by considering the variance inflation factors, conducting the condition number test and assessing the correlation estimates (Table 8.4 and 8.5). The following 3 criteria were used to assess multicollinearity: (a) variance inflation factors are considered moderately correlated between 1 to 5 and not correlated if below 1 (Marquandt 1970), (b)

when considering the eigenvalue the condition number test is conducted by finding the square root of the maximum eigenvalue divided by the minimum eigenvalue, values greater than 10 are considered contributors to multicollinearity (Belsley 1991) and (c) correlation estimates in the correlation matrix were assessed and estimates below 0.50 are not considered to be collinear (Belsley 1991).

### **3.3 Results**

#### **3.3.1 Impact of Volunteer Canola Interference on Soybean**

##### **3.3.1.1 Soybean Yield Loss**

A rectangular hyperbola yield loss model was used to describe percent crop yield loss in narrow and wide row-spaced seeded soybean in response to increasing densities of volunteer canola compared to yields in the respective weed-free treatments. The two parameter values used to describe yield loss are the *I*-value which represents the percent yield loss per weed at low weed densities and the *A*-value which is the maximum percent yield loss as weed density reaches infinity. At all site years except for narrow and wide row-spaced soybean at Carman in 2012 (not significant), the soybean yield loss in response to increasing volunteer canola densities conformed to the rectangular hyperbola model (Table 3.1). The narrow row-spaced soybean experiment with the lowest *I*-value was at Kelburn 2012 and the highest *I*-value in these experiments was over 5-times greater and was observed at Melita 2013. In narrow row-spaced soybean Kelburn 2012 had the largest *A*-value and Melita 2013 had the lowest *A*-value; the maximum theoretical crop yield loss ranged from 25.2 to 77.2 % with significant differences among these values. In narrow row-spaced soybean, the site-year with the lowest *I*-value estimates

(e.g., Kelburn 2012, Kelburn 2013, Carman 2013) tended to have among the greatest *A*-values. In wide row-spaced soybean Carman 2013 had the lowest *I*-value and Melita 2012 had the greatest *I*-value. This equated to a 37-fold range and 5.05 % absolute difference in soybean yield loss per individual volunteer canola plant at low densities. The wide row-spaced soybean experiment with the largest *A*-value was at Kelburn 2012 and the lowest *A*-value was observed at Melita 2013 with a difference of 56.6 % yield loss between these *A*-values.

The rectangular hyperbola model was different between narrow and wide row-spaced soybean in one-third of the experiments (two of the six site years, i.e., Melita 2012 and Kelburn 2013) (Table 3.1). In both cases, the difference was detected between the *I*-values only with greater *I*-values observed in wide row-spaced soybean indicating greater yield loss at low volunteer canola densities in wide row-spaced production systems than narrow row-spaced production systems. Narrow and wide row-spaced soybean experiments were combined for the analysis that examined differences in *I*- and *A*-values among site years. This reduced the possibility of type I errors among the estimates (only half as many required than when separating by row-spacing) and was justified as no differences between row spacing treatments were observed at most site years. Due to lack of fit, Carman 2012 was excluded from this analysis. Based on these single-degree-freedom estimates, the *A*-value, or maximum yield loss at Melita 2013 was significantly lower than all other *A*-values which were not different from each other (Table 3.1). *A*-values for wide and narrow row-spaced soybean were much lower at Melita 2013 than at all other sites indicating soybean were more competitive with volunteer canola at this site year than elsewhere. There also was a difference among *I*-values where the *I*-value of

Melita 2012 was significantly greater than those for Melita 2013 and Carman 2013. All other *I*-values were intermediate (data not shown).

At each location the soybean weed-free yields for narrow and wide row-spaced production were greater in 2012 than in 2013 (Table 3.1). There was a broad range in weed-free yields between narrow and wide row-spaced experiments in Carman for both 2012 and 2013 which had a weed-free yield of 3486 kg ha<sup>-1</sup> ranging to Melita which had a weed-free yield of 878 kg ha<sup>-1</sup>. Melita had 3.2 times more precipitation in July than the long-term average, and only 41 % of the long-term average precipitation.

**Table 3.1.** Weed free yields, parameter estimates for the hyperbolic yield loss model (*I*- and *A*-values) for each experiment and significance of non-linear regression for three locations in 2012 and 2013 for narrow and wide row-spaced seeded soybean. For yield, standard errors of the mean and 95 % confidence intervals for regression parameters are indicated in parenthesis.

Location	Year	Weed Free Yield	<i>I</i>	<i>A</i>
		kg ha <sup>-1</sup>	%	%
Narrow Row-Spacing				
Carman	2012	3486 (51.2)	<i>ns</i>	<i>ns</i>
Kelburn	2012	3401 (97.5)	0.90 (0.21, 1.60)	77.2 (52.4, 102.0)
Melita*	2012	1946 (76.5)	2.08 (1.17, 3.00)	66.6 (46.8, 86.5)
Carman	2013	1928 (35.7)	0.91 (-0.42, 2.24)	59.9 (20.3, 99.6)
Kelburn*	2013	1716 (43.5)	0.95 (0.30, 1.59)	66.5 (48.0, 85.0)
Melita	2013	1110 (59.7)	5.01 (-10.3, 20.3)	25.2 (14.1, 36.2)
Wide Row-Spacing				
Carman	2012	2736 (36.0)	<i>ns</i>	<i>ns</i>
Kelburn	2012	2711 (68.6)	1.31 (0.08, 2.55)	77.7 (52.9, 102.4)
Melita*	2012	2013 (51.9)	5.19 (1.79, 8.58)	58.0 (46.8, 69.9)
Carman	2013	1617 (135.5)	0.46 (-0.01, 0.93)	40.0 (20.3, 99.7)
Kelburn*	2013	1720 (56.2)	2.19 (0.95, 3.43)	67.6 (55.0, 80.2)
Melita	2013	878 (17.1)	0.53 (-0.62, 1.68)	21.2 (0.82, 41.7)

<sup>a</sup> *ns* indicates that the data did not conform to the hyperbolic yield loss model

\* indicates site years where a significant difference was observed between narrow and wide row-spaced seeded soybean. Significant differences were observed only between *I*-values single-degree-of-freedom estimates.

### 3.3.1.2 Volunteer Canola Action Threshold

The action threshold was determined from the parameter estimates from the hyperbolic yield loss model (Table 3.1) to determine what density of volunteer canola per known area would result in a pre-determined percentage (5 %) of yield loss in soybean. At narrow row-spacing, the experiment with the lowest estimated action threshold was at Melita in 2013 and the experiment with the largest estimate which was 5-times larger was observed at Carman in 2013 (Table 3.2). The action thresholds for the wide row-spaced soybean experiments varied more ranging from 1.1 to 12.4 plants m<sup>-2</sup>, suggesting more predictable interference between volunteer canola and soybean among site-years in narrow-row production. Average action thresholds were determined for all sites and a second time including only site-years that were not statistically significantly different (excluding Carman 2012 and Melita 2013). In these analyses, no differences between row-spacing were observed (p-values 0.4216 and 0.7193 for *I*- and *A*-values, respectively), suggesting little differences in the ability of soybean to compete with volunteer canola in the different production systems. Nevertheless, the average action threshold for narrow row-spaced soybean tended to be slightly greater than for wide row-spaced soybean, suggesting that overall, narrow row-spaced soybean may be marginally more competitive with volunteer canola than wide row-spaced soybean. The seemingly large, yet not significant different action thresholds between the narrow and wide row experiments at Carman and Melita 2013 were likely due to large variation within these experiment as shown by the large range in confidence intervals for the curve parameters at these locations (Table 3.1).

**Table 3.2.** Action threshold determined at 5 % yield loss for three locations in 2012 and 2013 in narrow and wide row-spaced seeded soybean.

Location	Year	Row-Spacing	
		Narrow	Wide
		plants m <sup>-2</sup>	
Carman	2012	ns	ns
Kelburn	2012	5.9	4.1
Melita*	2012	2.6	1.1
Carman	2013	6.0	12.4
Kelburn*	2013	5.7	2.5
Melita	2013	1.2	12.3
Combined model All		3.0	2.7
Combined model no C12, M13		3.2	2.5

<sup>a</sup> #m<sup>-2</sup> is the calculated density of volunteer canola per m<sup>-2</sup> required in order to result in 5 % yield loss

<sup>b</sup> ns indicates that the model was not significant

\* indicates site years where a significant difference was observed between narrow and wide row-spaced seeded soybean. Significant differences were observed only between *I*-values

### 3.3.1.3 Individual Growth and Development Parameters

Soybean growth and development parameters were measured to assist with explaining morphological changes associated with yield loss in soybean caused by interference with volunteer canola. Yield loss in soybean caused by volunteer canola interference was linked to reductions in soybean leaf area, soybean height and soybean branching in 10 of 12 site years and soybean biomass in 11 of 12 site years (Table 3.3).

The relationship between increasing densities of volunteer canola and soybean leaf area were linear except for one site year where this relationship was curvilinear and the narrow row-spaced soybean experiment in Carman 2012 was the only site year where no relationship was found. Slopes in narrow row-spaced soybean were less consistent in magnitude than the slopes in wide row-spaced soybean suggesting that soybean leaf area in response to increasing volunteer canola densities was more predictable under wide row-spaced production systems. Although significant, the experiments at Melita in 2012 and 2013 produced  $R^2$ -values (0.20 to 0.24) that were lower than all other experiments among which the coefficient of determination ranged between 0.37 and 0.52.

Soybean height decreased as volunteer canola densities increased in 10 of 12 experiments. At Melita in 2013, this relationship was not significant in both the narrow or the wide row-spaced production systems. Slopes were variable, but similar in narrow and wide row-spaced soybean suggesting that increasing densities of volunteer canola had a greater impact on soybean height than row-spacing. The coefficients of determination were similar to those observed for soybean leaf area.

The relationships between the number of branches on soybean and increasing densities of volunteer canola were negative and mostly linear in narrow row-spaced production and a positive quadratic relationship in the wide row-spaced production systems. Soybean

branching was not affected by increasing volunteer canola densities at Carman 2012 and Melita 2013 in narrow row-spaced production. Interestingly, the linear slope component for the branching relationships were lowest in the three Carman sites suggesting that increasing densities of volunteer canola had less effect on reducing soybean branches in Carman than at the other locations. In wide row-spaced production, the  $R^2$ -values covered a wider range and were more variable (ranging from 0.16 to 0.72) than in narrow row-spaced production (ranging from 0.31 to 0.60). The positive quadratic parameters in these relationships indicate that as volunteer canola densities increases, the rate at which the number of soybean branches decrease declines meaning that at some high density of volunteer canola the number of branches becomes 0.

Similar to soybean branching, the relationship of the response of soybean biomass to increasing densities of volunteer canola was mostly linear in narrow row-spaced production and mostly quadratic in wide row-spaced production. As the density of volunteer canola increased, soybean biomass decreased. Melita 2012 was the only site year where this relationship was not significant for both the narrow and wide row-spaced soybean production. In the quadratic relationships, the linear component was greater than in the linear relationships and ranged from 4.95 to 7.02 compared to 1.77 to 4.65 for the linear relationships. The coefficients of determination were smallest in Melita in 2013 for narrow and wide row-spaced production at 0.16 and 0.25, respectively, while coefficients of determination ranged from 0.34 to 0.78 for all other site-years.

**Table 3.3.** Regression equations describing the effect of volunteer canola density on soybean leaf area, soybean height, soybean branching and soybean biomass with the R<sup>2</sup> and p-values included for three locations in 2012 and 2013 for narrow and wide row-spaced seeded soybean. Soybean height, soybean branching and soybean biomass were measured at early pod development and soybean leaf area was measured at the 3-4 trifoliate stage.

Year	Location	Soybean LeafArea			Soybean Height			Soybean Branching			Soybean Biomass			
		Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value	
		cm <sup>2</sup> plant <sup>-1</sup>				cm plant <sup>-1</sup>				# branches plant <sup>-1</sup>				grams m <sup>-2</sup>
Narrow Row-Spacing														
2012	Carman	-	-	0.4721	y = 106.9 - 0.12x	0.55	0.0001	-	-	0.8519	y = 822.7 - 2.33x	0.34	0.0029	
2012	Kelburn	y = 168.6 - 0.29x	0.52	0.0001	y = 81.3 - 0.26x + 0.00078x <sup>2</sup>	0.62	0.0250	y = 8.0 - 0.020x	0.52	0.0001	y = 795.2 - 5.87x + 0.015x <sup>2</sup>	0.78	0.0073	
2012	Melita	y = 199.5 - 0.26x	0.24	0.0211	y = 89.7 - 0.29x + 0.0011x <sup>2</sup>	0.31	0.0286	y = 7.8 - 0.060x + 0.00023x <sup>2</sup>	0.43	0.0115	-	-	0.1885	
2013	Carman	y = 385.6 - 1.94x + 0.00067x <sup>2</sup>	0.37	0.0335	y = 70.5 - 0.032x	0.17	0.0298	y = 8.6 - 0.013x	0.31	0.0019	y = 802.4 - 2.67x	0.58	0.0001	
2013	Kelburn	y = 288.8 - 1.10x	0.38	0.0005	y = 62.9 - 0.040x	0.33	0.0014	y = 8.9 - 0.023x	0.60	0.0001	y = 953.2 - 4.65x	0.49	0.0001	
2013	Melita	y = 195.3 - 0.44x	0.21	0.0134	-	-	0.1028	-	-	0.1006	y = 574.9 - 7.02x + 0.041x <sup>2</sup>	0.25	0.036	
Wide Row-Spacing														
2012	Carman	y = 294.7 - 0.39x	0.48	0.0002	y = 96.5 - 0.050x	0.34	0.0030	y = 6.4 - 0.0075x	0.30	0.0054	y = 813.0 - 1.77x	0.51	0.0001	
2012	Kelburn	y = 168.4 - 0.24x	0.48	0.0002	y = 83.6 - 0.32x + 0.00084x <sup>2</sup>	0.72	0.0011	y = 8.2 - 0.057x + 0.00015x <sup>2</sup>	0.72	0.0002	y = 560.4 - 4.96x + 0.012x <sup>2</sup>	0.73	0.0011	
2012	Melita	y = 227.3 - 0.35x	0.23	0.0191	y = 84.8 - 0.40x + 0.0013x <sup>2</sup>	0.70	0.0005	y = 6.4 - 0.051x + 0.00019x <sup>2</sup>	0.54	0.0013	y = 542.8 - 6.22x + 0.020x <sup>2</sup>	0.67	0.0007	
2013	Carman	y = 294.6 - 0.49x	0.52	0.0001	y = 81.1 - 0.17x + 0.00051x <sup>2</sup>	0.41	0.0065	y = 6.8 - 0.0061x	0.16	0.0341	y = 810.4 - 5.67x + 0.015x <sup>2</sup>	0.56	0.0089	
2013	Kelburn	y = 222.7 - 0.54x	0.39	0.0004	y = 64.5 - 0.030x	0.33	0.0013	y = 8.4 - 0.039x + 0.000087x <sup>2</sup>	0.52	0.0296	y = 781.3 - 6.82x + 0.016x <sup>2</sup>	0.49	0.0147	
2013	Melita	y = 199.1 - 0.68x	0.20	0.0187	-	-	0.4595	y = 6.6 - 0.030x	0.29	0.0033	y = 395.9 - 2.54x	0.16	0.0336	

<sup>a</sup> if both linear and quadratic relationships were significant, the quadratic equation was selected

### 3.3.1.4 Multiple Regression

A multiple regression approach was used on explanatory variables taken at various developmental stages and standardized to unit variance to determine the relative importance of each growth and development variable on yield in soybean. The sign and magnitude of the estimates from this standardized multiple regression indicates the direction and relative importance of each soybean growth and development variable to yield loss related to volunteer canola interference. The models for narrow and wide row-spaced soybean produced similar coefficients of determination (Table 3.4). Based on minimized AIC and maximized adjusted  $R^2$  selection criteria, six variables for narrow row-spaced soybeans and seven variables for wide row-spaced soybean were considered as contributing variables to soybean yield in response to interference with increasing densities of volunteer canola. The six explanatory variables deemed important in multiple regression were the same for both narrow and wide row-spaced soybean suggesting that interference between volunteer canola and soybean is similar in time and physiological response in narrow and wide row-spaced soybean. The additional variable in wide row-spaced soybean not deemed important for explaining yield in narrow row-spaced soybean was soybean plant height at the 3-4 leaf stage.

The degree of significance, however, varied among the explanatory variables between narrow and wide row-spacing. In narrow row-spaced soybean, significant explanatory variables for yield under weed interference ranged in soybean development from the first trifoliolate until early pod development. The narrow row-spaced soybean variables with the highest estimates and, therefore, most influential to yield, included soybean biomass and soybean height at early pod-fill. For both of these variables, a significant linear response to increasing volunteer canola densities relationship was observed at 5 of 6 locations for

these experiments (Table of relationships: 3.3, 8.2 and 8.3). In wide row-spaced production, important soybean growth and development variables that were associated with yield in response to volunteer canola interference ranged from the first trifoliolate to post-harvest and only five of the seven variables produced estimates that were significant. The variables that were deemed to be the most important were height and branching at early pod-fill for which the number of significant linear relationships were 5 and 6, respectively (Table of relationships: 3.3, 8.2 and 8.3). Additionally, all the estimates were positive signifying that, for example, greater biomass accumulation, taller plants or plants with more branches will lead to greater soybean yields.

No substantial multicollinearity was observed among the explanatory variables including the same variables measured at different developmental stages.

Multicollinearity was determined by considering the variance inflation factors, conducting the condition number test and assessing the correlation estimates (Table 8.4 and 8.5). The narrow row-spaced soybean VIF's ranged from 1.04 to 1.82 and the wide row-spaced soybean VIF-s ranged from 1.03 to 2.55. The condition test in narrow row-spaced soybean was 2.51 and 3.23 in wide row-spaced soybean. The range in Pearson R correlation estimates in narrow row-spaced soybean was 0.070 to 0.39 and the range in wide row-spaced soybean was 0.093 to 0.33. Following these three criteria to weigh multicollinearity, multicollinearity among explanatory variables can appropriately be considered to have been inconsequential. This is important because as a result, individual variables are not considered dependent or related to each other allowing interpretation of each variable individually as they relate to the relative importance to growth and development variable on soybean yield in narrow and wide row-spaced soybean in response to increasing densities of volunteer canola.

**Table 3.4.** Standardized estimates for soybean variables at various soybean developmental stages used in multiple regression to determine the importance of each variable to yield in narrow and wide row-spaced seeded soybean due to volunteer canola interference. P-value of the standardized estimates, number of significant linear relationships (Table 3.3, 8.2 and 8.3) and model parameters ( $R^2$ , p-value, AIC) are included.

Variable	Soybean Developmental Stage	Estimate	p-value <sub>estimate</sub>	Significant Experiments (# / 6)	Model		
					Adjusted $R^2$	p-value <sub>model</sub>	AIC
Narrow Row-Spacing							
Density	1 Trifoliate	0.0449	0.2926	1	0.6488	<b>0.0001</b>	-208.73
Light Sensor Perpendicular	3-4 Trifoliate	0.0701	0.1244	4			
Leaf Area	3-4 Trifoliate	0.1864	<b>0.0006</b>	5			
Branching	Early Pod	0.1300	<b>0.0198</b>	4			
Height	Early Pod	0.2797	<b>0.0001</b>	5			
Biomass	Early Pod	0.3888	<b>0.0001</b>	5			
Wide Row-Spacing							
Density	1 Trifoliate	0.1477	<b>0.0022</b>	1	0.5853	<b>0.0001</b>	-160.85
Leaf Area	3-4 Trifoliate	0.0952	0.1212	6			
Light Sensor Perpendicular	3-4 Trifoliate	0.1129	<b>0.0331</b>	3			
Height	3-4 Trifoliate	0.1458	<b>0.0050</b>	3			
Biomass	Early Pod	0.0934	0.2215	6			
Branching	Early Pod	0.2514	<b>0.0003</b>	6			
Height	Early Pod	0.3348	<b>0.0001</b>	5			

### 3.3.1.5 Soybean Seed Characteristics

Soybean seed characteristics were measured to determine if these were affected by interference from increasing densities of volunteer canola and their relationship to yield loss. The yield loss in soybean caused by the interference of volunteer canola was linked to changes in soybean individual seed weight in 2 of the 12 site years and to soybean individual seed area in 5 of 12 site years (Table 3.5). All significant seed weight regression equations were linear in Kelburn 2013 for both narrow and wide row-spaced seeded soybean. Soybean seed weight for narrow row-spaced soybean in Kelburn 2013 and wide row-spaced soybean in Kelburn 2013 decreased with increasing volunteer canola densities; there was no significant relationship for 10 of the 12 site years. Three of the 5 significant seed area regression equations were determined to be quadratic, the two exceptions that were linear were the narrow row-spaced seeded soybean in Kelburn 2012 and the wide row-spaced seeded soybean in Kelburn 2013. The individual seed area for soybean decreased for narrow row-spaced seeded soybean in Kelburn 2012 and Kelburn 2013 as a result of increasing volunteer canola densities; there was no significant relationship for 4 of the 6 narrow row-spaced site years. In contrast, the area of individual soybean seeds of wide row-spaced seeded soybean increased in Kelburn 2012, Melita 2012 and Kelburn 2013 as a result of increasing volunteer canola densities; there was no significant relationship for 3 of the 6 wide row-spaced site years.

**Table 3.5.** Regression equations describing the effect of volunteer canola density on soybean seed weight and soybean seed area with R<sup>2</sup> and p-values included for three locations for 2012 and 2013 in narrow and wide row-spaced soybean.

Year	Location	Soybean Individual Seed Weight			Soybean Individual Seed Area		
		Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value
		(grams)			(cm <sup>2</sup> )		
<b>Narrow Row-Spacing</b>							
2012	Carman	-	-	0.2186	-	-	0.4650
2012	Kelburn	-	-	0.2103	$y = 0.38 - 0.00099x$	0.48	0.0002
2012	Melita	-	-	0.1594	-	-	0.7549
2013	Carman	-	-	0.3349	-	-	0.4349
2013	Kelburn	$y = 0.18 - 0.00015x$	0.55	0.0001	$y = 0.38 + 0.00038x - 0.0000010x^2$	0.60	0.0256
2013	Melita	-	-	0.4463	-	-	0.2956
<b>Wide Row-Spacing</b>							
2012	Carman	-	-	0.6872	-	-	0.7566
2012	Kelburn	-	-	0.3215	$y = 0.38 - 0.00021x + 0.0000069x^2$	0.28	0.0185
2012	Melita	-	-	0.0737	$y = 0.39 - 0.00025x + 0.0000011x^2$	0.36	0.0032
2013	Carman	-	-	0.3551	-	-	0.1817
2013	Kelburn	$y = 0.19 - 0.000072x$	0.36	0.0007	$y = 0.40 + 0.00075x$	0.27	0.0043
2013	Melita	-	-	0.1784	-	-	0.1914

<sup>a</sup> if both linear and quadratic relationships were significant, the quadratic equation was selected

### **3.3.2 Effect of Increasing Densities of Volunteer Canola on Volunteer Canola**

#### **3.3.2.1 Volunteer Canola Recruitment, Survivorship and Seed Return**

Volunteer canola seedling recruitment and survivorship at the highest volunteer canola target densities were compared among sites and row-spacing to determine whether volunteer canola recruitment or survivorship varied among sites and years and may help explain the results when a lack of convergence of the yield loss models was observed as in Carman 2012. Volunteer canola recruitment is reported only for the 320 seeds m<sup>-2</sup> density which was the highest density treatment that was common to all site-years in 2012 and 2013. The same analysis was conducted for the 640 seed m<sup>-2</sup> density but for 2013 only. Seedling recruitment in the 320 seeds m<sup>-2</sup> treatment was different between 2012 and 2013, but did not vary among sites and row-spacing treatments within each year (Table 3.6). At the 640 seed m<sup>-2</sup> densities in 2013 only, recruitment was lower at Melita than at the other locations, which differed from the observations at the 320 seeds m<sup>-2</sup> density.

Survivorship of volunteer canola was more variable among sites and years than recruitment (Table 3.6). The survivorship is the proportion of the density of volunteer canola seedlings that competed for space and resources at the V3 to V4 stage compared to the plant densities at the end of the growing season. No consistent trend was observed among sites and years indicating that weather conditions likely played a significant role in volunteer canola survivorship. Soybean competitive ability also may have impacted volunteer canola survivorship as it appears overall survivorship was lower in narrow row-spaced soybean than in wide row-spaced soybean. Survivorship greater than 100% likely was due to uneven density distribution of the volunteers in each plot due to hand seeding and not counting the same area in each plot at the 3-4 leaf soybean developmental stage

and at soybean maturity. In the 640 seeds m<sup>-2</sup> treatment used in 2013 only, survivorship at Melita was less than half that at either Carman or Kelburn.

**Table 3.6.** The final density of volunteer canola plant recruitment, the survivorship (density at maturity/density at 3-4 soybean trifoliolate developmental stage) and the seed return for the two highest densities applied in each experiment for three locations in 2012 and 2013 in narrow and wide row-spaced seeded soybean. For each seeding density the standard error of the mean is indicated in parenthesis.

Year	Location	Final Density (plants m <sup>-2</sup> )						Survivorship (%)						Seed Return (grams m <sup>-2</sup> )											
		Seeding Density												Seeding Density											
		320 seeds m <sup>-2</sup>			640 seeds m <sup>-2</sup>			320 seeds m <sup>-2</sup>			640 seeds m <sup>-2</sup>			320 seeds m <sup>-2</sup>			640 seeds m <sup>-2</sup>								
Narrow Row-Spacing																									
2012	Carman	122	(11.6)	a	-	-	-	24	(4.2)	bc	-	-	-	-	-	-	-								
2012	Kelburn	164	(15.4)	a	-	-	-	63	(6.5)	ab	-	-	125.8	(5.0)	a	-	-								
2012	Melita	122	(19.1)	a	-	-	-	118	(19.0)	a	-	-	67.4	(2.4)	bd	-	-								
2013	Carman	91	(10.2)	b	188	(10.8)	a	54	(8.0)	abc	53	(3.5)	a	117.6	(12.6)	ab	186.3 (5.6) a								
2013	Kelburn	65	(3.0)	b	171	(12.1)	a	32	(4.1)	c	55	(9.6)	a	99.8	(4.2)	ab	112.5 (7.2) a								
2013	Melita	80	(8.8)	b	100	(12.6)	b	23	(3.1)	c	23	(4.4)	b	88.0	(4.2)	bc	107.7 (6.5) b								
Wide Row-Spacing																									
2012	Carman	211	(14.8)	a	-	-	-	54	(5.6)	bc	-	-	32.2	(7.5)	d	-	-								
2012	Kelburn	227	(16.0)	a	-	-	-	110	(6.7)	ab	-	-	148.4	(7.1)	a	-	-								
2012	Melita	117	(18.9)	a	-	-	-	82	(15.7)	a	-	-	70.3	(2.8)	bd	-	-								
2013	Carman	107	(15.6)	b	173	(25.9)	a	51	(9.3)	abc	51	(5.3)	a	98.0	(4.5)	ab	123.8 (5.4) a								
2013	Kelburn	76	(2.1)	b	265	(15.8)	a	40	(7.0)	c	58	(4.6)	a	128.0	(6.3)	ab	183.1 (9.1) a								
2013	Melita	54	(5.4)	b	49	(4.3)	b	25	(2.4)	c	19	(3.1)	b	83.2	(2.6)	bc	71.3 (3.6) b								

<sup>a</sup> #m<sup>-2</sup> is the measured density of volunteer canola that recruited m<sup>-2</sup>

<sup>b</sup> means were compared across columns according to LSD at the 0.05 level significance

<sup>c</sup> LSD means were averaged between row-spacing

### **3.3.2.2. Volunteer Canola Growth and Development**

Several volunteer canola growth and development parameters were measured in order to explain the effect on volunteer canola due to increased densities of volunteer canola in soybean. Volunteer canola interference was linked to increasing densities of volunteer canola in soybean leading to significant regressions in branching, biomass and seedweight in volunteer canola for 10 of the 12 site years and volunteer canola height for 7 of the 12 site years (Table 3.7). The relationship between increasing volunteer canola density and volunteer canola seed return was quadratic in 4 narrow row-spaced and 3 wide row-spaced experiments and similar to soybean yield loss, no relationship in either row-spacing was found at Carman in 2012. In narrow row-spaced soybean, the linear component of the slopes tended to be greater than in wide row-spaced soybean suggesting increased volunteer canola seed return for an equivalent increase in volunteer canola plant density. Coefficients of determination were similar in narrow and wide row-spaced production systems and ranged from 0.30 to 0.88. The lowest coefficients of determination were observed in the relationships for Melita in 2013 with only significant linear components.

Several volunteer canola growth and development parameters (plant height, branching, biomass) were measured to help explain the effect of increasing volunteer canola density at maturity on volunteer canola seed return when in soybean. The relationships between volunteer canola biomass and increasing volunteer canola density were similar (positive slopes and coefficients of determination) to those observed for volunteer canola seedweight by site year. Volunteer canola branching and volunteer canola height decreased with increasing volunteer canola densities. The coefficients of determination for each regression appears to be relatively less than the other growth and development

parameters for both narrow and wide row-spacing; combined with only linear relationships in 7 of 12 site years, volunteer canola height does not appear to be an important descriptor of increasing volunteer canola in soybean. The same ten experiments that showed significant regressions for volunteer canola biomass and seed return also resulted in significant relationships for branching of volunteer canola. There was, however, a difference in the type or relationship between narrow and wide row-spaced production systems with respect to branching. Branching was related primarily linearly to volunteer canola density in narrow row-spaced soybean while in wide-row soybean, the relationship between branching and volunteer canola density was principally quadratic indicating that the number of branches produced by volunteer canola in conjunction with increasing volunteer canola densities would approach a minimum value. The coefficients of determination for volunteer canola branching were in the same range as those for biomass and seed return. The number of significant relationships (3 narrow row-spaced and 4 wide row-spaced experiments) and their coefficients of determination (0.17 to 0.34) were lowest for volunteer canola height, suggesting that volunteer canola height is less important to volunteer canola seed return with increasing density than branching or biomass. Volunteer canola biomass and seed return as they relate to volunteer canola density were more consistent in narrow row-spacing than in wide row-spacing. Similarly, as described in the action threshold and row width discussion earlier, volunteer canola growth and development parameters seemed more variable under wide row-spacing, the opposite appears to be true for volunteer canola branching where regression is more variable and less significant under narrow row-spacing. Volunteer canola height was not affected by increasing volunteer canola density at Carman in 2012, Kelburn in 2013 and

Melita in 2013 in narrow row-spaced experiments and at Carman in 2012 and Kelburn in 2012 in wide row-spaced seeded production systems.

**Table 3.7.** Regression equations describing the effect of volunteer canola density on canola height, canola branching, canola biomass and canola seed return with R<sup>2</sup> and p-values included for three locations for 2012 and 2013 in narrow and wide row-spaced seeded soybean.

Year	Location	Height			Branching			Biomass			Seed Return		
		Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value	Equation	R <sup>2</sup>	p-value
		cm				# branches plant <sup>-1</sup>				grams m <sup>-2</sup>			
Narrow Row-Spacing													
2012	Carman	-	-	0.2581	-	-	0.5769	-	-	0.0894	-	-	0.5526
2012	Kelburn	$y = 88.75 - 0.051x$	0.21	0.0245	$y = 2.85 - 0.0067x$	0.33	0.0032	$y = 122.43 + 1.71x$	0.66	0.0001	$y = 23.59 + 1.08x - 0.0025x^2$	0.70	0.0396
2012	Melita	$y = 98.25 - 0.074x$	0.29	0.0100	$y = 3.92 - 0.013x$	0.49	0.0003	$y = 65.93 + 4.21x - 0.0041x^2$	0.74	0.0012	$y = 12.53 + 1.07x - 0.0035x^2$	0.76	0.0004
2013	Carman	$y = 80.24 - 0.041x$	0.31	0.0021	$y = 2.35 - 0.0085x$	0.30	0.0025	$y = 83.59 + 3.91x - 0.0090x^2$	0.86	0.0042	$y = 23.78 + 1.52x - 0.0033x^2$	0.88	0.0032
2013	Kelburn	-	-	0.6182	$y = 3.38 - 0.015x$	0.44	0.0001	$y = 153.34 + 3.61x - 0.011x^2$	0.44	0.0225	$y = 32.71 + 1.26x - 0.0039x^2$	0.64	0.0029
2013	Melita	-	-	0.7094	$y = 4.09 - 0.00029x + 0.00029x^2$	0.65	0.0031	$y = 243.47 + 1.30x$	0.28	0.0040	$y = 60.60 + 0.47x$	0.35	0.0009
Wide Row-Spacing													
2012	Carman	-	-	0.4520	-	-	0.4055	-	-	0.4892	-	-	0.4055
2012	Kelburn	-	-	0.1722	$y = 3.59 - 0.023x + 0.000050x^2$	0.74	0.01040	$y = 149.91 + 1.15x$	0.58	0.0001	$y = 49.69 + 0.38x$	0.57	0.0001
2012	Melita	$y = 99.74 - 0.093x$	0.34	0.0027	$y = 4.98 - 0.045x + 0.00012x^2$	0.72	0.0092	$y = 184.59 + 0.75x$	0.19	0.0359	$y = 35.80 + 0.69x - 0.0022x^2$	0.51	0.0202
2013	Carman	$y = 78.38 - 0.024x$	0.17	0.0292	$y = 2.27 - 0.0070x$	0.27	0.0046	$y = 107.14 + 2.52x - 0.0056x^2$	0.61	0.0288	$y = 31.21 + 0.92x - 0.0021x^2$	0.64	0.0108
2013	Kelburn	$y = 85.38 - 0.031x$	0.19	0.0196	$y = 4.25 - 0.043x + 0.00010x^2$	0.64	0.0009	$y = 258.43 + 1.11x$	0.53	0.0001	$y = 46.22 + 0.99x - 0.0016x^2$	0.78	0.0326
2013	Melita	$y = 96.37 - 0.084x$	0.21	0.0131	$y = 4.77 - 0.095x + 0.00067x^2$	0.77	0.0198	$y = 207.30 + 1.47x$	0.16	0.0372	$y = 50.47 + 0.67x$	0.30	0.0024

<sup>a</sup> if both linear and quadratic relationships were significant, the quadratic equation was selected

### 3.3.3 Environmental Conditions

Daily air temperature and precipitation data are recorded by Environment Canada and MAFRD weather stations, data from the closest stations to the experimental sites was used to summarize monthly means for temperatures and precipitation throughout each growing season (Table 3.8). Long-term 30-year averages (1981-2010) for all sites were determined from Environment Canada data, except for Kelburn where long-term averages were determined from Glenlea data ending in 2002. Air temperature (°C) and precipitation (mm) monthly means were determined starting in May until September including the average air temperature for the growing season.

The seasonal air temperature at Carman in 2012 was 0.7 °C above the long-term average and 0.1 °C above the long-term average in 2013 (Table 3.8). In 2012, the seasonal air temperature at Kelburn was 1.3 °C above the long-term average and 1°C above the long-term average in 2013. The seasonal air temperature at Melita for 2012 was above the long-term average at 0.6 °C and 0.3 °C above the long-term average in 2013. At all three locations in 2012, July was the warmest month and deviated most from the long-term average whereas in 2013, September deviated most from the long-term average and also was warmer than average.

In 2012, the seasonal precipitation at Carman was 31 % below the long-term average and 19 % below the long-term average in 2013. At this location, September was relatively the driest month where precipitation was 94 % below the long-term average of 49 mm. The seasonal precipitation in Kelburn for 2012 was 38 % below the long-term average in 2012 and 7 % below the long-term average in 2013. Melita was the location with the most divergent precipitation between the two growing seasons. The seasonal precipitation in Melita for 2012 was 44 % below the long-term average and 19 % above the long-term

average in 2013. These differences in precipitation among locations can affect the competitive outcome between crops and weeds.

**Table 3.8.** Monthly averages and long-term averages for air temperature and precipitation during the growing season (May to September) at three locations in 2012 and 2013.

Location	Year	May	June	July	August	September	Growing season
<u>Air Temperature (°C)</u>							<u>Average</u>
Carman <sup>1</sup>	2012	12.2	17.7	21.9	19.0	12.6	16.7
	2013	10.4	17.7	18.6	18.7	15.1	16.1
Long-term Average <sup>2</sup>		11.6	17.2	19.4	18.5	13.4	16.0
Kelburn <sup>3</sup>	2012	13.0	18.2	22.5	19.7	13.1	17.3
	2013	11.6	18.4	19.3	19.8	16.1	17.0
Long-term Average <sup>4</sup>		12.2	17.0	19.4	18.8	12.5	16.0
Melita <sup>1</sup>	2012	11.8	17.4	21.5	18.7	13.4	16.6
	2013	11.2	17.0	18.7	19.0	15.8	16.3
Long-term Average <sup>2</sup>		12.6	17.1	19.5	18.5	12.2	16.0
<u>Precipitation (mm)</u>							<u>Total</u>
Carman <sup>1</sup>	2012	60.5	86.2	27.8	47.2	2.9	224.6
	2013	111.0	50.6	49.0	59.4	29.9	299.9
Long-term Average <sup>2</sup>		69.6	96.4	78.6	74.8	49.0	368.4
Kelburn <sup>3</sup>	2012	71.9	64.9	32.4	57.9	3.8	230.9
	2013	87.3	60.8	90.3	75.4	33.1	346.9
Long-term Average <sup>4</sup>		59.8	99.7	91.7	72.4	48.9	372.5
Melita <sup>1</sup>	2012	28.9	66.2	44.6	26.6	5.4	171.7
	2013	51.2	78.4	141.0	24.0	73.8	368.4
Long-term Average <sup>2</sup>		47.8	85.3	67.4	58.5	50.7	309.7

<sup>1</sup> Environment Canada monthly means

<sup>2</sup> Environment Canada 30 year averages

<sup>3</sup> Manitoba Agriculture, Food and Rural Development Ag-Weather program

<sup>4</sup> Environment Canada 30 year (1981-2010) averages from Glenlea ending 2002

### 3.3.4 Soil Characteristics and Nutrient Analysis

The soil characteristics at Carman, Kelburn and Melita in 2012 and 2013 were recorded to determine if any effect on soybean or volunteer canola growth and development can be attributed to the properties of the different soils. Soil texture from Carman and Melita was a fine sandy clay where as soil from Kelburn (situated within 0.5 km of the Red River) was a clay soil (Table 3.9). The Carman soil was a Rignold Gleyed Black, at Kelburn the soil was a Orthic Dark Gray Chernozem and at Melita, the soil was a Gleyed Black Chernozem. On average, soil pH at Carman was the lowest at 6.5, followed by Kelburn with an average soil pH of 7.2, and the highest average pH of 8.0 is from Kelburn. The percent organic matter at Kelburn in 2012 and 2013 was much greater than that at Carman.

The soil analysis, which was collected in either the fall or the spring, for four macro-nutrients (nitrogen-phosphorus-potassium-sulphur) were determined as residual soil nutrients, in particular residual soil nitrate may impact the interference between volunteer canola and soybean. There was a broad range in residual spring soil nitrate ranging from 15 to 75 kg ha<sup>-1</sup> (Table 3.9). Residual phosphorus (Olsen-P) and potassium were greater in Kelburn for both site years than the other 4 site years.

**Table 3.9.** Soil texture, pH, organic matter and the soil analysis for nitrate, phosphorus, potassium and sulfur at three locations in 2012 and 2013

Location	Year	Soil Texture	pH	Organic Matter	Nitrate-N <sup>1</sup>	Phosphorus	Potassium	Sulphur <sup>1</sup>
				g kg <sup>-1</sup>	kg ha <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	kg ha <sup>-1</sup>
Carman	2012	fine sandy loam	7.1	*	24	12	282	121
	2013	fine sandy loam	5.9	25	37	8	170	63
Kelburn	2012	Clay	6.7	68	75	26	>510	24
	2013	Clay	7.7	78	68	81	646	128
Melita	2012	fine sandy loam	8.1	*	49	7	221	99
	2013	fine sandy loam	7.9	*	15	4	88	47

<sup>1</sup> 0-60 cm soil measurement

\* information not available

### **3.4 Discussion**

#### **3.4.1 Action Threshold for Volunteer Canola Management in Soybean**

This study showed that volunteer canola is very competitive with soybean which led to significant yield losses at 5 of 6 site years. These findings are similar to other studies that have examined yield loss in soybean in response to weed interference that also describe how relatively low densities of weeds such as velvetleaf (*Abutilon theophrasti* Medic.) in Michigan (Dekker and Meggit 1983) and the interference of five different weeds in North Carolina (Shurtleff and Coble 1985) caused significant yield loss and impacts on growth and development parameters in soybean. This study is the first to describe the relationship between yield loss in soybean and volunteer canola density. To compare, the relationship between yield loss in wheat (*Triticum aestivum* L.) and wild oat in western Canada needs to be examined. Wild oat is considered among the most competitive and abundant weeds in western Canada (Thomas 1991) while wheat is one of the more competitive crops in this region (eg. Holman et al. 2004). To achieve 5 % yield loss in wheat, an average of 8 wild oat plants  $m^{-2}$  are required when at the same developmental stage as wheat and nearly 14 wild oat plants  $m^{-2}$  are required if the wild oat is a full leaf stage behind wheat (O'Donovan et al. 1985). On average about 3.0 volunteer canola plants  $m^{-2}$  were required to cause 5% yield loss in soybean indicating that volunteer canola causes greater yield loss in soybean per individual than the wild oat in wheat. For context, it is recommended that seedbank densities of wild oat should be kept low to minimize the impact of this weed on crop yield. Uncontrolled, wild oat populations increased to 271 plants  $m^{-2}$  over 4 years in continuous wheat production when no herbicides were applied (O'Donovan 1988). In comparison, canola harvest losses are substantial and have been recorded at an average of 4 447 to 6 429 seeds  $m^{-2}$  (Cavaliere et al. 2016) or as high as 13 900 seeds  $m^{-2}$

(Gulden et al. 2003a). As a result, even low persistence and subsequent recruitment of volunteer canola can cause significant yield losses in soybean and as a result monitoring and managing volunteer canola seedling population densities is critical to minimizing yield loss in soybean from this weed. Given the high seedbank additions of volunteer canola at harvest, even low levels of adventitious presence of herbicide-resistance traits such as glyphosate-resistance in glufosinate-resistant canola (Friesen et al. 2003) could result in problematic population densities of volunteer canola in soybean production when unmanaged.

Collectively, the largest high action thresholds were observed at the Carman experimental site suggesting that soybeans at Carman were more competitive with volunteer canola than soybeans at Melita or Kelburn. This included 2012, where soybean at Carman were so competitive with volunteer canola that an action threshold could not be determined. Environmental factors likely contributed to the results observed at Carman in 2012. Canola seed was spread on the surface of the plots, with some incorporation of seeds in the drill row during planting of soybean; in 2012 at Carman the first rain was received 7 days after planting and average temperatures at planting until rainfall were 19.1°C. Volunteer canola likely emerged about a week after the soybean crop and a study by O'Donovan (1992) in Alberta found that when 20 barley (*Hordeum vulgare* L.) plants m<sup>-2</sup> emerged 8 days earlier than canola, canola yield was reduced by about 30 %. Density and time of emergence were identified as being critical for canola competition. A delay in volunteer canola germination in this study likely contributed to the lack of convergence of the yield loss models at Carman in 2012. The competitive ability in narrow and wide row-spaced production likely out-competed the late-germinating volunteer canola to the point where the yield loss data was not great enough for the rectangular hyperbola model

(Cousens 1985) to detect any significance for the site year. Another contributing factor could be a result of the trial placement in 2012. The trial was located within a relatively small field (about 2 hectares) with treelines on three sides of the field; this area may have experienced a microclimate with reduced wind and increased heat unit accumulation. Soybean was more competitive than expected because the soybean over-towered the volunteer canola in this field experiment, which was not the case for the other site years, and in farmers commercial fields.

### **3.4.2 Comparing the Action Threshold of Volunteer Canola in Soybean by Row Width**

The effect of row-spacing and the ensuing action threshold to control volunteer canola in soybean was less prominent than expected. There was a general lack of difference between narrow and wide row-spacing. Narrow row-spaced seeded soybean were expected to be more competitive with volunteer canola than at wide row-spacing and although this was observed at two site years, this response was not consistent throughout this study indicating that row-spacing appears to have limited impact on volunteer canola competition with soybean. The explanatory variables did not provide a clear explanation why the differences in soybean yield loss between the row-spacings were observed at these two site-years only.

Canola is very competitive early in the growing season as canola can thrive under cool days with low growing-degree day conditions (Harker et al. 2011) while soybean prefers warmer temperatures. This may explain the overall lack of response in soybean performance between row-spacing where by the time soybean begins the phase of rapid biomass accumulation, volunteer canola has already maximized space capture,

irrespective of soybean spatial arrangement. Bell et al. (2015) also observed that factors such as row-spacing and seeding rate in soybean may be less effective to compete with more competitive weeds such as palmer amaranth (*Amaranthus palmeri* S. Wats.) or in this case volunteer canola. Both of these weed species can quickly over-tower the crop; weeds that are taller are intrinsically not required to forfeit regular growth and development to be better at competing with the crop for resources (Olsen et al. 2006). Even though canola is a more competitive crop, it was expected that soybean seeded to narrow row-spacing would achieve higher yields and reduce the yield-loss penalty by volunteer canola interference. A study by Puricelli et al. (2003) showed that weed-free yields in soybean grown at different row-spacing tended to be similar. Due to the shorter growing seasons in northern regions, crops planted to narrow row-spacing capture space more quickly and as a result tend to produce more yield than when grown at wide row-spacing (Harder et al. 2007). Puricelli et al. (2003) also showed that under weed interference, yields for narrow row-spaced soybeans (35 cm) were greater than yields of soybeans grown at wide row-spacing (70 cm).

### **3.4.3 Impact of Volunteer Canola Interference on Soybean Growth and Development**

Soybean height and the number of branches at the early pod filling stage were found to be important explanatory variables contributing to soybean yield when under interference with volunteer canola. Growth and development variables measured at the early pod stage had the highest estimates indicating that these variables contributed more to predicting soybean yield from interference at increasing densities of volunteer canola (Table 3.4). Positive correlations between crop height and crop yield has been reported for other

major crop species including corn (Yin et al. 2011), wheat (Law et al. 1978) and rice (Wei et al. 2010). In soybean, a study in Illinois found that tall-type soybeans consistently yielded about 5 % more than normal-height soybeans (Hicks et al. 1969). One factor outlined in a study in North Carolina found that seed yield and plant height were positively correlated to photosynthetically active radiation such that taller plants were able to intercept more light (Wells et al. 1993). In this study, under interference with volunteer canola, soybean height was the most important growth parameter to describe soybean yield in both row-spacing treatments. Jannink et al. (2014) demonstrated that soybean selected for early height development can result in increased yield by increasing the ability to suppress weeds. Similarly, branching has been shown to be highly correlated to seed yield in soybean, the number of branches and length of the branches were significant in a study by Board (1987) in Louisiana. In a narrow row-spaced soybean field, soybean height at the first trifoliolate was influenced by volunteer canola and shown to be a significant growth parameter, more research, including more sampling will need to be completed to determine if early season height can be used as a tool to predict soybean yield. Green-Tracewics et al. (2011) showed that weed-interference induced shade avoidance in soybean and had a direct impact on the reduction of seed yield by reducing branching. Likewise, in wheat, the number of tillers per plant were reduced, as well as several other growth parameters including plant height, leaf area, biomass and yield were reduced when grown in competition with green foxtail in Manitoba (Blackshaw et al. 1981). Results from the experiment indicate that the number of soybean branches as well as soybean height were the two most important growth parameters when describing soybean yield during interference with volunteer canola in narrow and wide row-spaced soybean.

Height and branching growth parameters were not significant for 3 of the 4 Melita experiments in 2012 or 2013, potentially due to excessive moisture at the critical flowering stage. In Melita in 2013, soybean in July received twice as much rain as the long-term average. A study conducted in North Carolina found that excess moisture during early pod formation had a greater impact on reducing soybean yield than at any other stage of soybean development (Sionit and Kramer 1977). Furthermore, the coefficients of determination were lower in the replicated trial in 2013 than in 2012 for 6 of the 8 significant experiments between the height and branching growth parameters. Increased moisture has a greater impact than the relationship of height and branching to soybean and resulted in greater inconsistency in these parameters.

During May and July in 2013, average air temperatures were below those observed in 2012 and the long-term average at all sites. In addition, precipitation in May of 2013 was above both the precipitation in May of 2012 and the long-term average at all sites during that month. Cool-wet soils in May during planting likely impacted soybean emergence and vigor (e.g., Muendel 1986). Later during the growing season, Ohnishi et al. (2010) found that lower temperatures, 3-4 days before soybean flowering can impact pollen development likely affecting pod set and seed yield. Excess rainfall and lower temperatures may have contributed to reduced coefficients of determination in 2013 compared to 2012 and to the non-significant regression equations for height and branching in Melita 2013.

Soybean parameters related to soybean yield under volunteer canola interference included soybean leaf area, soybean biomass and light absorption (Appendix 8.2) were all shown to be significant descriptors of soybean yield, however, all were considered somewhat less important descriptors of soybean yield under volunteer canola interference

compared with soybean height and soybean branching (Table 3.3 and Table 3.4). Leaf area slopes among these relationships were less consistent in narrow row-spaced soybean than slopes in wide row-spaced soybean suggesting that soybean leaf area is more stable when soybeans were planted to wide row-spacing. Shurtleff and Coble (1985) found that different weeds impact soybean leaf area differently when growing at different distances from the row. Perhaps, soybean leaf area planted to wide row-spacing was less responsive to volunteer canola because volunteer canola could occupy the available space between the wide row-spaced soybean and was competing less with soybeans that were in closer proximity to each other since rows were further apart. In other words, there may have been more intra-specific interference within volunteer canola and within soybean in the wide row-spaced production system due to the different spatial arrangement of the two plant species.

Furthermore, light interception determined with the light sensor either parallel or perpendicular to the soybean row was not very different among increasing volunteer canola densities in this study. As mentioned earlier, reductions from volunteer canola interference were seen in soybean growth and development even at early soybean crop stages. Therefore, increased volunteer canola densities could have a greater impact on soybeans at the early soybean crop stages (leading to thinner and shorter plants) potentially offsetting the overall light penetration at the 3-4 trifoliolate stage by reducing the overall variation between plots at different densities volunteer canola because the soybean crop would cover more or less space somewhat proportionally to the amount of competition from volunteer canola. A study by Marwat and Nafziger (1990) in Illinois showed that there was a difference in light interception when soybean was grown alone than when soybean was grown with cocklebur (2 % increase in overall light interception)

and with velvetleaf (4 % increase); weeds also experience growth reductions under interference (Legere et al. 1989). Light interception appears to be influenced by several factors.

Soybean seed characteristic including seed weight and area per seed did not respond consistently to increasing densities of volunteer canola. A study by Bergonia et al. (1991) describing that, as expected, soybean seed weight decreased under increasing levels of competition with velvetleaf in Missouri where the negative relationship found in this study suggests that as volunteer canola interference increases, seed weight decreases. A larger sample size across more densities may be required to link resulting soybean seed size to different densities of volunteer canola interference.

#### **3.4.4 Volunteer Canola Recruitment, Survivorship and Seed Return**

Seed return of volunteer canola was density dependent. Using the determined action threshold densities and the density dependent equations for volunteer canola seed return (equations not shown), volunteer canola seed return ranged from 13 to 61 kg ha<sup>-1</sup> at the 5 % yield loss threshold, this is about 10 to 30 % of the average seedbank additions incurred during harvest of a canola crop (Cavalieri et al. 2016). Although these seedbank additions may contribute to future volunteer canola populations, these alone do not appear to be reason enough to warrant management of volunteer canola at densities below the action thresholds. For this study, canola seed return may not have been accounted for in its entirety; there were pre-harvest seed losses as canola stayed in the field much longer than it normally would because the canola was only harvested at the same time as the soybean crop.

The ability and ease with which volunteer canola can be managed in subsequent crops should be considered when assessing the additional costs for managing volunteer canola in soybean fields. However, dense volunteer canola populations resulted in significant seed production by volunteer canola in soybean fields. Volunteer canola seed production ranged from about 700 to 1400 kg ha<sup>-1</sup> (Table 3.6). Average harvest losses in canola crops in western Canada are much lower (e.g., Cavalieri et al. 2016) than maximum seed production observed in these soybean experiments and therefore, unmanaged, high-density populations of volunteer canola can result in significant seedbank additions in soybean production that may lead to persisting volunteer canola populations (Gulden et al. 2003a).

There was no apparent conflict with resident volunteer canola populations contributing to overly high or variable volunteer canola plant densities in these experiments as these would have produced noticeably different recruitment values in the low density treatments that would suggest a persistent volunteer canola population from a previous canola crop was not an issue. As previously mentioned, in 2013, below normal temperatures and increased rainfall early in the growing season likely impacted volunteer canola recruitment and growth. This weather effect appeared to influence the survivorship of canola as well. Early season densities of volunteer canola were compared to late season densities of volunteer canola and in 2013 the combination of soybean competition and volunteer canola self-thinning reduced the survivorship of volunteer canola. Volunteer canola survivorship did not seem to be influenced by row-spacing but by year and by location.

### **3.4.5 Soil Analysis**

The soil analysis for four macro-nutrients (nitrogen-phosphorus-potassium-sulphur) were determined as residual soil nutrients, in particular residual soil nitrate may impact the interference between volunteer canola and soybean. In low soil nitrate situations, soybean is expected to compete more effectively with volunteer canola as volunteer canola growth and development would be limited by nitrogen while soybean is capable of fixing atmospheric N and growth and development would not be nitrogen limited. Kelburn, in both years, and Melita, in 2012, had substantial residual spring soil nitrate content (Table 3.8). In general, these site-years were associated with amongst the lowest action thresholds determined in these experiments suggesting that residual nitrogen availability may play a role in driving competition between volunteer canola and soybean and therefore also the action thresholds, although sufficient data was not collected in these experiments to make firm conclusions on this (Table 3.9). Soil pH or soil texture had no apparent impact on competition dynamics (Table 3.9).

### **3.5 Conclusion**

This study showed that volunteer canola is a highly competitive weed in soybean planted at either narrow or wide row-spacing. On average, in 2012 and 2013 the action threshold for volunteer canola in soybean was 3.0 plants m<sup>-2</sup> in narrow and 2.7 plants m<sup>-2</sup> in wide row-spaced soybean, however, these were not significantly different. In addition, when describing yield in soybean under interference with increasing densities of volunteer canola, height and the number of branches at early pod fill were found to be critical variables. Though, self-thinning and some level of soybean competitive ability led

to reduced volunteer canola survivorship densities by up to 81 % by the end of the growing season. Volunteer canola was still able to set large amounts of seed, which should impact management decisions, likely reducing action thresholds by considering future volunteer canola populations and potential yield loss to subsequent crops.

## **4.0 Relating Early-Season Volunteer Canola and Soybean Ground Cover to Yield Loss in Soybean Using Image Analysis**

*Abstract.* Early season digital image analysis was investigated as a potential predictive tool to estimate yield loss in soybean from volunteer canola interference. Digital images were captured from 6 paired field experiments across southern Manitoba seeded to eight densities of volunteer canola in narrow and wide row-spaced soybean. Ground cover was determined and linear regression was used to relate volunteer canola ground cover to soybean yield loss from volunteer canola interference. The data were fit to linear regression; slopes ranged from 0.26 to 2.79 % soybean yield loss % groundcover<sup>-1</sup> for both narrow and wide row-spaced soybean. A difference in slopes between narrow and wide row-spacing was observed suggesting that separate models would be required for narrow and wide row-spaced soybean. This method shows promise relating soybean yield loss to total ground cover and could be developed to serve as a decision making tool for managing volunteer canola in soybean.

## 4.1 Introduction

Competition by weeds for resources such as light, nutrients and water can lead to substantial crop yield loss. Predicting the effects and outcome of weed competition on crop yield loss is an integral part of future integrated weed management programs (Kropff 1988). The methodologies used to measure plant growth and development tend to be labor intensive, time consuming, costly, and can be destructive to the plant (Ngouajio et al. 1999). Current practices such as scouting for weeds may be inexact due to the fact that weeds tend to have a patchy distribution (Wiles et al. 1993). Kropff et al. (1988) suggested that digital image analysis may be an appropriate and efficient method to predict weed interference with crops to overcome the obstacles of manual sampling.

Digital image analysis can be an efficient real-time, non-destructive assessment of early stage canopy development and can also characterize growth and determine crop nitrogen nutrition status (Bumgarner et al. 2012; Lee and Lee 2013). Image analysis has been shown to be effective at describing weed populations and assess plant diseases (Ngouajio et al. 1999). Ngouajio et al. (1999) suggested that the quality of yield loss predictions may be reduced when digital images are captured and analysed too early in the season, that weeds often have different times of emergence and as a result could cause different leaf cover estimates based on weed population composition and their respective competitive ability at different developmental stages. However, Lati et al. (2011) found linear relationships between ground cover and plant biomass regardless of the developmental stage of the weed community. An example of the potential impact of using digital image analysis is the modernization of visual sugarcane yield forecasts in Brazil that had an error rate of 9.1 %; when digital image analysis was implemented, the yield

forecast error rate decreased to 2.6 %, indicating that digital image analysis can be used as a reliable predictive tool for the estimation of sugarcane yield (Almeida et al. 2006).

The large proportion of hectares seeded to herbicide-resistant canola and the recent increase in the hectares planted to glyphosate-resistant soybean has contributed to volunteer canola becoming a prominent weed issue in Western Canada (Johnson et al. 2004). Even before herbicide-resistant canola was commercialized, volunteer canola was ranked the most abundant weed in zero-till and conventionally tilled fields in a pre-seed weed survey conducted in Manitoba in the spring of 1994 (Thomas et al. 1997). The benefits of easy and effective weed management contributing to increased yield in herbicide-resistant canola have led to a high proportion of canola fields seeded to herbicide-resistant genotypes in Western Canada (Harker et al. 2006). Continual high seed losses at harvest (Cavaliere et al. 2016; Gulden et al. 2003b) contribute to the significance of volunteer canola populations in subsequent crops in the rotation.

Unmanaged, these can lead to significant yield losses in soybean (Chapter 3).

The objective of this study was to determine whether there was a relationship between early season total ground cover in narrow and wide row-spaced soybean with increasing volunteer canola densities and soybean yield loss in response to interference with volunteer canola using digital image analysis. The specific hypotheses tested in this experiment were i) the relationship between early season ground cover and soybean yield loss in response to increasing volunteer canola density is linear and ii) the slopes of this relationship are greater in narrow row soybean compared to wide row soybean.

## 4.2 Materials and Methods

The experiments described in Chapter 3 were also used for this study. In brief, six paired randomized complete block, additive design experiments were established in 2012 and 2013 near Kelburn Farms, MB (49°41'15.782", 97°7'22.334"), at the Ian N. Morrison Research Farm near Carman, MB (49°30'3.471", 98°1'49.992") and near Melita, MB (49°15'47.099", 100°59'39.631"). Dekalb 23-10RY soybean seed was used to plant six paired experiments to narrow row-spacing (25 cm) and six experiments to wide row-spacing (75 cm) at a target density of 445 000 plants ha<sup>-1</sup> for both row types. Prior to seeding the soybean crop, volunteer canola (73-45 RR, bare seed) was broadcast over each plot at one of seven densities in 2012 (0, 10, 20, 40, 80, 160, 320 seeds m<sup>-2</sup>). In 2013, one more volunteer canola density was added (640 seeds m<sup>-2</sup>) for a total of eight different treatments. Each plot was 3 meters wide by 8 meters long and treatments were replicated 4 times.

Two 25 cm by 25 cm digital images of a representative area in each plot were captured (Pentax K-1 digital camera) when the soybean crop had reached the first trifoliolate developmental stage (BBCH 102). One soybean row passed through the quadrat for each image. The area within the quadrat was subjected to digital image analysis using Assess 2.0 (APS Press, St. Paul, MN, USA) to determine the total ground cover (soybean plus volunteer canola) as a percentage of total area in the image. When present, all plants other than volunteer canola or soybean were removed from the area inside the quadrat before the image was captured.

Ground cover for 0 seeds m<sup>-2</sup> treatments were averaged for each experiment at each site year then subtracted from the ground cover from each treatment within the corresponding site year to determine the potential yield loss from occurring volunteer

canola alone, assuming that soybean seedling densities were the same in all quadrats. Soybean yield loss (the percentage yield loss due to volunteer canola interference) data from Chapter 3 were related to percent ground cover using linear and quadratic regression analysis. The GLM procedure was used to extract linear and quadratic components and the more appropriate model was then chosen to determine the slopes for each replicate for each row-spacing experiment at each location. The first model (Table 8.6) did not have a defined intercept meaning that, for example, at 0 % ground cover, regressions could predict yield loss. The regression model was then re-fit to force the intercept through zero as subtraction of all other ground cover negated the need to estimate an intercept. These slopes and intercepts were then subjected to Mixed model analysis where sites and years were considered fixed effects and replicates were considered the random effect. Assumptions of ANOVA were tested as outlined in the previous chapter. Means of slopes were separated using Fisher's protected least significant difference because of the comparisonwise error rate. In addition, correlation analyses were conducted on site-year means of significant regression slopes and the developmental stage of volunteer canola, soybean and their difference (volunteer canola - soybean), as well as *I*- and *A*-values from the previous Chapter. Slopes between row-spacings were compared with a single degree of freedom estimate to determine if there was a difference between narrow and wide row-spaced soybean.

### **4.3 Results and Discussion**

Linear regression was sufficient to describe soybean yield loss in response to interference from volunteer canola using early season total ground cover data and this

relationship was significant at 5 narrow and 6 wide row-seeded site years (Table 4.1). For the significant regressions, the coefficients of determination ranged from 0.00 to 0.64 and the slopes ranged from 0.26 to 2.79 % soybean yield loss % groundcover<sup>-1</sup>. The slopes were compared to determine the universality of the relationship between early-season additional groundcover resulting from volunteer canola and soybean yield loss. The smallest (Carman in 2012) and largest (Melita in 2012) slopes were observed in narrow row-spaced soybean. All other slopes ranged between these and showed various degrees of differences among them. The range in slopes among wide row-spaced soybean experiments was less than among narrow row-spaced soybean experiments. The difference in slopes between narrow and wide row-spacing suggests that there would likely be a need to have separate models for narrow and wide row-spaced soybean.

Overall, the average slope observed for narrow row-spacing soybean was greater than for wide row-spacing soybean (Table 4.1). The small quadrat 25 cm by 25 cm used for the images, may have contributed to this difference in slopes as the spatial arrangement of soybean plants was different between the row-spacing treatments. Both narrow and wide row-spaced soybean were planted at the same population density; however, due to the difference in spatial arrangement, soybean plants in wide row-spacing would be subject to leaf-overlap in the row before soybean planted to narrow row-spacing and this may have contributed to less interference with volunteer canola even at these early developmental stages.

**Table 4.1** Regression equation parameters: Slopes, regression coefficients, p-values for each regression, soybean and canola developmental stages at the time the digital images were taken for each site year and row spacing experiment. Slopes followed by different letters are significantly different and standard errors of the slope are indicated in parentheses.

Location	Row-spacing	Year	Slope		R <sup>2</sup>	p-value <sup>a</sup>	Soybean Stage <sup>b</sup>	Canola Stage <sup>c</sup>
			% soybean yield loss	% groundcover <sup>-1</sup>				
<b>Carman</b>	Narrow	2012	0.10 (0.05)	H	0.05	0.0558	0.75	cotyledon
		2013	0.78 (0.05)	CDE	0.64	<b>0.0001</b>	1.25	2.5
	Wide	2012	0.31 (0.07)	GH	0.21	<b>0.0001</b>	0.75	cotyledon
		2013	0.32 (0.11)	FHG	0.00	<b>0.0061</b>	1.50	3.0
<b>Kelburn</b>	Narrow	2012	0.62 (0.05)	DEF	0.47	<b>0.0001</b>	1.25	3.0
		2013	1.38 (0.11)	B	0.54	<b>0.0001</b>	0.75	1.5
	Wide	2012	0.63 (0.05)	DEF	0.39	<b>0.0001</b>	1.25	3.0
		2013	1.11 (0.10)	C	0.07	<b>0.0001</b>	1.00	3.0
<b>Melita</b>	Narrow	2012	2.79 (0.20)	A	0.52	<b>0.0001</b>	0.50	1.0
		2013	0.47 (0.06)	EFG	0.15	<b>0.0001</b>	1.25	3.0
	Wide	2012	0.87 (0.06)	BCD	0.50	<b>0.0001</b>	1.00	2.5
		2013	0.26 (0.08)	FGH	0.13	<b>0.0048</b>	1.00	3.0
<b>Narrow vs. Wide Avg</b>			0.43**** <sup>d</sup>					

a significant p-values are bolded

b soybean development stage refers to the number of trifoliates

c canola development stage refers to the number of true leaves unless otherwise noted. For correlation analysis, the cotyledon stage was designated as 0.5.

d single degree freedom contrast (\*\*\* = p<0.001)

The significant differences among slopes (Table 4.1) indicate that a single, universal equation to predict soybean yield losses caused by volunteer canola from early season ground cover only may not be applicable in all instances. Some of the variation observed among site years in this study may have been related to the absolute and relative differences in developmental stages of the crop and weed when the images were obtained. The developmental stage of soybean ranged from the first trifoliolate (half- to full-leaf expansion) to the second trifoliolate (max. half-leaf expansion) when the images were captured. At this time, the developmental stages of volunteer canola ranged from cotyledons to the 3-leaf stage among the experiments. The developmental stage of volunteer canola was similar within each experiment. Weak negative relationships were found between the developmental stage of volunteer canola (Pearson  $R = -0.66$ ,  $p$ -values = 0.0372) or the developmental stage of soybean (Pearson  $R = -0.76$ ,  $p$ -values = 0.0111) at which the ground cover images were taken and the slopes of the significant regression lines. A study found similar results as there was a negative relationship between ground cover and developmental stage; the negative relationship weakened at more advanced developmental stages (Ngouajio et al. 1998). Timing of image capture can be critical; if too early, plants may not have emerged and if too late, it may result in too much ground cover to detect any differences (Andreason et al. 1997).

The large slope values for narrow row-spaced soybeans at Kelburn in 2013 and at Melita in 2012 may have been influenced by the early developmental stage of soybean at the time of image capture. This suggests that this method may be more sensitive to detecting soybean yield loss at early developmental stages. Findings by Ali et al. (2014) described that significant regression analysis was observed to be able to measure early-season leaf cover and predict yield loss later on during the growing season. Defining what

developmental stage 'early-season' is appears to be critical to detect and interpret yield loss later in the season. For example, among the significant relationships in Table 4.1, in 5 of the 6 site years with the lowest slopes, volunteer canola was among the most advanced in development stage at the time the images were captured. This, likely in conjunction with other factors, contributed to the reduced sensitivity (slope) in detecting yield loss in soybean. Capturing the images too early or too late presents separate, but important challenges. For images captured at the cotyledon stage, it is possible that not all volunteer canola seedlings had recruited and image resolution needs to be of sufficient quality to accurately capture and quantify the seedlings. For images captured at the 3-leaf stage of volunteer canola, overlap among leaves of volunteer canola and soybean may have interfered with and underestimated the size of the volunteer canola population (e.g., Ngouajio et al. 1998).

The relative difference in developmental stages between volunteer canola and soybean (absolute value of volunteer canola stage – soybean stage) had no impact on the regression parameter (slopes) in this study. This is interesting as the importance of the relative time of emergence to the outcome of crop weed interference in western Canada is well documented (Blackshaw 1993; O'Donovan et al. 2008; Willenborg et al. 2005). Research by Willenborg et al. (2005) found that the effect of relative time of emergence for wild oats varied between site years but that in cultivated oats the relative time of emergence of wild oats had a similar influence on important factors such as cultivated oat yield loss and wild oat seed production. More research on understanding the effects of relative time of emergence, variable environmental conditions, and importance of the developmental stages of the crop and weeds at the time the images are taken is warranted

to further refine the relationship between early season ground cover and soybean yield loss.

The previous study (Chapter 3) showed no rectangular hyperbola relationship between increasing volunteer canola density and soybean yield loss and reasons for this (poor volunteer canola recruitment and high soybean competition) are discussed in Chapter 3. Lack of a relationship between increasing density of volunteer canola and soybean yield loss is not the explanation for the lack of a relationship between early-season groundcover and yield loss in soybean for narrow row-spaced soybean at Carman in 2013 in this study. In this experiment, however, both volunteer canola (3-leaf stage) and soybean (1.5 trifoliates) were at the largest developmental stages observed in this study and this likely contributed to the lack of a relationship between early-season groundcover caused by volunteer canola and soybean yield loss. This further emphasizes the importance of image capture and decision making at the most appropriate developmental stage (Rasmussen and Norremark 2006) to maximize the chance for detecting the relationship between yield loss in soybean and additional groundcover due to volunteer canola.

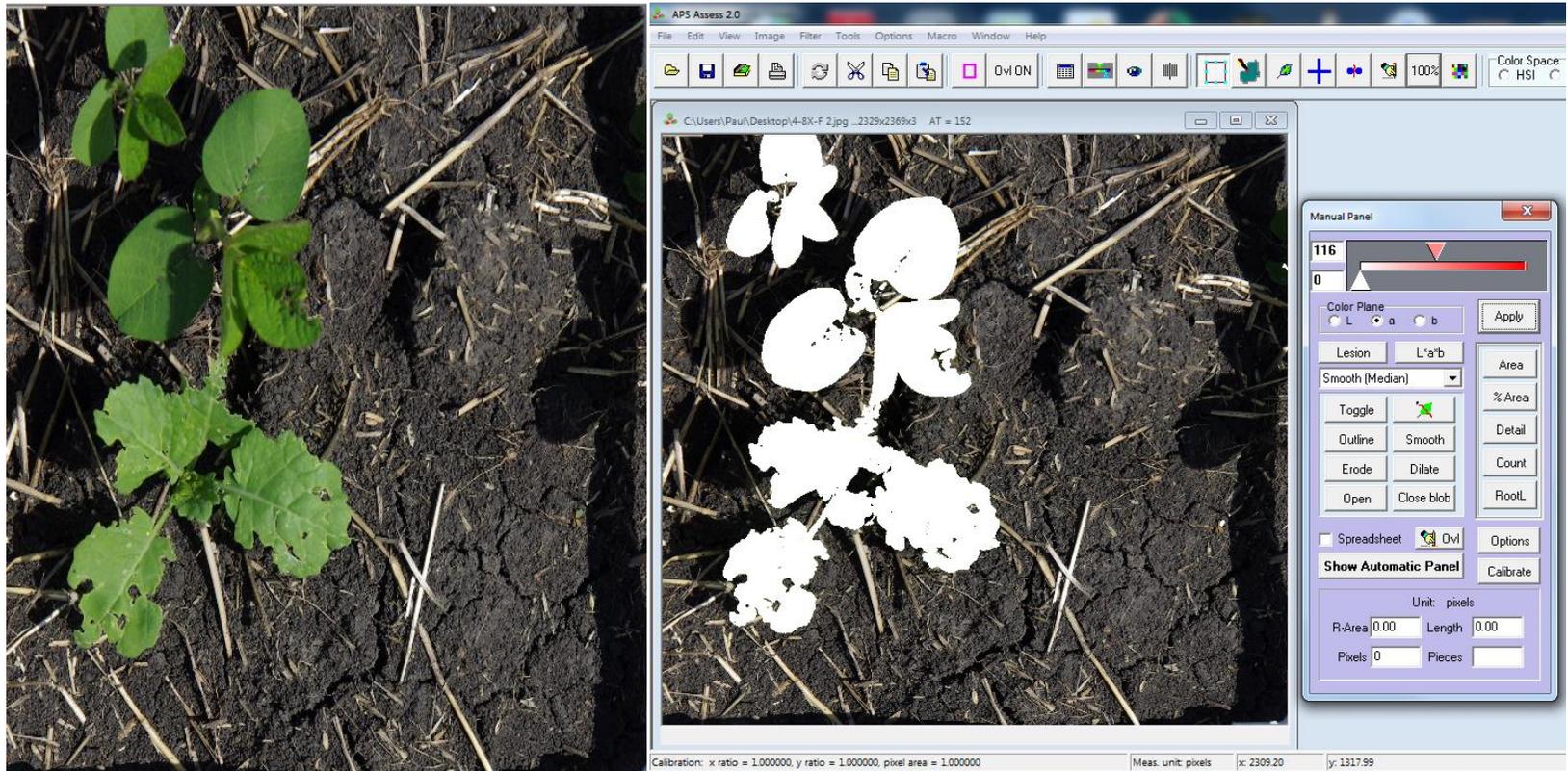
There are some additional considerations to be aware of while conducting this type of research in the field. Weeds not considered in the model need to be removed to ensure that any additional groundcover related to yield loss in soybean by the target weed species only. Natural, uncontrolled weed communities can lead to very high variance in the data (Ali et al. 2014; Ngouajio et al. 1998; Swanton et al. 1999). Weed communities with similar composition at different locations or with different times of emergence can impact the model by altering the competitive ability of the community (Cowen et al. 1998). Amount of light and shadows have no apparent impact for Assess 2.0 to separate plant ground cover from the soil and plant residues (Figure 4.1). Groundcover images are best

captured on days with no wind or rain which may cause leaf orientation to differ substantially from their 'natural' position. Capturing the image from an area that visually represents the mean volunteer canola density is essential to determination of a reliable yield loss estimate from early-season groundcover. Increasing the number of digital images captured per experimental unit should be considered to increase sample size, to better capture inherent spatial variation, reduce variation and contribute to increased coefficients of determination for the regression analysis.

The analysis of digital images to be used as a decision making tool for weed management is a concept that is actively being developed and is still a relatively new initiative that is not well understood at this point in time (Shaner and Beckie 2013). Critical challenges include: identification, quantification, analysis, interpretation (Shaner and Beckie 2013) and interference from plant residues and in a study by Andreason et al (1997), algae and moss in the background of the crop and weed canopy. The suitability of image analysis is being considered across crops to detect a wide array of factors including soil fertility, nitrogen levels in the crop, soil moisture, yield and many others. An example is in viticulture where Arno et al. (2009) describe image analysis as a tool to determine yield and vigor to harvest grapes into better defined zones to produce higher quality wines by not using grapes made up of varying qualities.

#### **4.4 Conclusion**

The results of this research clearly showed that digital image analysis has the potential to predict soybean yield loss caused by volunteer canola interference early in the growing season in both narrow and wide row-spaced soybean production systems. The large



**Figure 4.1.** The cropped digital image (left) used for analysis in Assess 2.0. The image on the right shows the separation of the canopy from the ground and residue. The color of the canopy is separated accordingly such that any shadows or residue do not interfere with the crop/weed interaction; multiple shades of green can be separated and included as canopy cover.

variation among slopes indicated that a number of factors affect the relationship between soybean final yield and additional early-season groundcover from volunteer canola. More research is needed to determine when and how digital images are captured including height (Ngouajio et al. 1999) and the effect that this may have on the slope of linear regression equations.

## 5.0 General Discussion

### 5.1 Volunteer Canola Interference with Soybean

The experiments were conducted to quantify the magnitude of yield loss in soybean from increasing densities of volunteer canola seeded to narrow and wide row-spacing while assessing the impact on soybean growth and development at various stages. Furthermore, yield loss was used to develop an action threshold in narrow and wide row-spaced soybean for managing volunteer canola. Digital images were captured at the 3-4 soybean trifoliolate stage to determine if there was a link between early-season total ground cover and yield loss in soybean from volunteer canola.

Row-spacing appeared to have an impact on the interference between volunteer canola and soybean. Due to the influence of timing of precipitation and temperature at certain critical stages of the experiment for some site years, the influence of either soybean or volunteer canola appeared to be more prevailing under different environmental conditions in narrow and wide row-spacing. About one third of experiments were significantly different between the narrow and wide row-spacing while the other two thirds did not show any differences between row-spacing. When considering all the site years in one model (except for narrow and wide row-spaced seeded soybeans at Carman in 2012), narrow row-spaced soybean had an action threshold of 3.0 plants  $m^{-2}$  while wide row-spaced soybean had an action threshold of 2.7 volunteer canola plants  $m^{-2}$  which would result in 5 % soybean yield loss. When the model was calculated again without sites from Carman in 2012 (did not conform to rectangular hyperbola) and Melita in 2013 (was significantly different from all other sites) the narrow row-spaced soybean action threshold was 3.2 and the wide row-spaced soybean was 2.5 volunteer canola plants at 5 % soybean yield loss.

The link between volunteer canola interference and soybean growth and development were evident where soybean height and soybean branching were the 2 most important descriptors of the relationship for soybean yield loss from increasing densities of volunteer canola. Other parameters such as soybean leaf area and soybean height at early developmental stages proved to be important in describing the interaction between soybean yield loss and volunteer canola interference. Furthermore, early-season soybean and volunteer canola ground cover was measured by capturing and processing digital images. Early-season ground cover comparing soybean yield loss to percent ground cover was significant in 10 of 12 site years but somewhat variable between site years and between row-spacing. Digital image analysis showed the potential to be used as a predictive tool to forecast soybean yield loss caused by volunteer canola interference at the 3-4 soybean trifoliolate developmental stage in narrow and wide seeded soybean row-spacing.

## **5.2 The Impact on the Soybean Expansion into Western Canada**

The purpose of this study was to enable producers to make more informed management decisions to control volunteer canola in soybean based on volunteer canola population densities (Figure 8.1). Looking forward, this research will support future studies as seeded soybean hectares continue to increase into Western Canada in future years. The increase in seeded hectares in Manitoba has mostly been from producers increasing their proportion of seeded hectares in the western part of the province and from some producers growing soybeans for the first time in western and northern parts of the province. As the relative maturity of soybeans continue to decrease there will likely continue to be more producers that adopt soybeans into their crop rotation. This research will be particularly important in these relatively ‘new’

production areas where soybeans are not widely grown at this point in time because canola tends to be one of the more valuable crops in the rotation in these areas; these new producers will undoubtedly be faced with the same issues producers in Manitoba face with the interference of volunteer canola in soybean for the several reasons previously mentioned.

### **5.3 Future Research**

This is the first study quantifying the interference of volunteer canola on soybean. The product of this research will act as a baseline for other studies evaluating the interaction between soybean, the third most seeded crop (Statistics Canada 2016), and volunteer canola, the fifth most abundant weed in the province of Manitoba (Leeson et al. 2016). When considering future studies assessing the interaction of these 2 crops, learnings from this study could likely benefit the outcome of those studies. For example, in 2013 (the second year of the study) a higher density of volunteer canola was included to determine maximum yield loss and high densities of volunteer canola. Following this study, a recommendation would be to include an even greater density of volunteer canola, either 960 or 1 280 seeds m<sup>-2</sup>. Additionally, the canola seeds that were applied to the soil should be incorporated into the soil, even by light tillage. In some instances rainfall delays or other adverse weather conditions likely impacted the relationship between soybean yield loss and volunteer canola competition. Improving the consistency of emergence of volunteer canola relative to soybean planting would remove some of the variability by site year based on seasonal environmental conditions.

Results from this study suggest a difference between the effect of volunteer canola on soybean yield loss in Melita compared with the Carman or Kelburn site. Future research will need to evaluate the relationship between these 2 crops by region to grasp how they interact

under different environmental and production conditions. Studies should also include other economically important weeds that are relatively abundant by region as seeded soybean hectares continue to increase in Western Canada. This study evaluated a C3 weed species and a C3 crop species that prefers warm temperatures; a study examining economically-important C4 weed species common to Manitoba such as weeds from the *Amaranthus* family or foxtail (*Setaria* spp.) or barnyardgrass (*Echinochloa* spp.) would likely yield different results. The growth habit from these species tends to be more similar during the early parts of the season as soybean. These weed species would have slower development in the spring leading to less of a competitive advantage during a critical developmental period. Further, more work needs to be done to evaluate glyphosate-resistant cropping systems and the resulting interference from glyphosate-resistant weeds. A survey by Beckie et al. (2014) across Saskatchewan and Manitoba identified glyphosate-resistant populations of kochia in previously cropped corn and soybean fields. The onset of dicamba-resistant soybean varieties will provide alternative management options, although some farmers will continue to rely on glyphosate-resistant varieties and an effort needs to be made to assess the agronomic and economic impact for those glyphosate-resistant weeds that flourish in glyphosate-resistant cropping systems.

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## 7.0 Appendix

### Extra Sums Squared Method

The extra sums of squared method used by Lindquist et al. (1996) was used to determine if there were differences for the *I*- and *A*-values between site years. The sequential, selective removal of many site years (8 or 9 site years of 11 site years) from the model was required for site years to be considered the same indicating that the range of *I*- and *A*-values across site years was highly variable (Table 8.1 in Appendix). Experiments were considered outliers if their *I*- or *A*-values from hyperbolic yield loss equation had values that were far from the average. Experiments were removed in different combinations and quantities over 20 model runs yielding 2 model runs that suggest experiments can be considered different. The result suggests that the differences are too variable to use this analysis. 20 model runs with each combination and p-value are found on Table. 8.1. Two groups of experiments that are considered different were found when all but 2 and 3 experiments remained; with so many experiments removed these findings are no longer meaningful. There are a lot of differences and so the sites cannot be combined to determine if they are different.

**Table 8.1.** Twenty different model runs were performed using the Lindquist method using a variation of site years to produce a  $p\text{-value}_{\text{model}}$  for narrow and wide row-spaced seeded soybean production at three locations in 2012 and 2013.

Row Spacing		Narrow						Wide						$p\text{-value}_{\text{model}}$
Year		2012			2013			2012			2013			
Location	Carman	Kelburn	Melita											
Model Run #														
1	ns												<0.0001	
2	↓		X				X						<0.0001	
3							X			X		X	<0.0001	
4				X						X		X	<0.0001	
5							X			X		X	<0.0001	
6				X			X		X		X		<0.0001	
7			X	X	X	X	X			X		X	<0.0001	
8				X			X	X					0.0002	
9				X			X	X		X	X	X	0.0007	
10				X			X	X				X	0.0009	
11				X			X	X		X		X	0.0011	
12				X			X	X	X			X	0.0012	
13				X			X	X	X	X		X	0.0012	
14				X		X	X	X	X			X	0.0017	
15				X		X	X	X	X			X	0.0037	
16				X			X	X		X	X	X	0.0077	
17				X	X		X	X		X	X	X	0.0195	
18				X			X	X	X	X	X	X	0.0454	
19			X	X	X	X	X	X		X		X	0.0966	
20			X	X	X	X	X	X	X	X		X	0.2515	

<sup>a</sup> ns indicated that this site year was not significant and therefore was not used as part of the model

<sup>b</sup> 'X' indicates that the site year was removed from the model

**Table 8.2.** Regression equations describing the effect of light interception from volunteer canola density on soybean parallel and perpendicular to the direction of the soybean seedrow with the R<sup>2</sup> and p-values included for three locations in 2012 and 2013 in narrow and wide row-spaced seeded soybean.

Year	Location	Parallel to Seedrow			Perpendicular to Seedrow		
		Equation	R <sup>2</sup>	p value	Equation	R <sup>2</sup>	p value
Narrow Row-Spacing							
2012	Carman	-	-	0.8759	$y = 0.48 - 0.0011x$	0.23	0.0178
2012	Kelburn	$y = 0.66 - 0.0018x$	0.39	0.0010	$y = 0.65 - 0.0019x$	0.59	0.0001
2012	Melita	-	-	0.4906	-	-	0.0651
2013	Carman	-	-	0.5021	-	-	0.1903
2013	Kelburn	$y = 0.42 - 0.00097x$	0.30	0.0024	$y = 0.51 - 0.0011x$	0.25	0.0067
2013	Melita	$y = 0.49 - 0.0019x$	0.32	0.0017	$y = 0.55 - 0.0015x$	0.27	0.0046
Wide Row-Spacing							
2012	Carman	-	-	0.2252	-	-	0.6697
2012	Kelburn	$y = 0.31 - 0.00060x$	0.18	0.0385	$y = 0.56 - 0.0010x$	0.27	0.0095
2012	Melita	$y = 0.22 - 0.0020x + 0.0000081x^2$	0.28	0.0274	-	-	0.3372
2013	Carman	-	-	0.3475	-	-	0.2788
2013	Kelburn	-	-	0.2888	$y = 0.49 - 0.0010x$	0.29	0.0032
2013	Melita	-	-	0.7852	$y = 0.60 - 0.0019x$	0.16	0.0370

<sup>a</sup> if both linear and quadratic relationships were significant, the quadratic equation was selected

**Table 8.3.** Regression equations describing the effect of volunteer canola density on soybean height (cm) with the R<sup>2</sup> and p-values included for three locations in 2012 and 2013 in narrow and wide row-spaced seeded soybean.

Year	Location	Soybean Height - 1 Trifoliolate			Soybean Height - 3-4 Trifoliolate		
		Equation	R <sup>2</sup>	p value	Equation	R <sup>2</sup>	p value
Narrow Row-Spacing							
2012	Carman	-	-	0.3040	$y = 106.9 - 0.12x$	0.55	0.0001
2012	Kelburn	-	-	0.2963	$y = 81.3 - 0.26x + 0.00078x^2$	0.62	0.0250
2012	Melita	-	-	0.1002	$y = 89.7 - 0.29x + 0.0011x^2$	0.31	0.0286
2013	Carman	$y = 32.13 - 0.017x$	0.24	0.0081	$y = 70.5 - 0.032x$	0.17	0.0298
2013	Kelburn	-	-	0.1635	$y = 62.9 - 0.040x$	0.33	0.0014
2013	Melita	-	-	0.0573	-	-	0.1028
Wide Row-Spacing							
2012	Carman	-	-	0.6872	$y = 96.5 - 0.050x$	0.34	0.0030
2012	Kelburn	$y = 27.03 - 0.070x - 0.00025x^2$	0.27	0.0141	$y = 83.6 - 0.32x + 0.00084x^2$	0.72	0.0011
2012	Melita	-	-	0.9635	$y = 84.8 - 0.40x + 0.0013x^2$	0.70	0.0005
2013	Carman	$y = 35.86 - 0.011x$	0.15	0.0404	$y = 81.1 - 0.17x + 0.00051x^2$	0.41	0.0065
2013	Kelburn	$y = 28.04 - 0.049x + 0.00013x^2$	0.22	0.0496	$y = 64.5 - 0.030x$	0.33	0.0013
2013	Melita	-	-	0.9132	-	-	0.4595

<sup>a</sup> if both linear and quadratic relationships were significant, the quadratic equation was selected

**Table 8.4** Parameter Estimates Table for Multiple Regression

Variable	Stage	Parameter Estimate	p-value <sub>estimate</sub>	Variance Inflation Factor
Narrow Row-Spacing				
Intercept		-0.0034	0.9342	0
Density	1 Trifoliolate	0.0449	0.2926	1.0430
Light Sensor Perpendicular	3-4 Trifoliolate	0.0701	0.1244	1.1812
Leaf Area	3-4 Trifoliolate	0.1864	0.0006	1.6436
Height	Early Pod	0.2797	0.0001	1.3651
Branching	Early Pod	0.1300	0.0198	1.7438
Biomass	Early Pod	0.3888	0.0001	1.8208
Wide Row-Spacing				
Intercept		-0.0196	0.6751	0
Density	1 Trifoliolate	0.1477	0.0022	1.0269
Height	3-4 Trifoliolate	0.1458	0.0050	1.1462
Light Sensor Perpendicular	3-4 Trifoliolate	0.1129	0.0331	1.2222
Leaf Area	3-4 Trifoliolate	0.0952	0.1212	1.7081
Height	Early Pod	0.3348	0.0001	2.2099
Branching	Early Pod	0.2514	0.0003	2.0303
Biomass	Early Pod	0.0934	0.2215	2.5481

**Table 8.5** Multicollinearity Table for Multiple Regression

#	Eigenvalue	Condition Index	Intercept	Density	Height	Light Sensor Perpendicular	Leaf Area	Height	Branching	Biomass
Stage				1 Trifoliolate	3-4 Trifoliolate	3-4 Trifoliolate	3-4 Trifoliolate	Early Pod	Early Pod	Early Pod
Narrow Row-Spacing										
1	2.6982	1.0000	1.9E-06	0.0067	-	0.0330	0.0506	0.0456	0.0499	0.0507
2	1.0112	1.6333	0.0080	0.7701	-	0.1140	0.0008	0.0180	0.0094	0.0003
3	1.0006	1.6422	0.9890	0.0049	-	0.0030	0.0001	0.0005	5.01E-08	0.0002
4	0.7950	1.8423	0.0012	0.1719	-	0.6610	0.0002	0.1824	0.0250	0.0020
5	0.6176	2.0902	0.0002	0.0020	-	0.1730	0.2602	0.6383	0.0566	0.0082
6	0.4506	2.4471	0.0006	0.0084	-	0.0031	0.6880	0.1045	0.3322	0.1940
7	0.4267	2.5146	0.0011	0.0361	-	0.0129	1.1E-06	0.0108	0.5269	0.7446
Wide Row-Spacing										
1	3.1099	1.0000	4.5E-05	0.0001	0.0142	0.0212	0.0347	0.0333	0.0333	0.0309
2	1.1326	1.6571	0.0002	0.3997	0.2448	0.1232	0.0169	0.0013	0.0038	0.0002
3	1.0246	1.7422	0.8261	0.0779	0.0422	0.0107	0.0004	0.0003	9.7E-05	2.1E-08
4	0.8723	1.8881	0.1557	0.4855	0.1904	0.1953	0.0106	6.8E-05	0.0007	0.0052
5	0.7028	2.1036	0.0098	0.0085	0.4264	0.5792	0.0149	0.0255	0.0527	0.0070
6	0.5052	2.4812	0.0002	0.0082	0.0791	0.0123	0.7561	0.0031	0.2664	0.0100
7	0.3553	2.9585	0.0002	0.0001	0.0028	0.0072	0.1156	0.8425	0.3585	0.0052
8	0.2973	3.2343	0.0078	0.0201	0.0002	0.0508	0.0510	0.0941	0.2845	0.9416

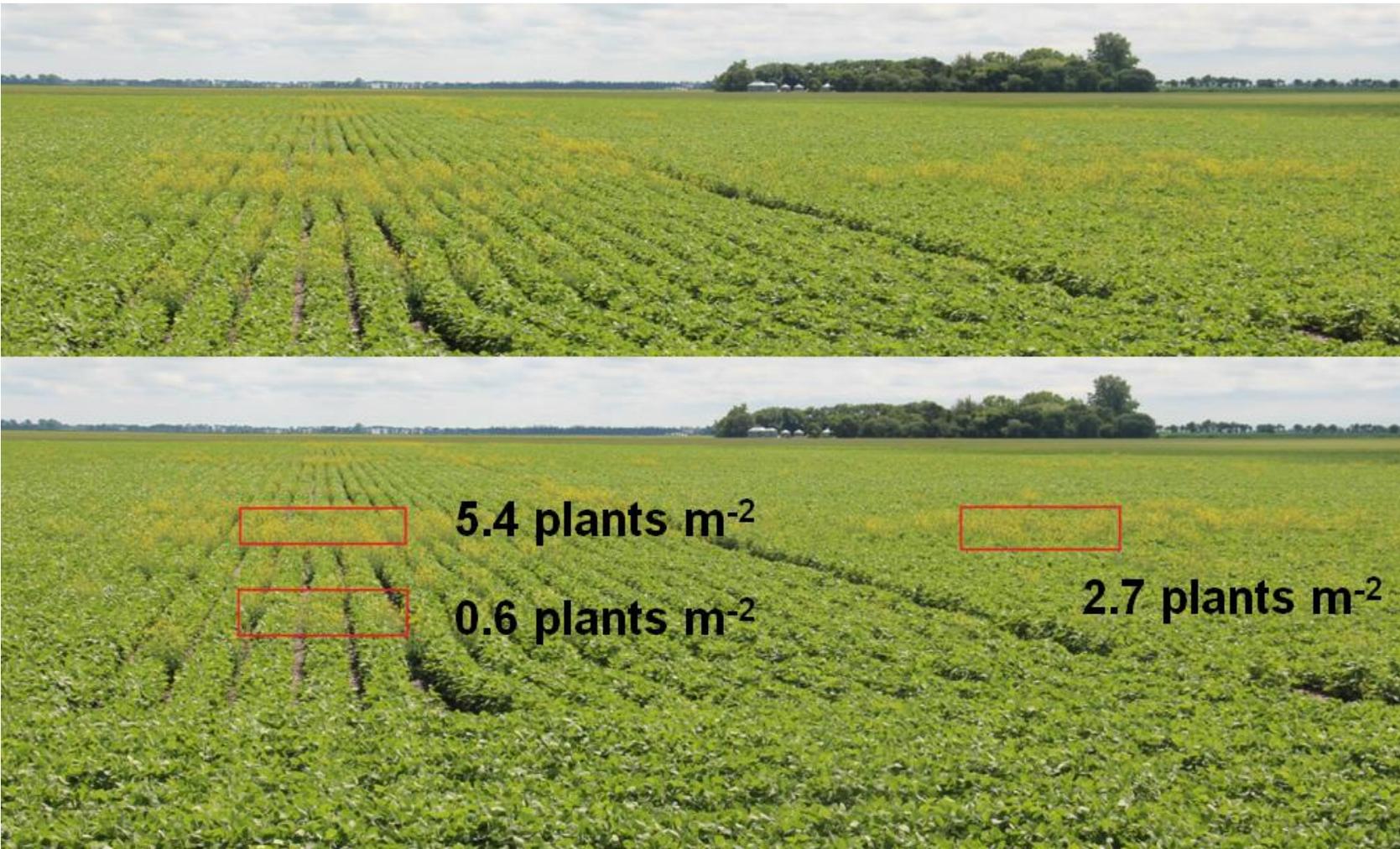
**Table 8.6.** Regression equation parameters: Slopes, intercepts, regression coefficients, p-values for each regression, soybean and canola developmental stages at the time the digital images were taken for each site year and row-spacing experiment. Confidence intervals (95%) of the estimates are indicated in parentheses.

Location	Row-space	Year	Slope	Intercept	R <sup>2</sup>	p-value <sup>a</sup>	Soybean Stage <sup>b</sup>	Canola Stage <sup>c</sup>
Carman	Narrow	2012	0.26 (-0.15, 0.66)	0.86 (-2.76, 4.48)	0.07	0.2085	0.75	cotyledon
		2013	1.05 (0.74, 1.36)	8.21 (1.18, 15.23)	0.66	<b>0.0001</b>	1.25	2.5
	Wide	2012	1.08 (0.56, 1.60)	7.15 (2.36, 11.95)	0.49	<b>0.0003</b>	0.75	cotyledon
		2013	-0.25 (-1.67, 1.17)	17.99 (0.71, 35.28)	0.01	0.7237	1.50	3.0
Kelburn	Narrow	2012	0.96 (0.55, 1.36)	7.16 (-6.27, 20.59)	0.53	<b>0.0001</b>	1.25	3.0
		2013	1.72 (1.07, 2.37)	6.06 (-3.90, 16.01)	0.53	<b>0.0001</b>	0.75	1.5
	Wide	2012	1.08 (0.47, 1.70)	22.97 (10.07, 35.87)	0.39	<b>0.0014</b>	1.25	3.0
		2013	0.67 (0.11, 1.23)	29.20 (18.04, 40.36)	0.19	<b>0.0219</b>	1.00	3.0
Melita	Narrow	2012	2.30 (1.29, 3.31)	18.07 (9.21, 26.92)	0.50	<b>0.0001</b>	0.50	1.0
		2013	0.41 (0.03, 0.78)	9.13 (-2.49, 20.74)	0.16	<b>0.0334</b>	1.25	3.0
	Wide	2012	1.39 (0.88, 1.90)	23.12 (16.08, 30.17)	0.59	<b>0.0001</b>	1.00	2.5
		2013	0.82 (0.16, 1.49)	-3.14 (-15.15, 8.86)	0.26	<b>0.0181</b>	1.00	3.0
LSD <sub>0.05</sub>			0.88	12.23				

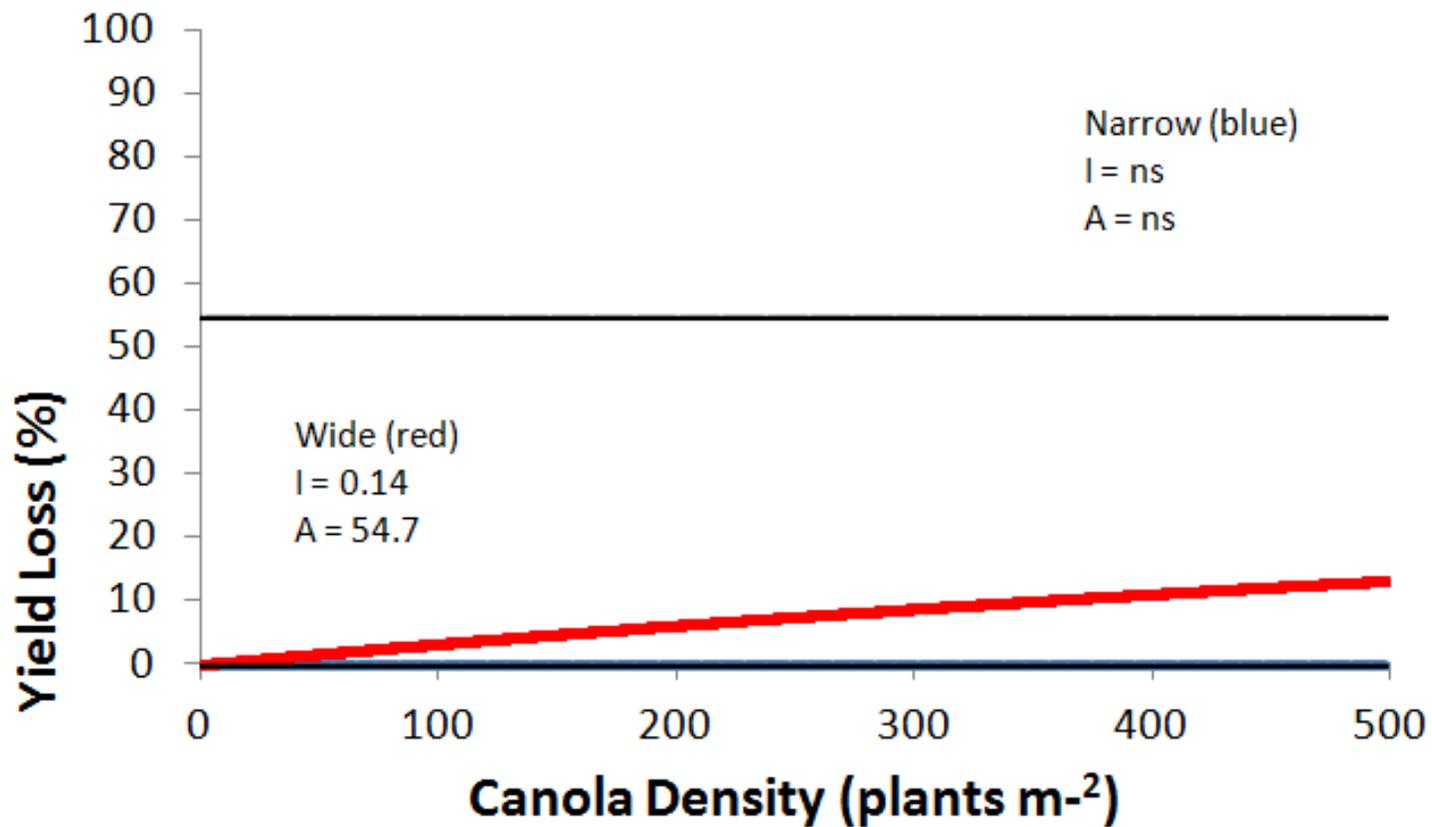
a significant p-values are bolded

b soybean development stage refers to the number of trifoliates

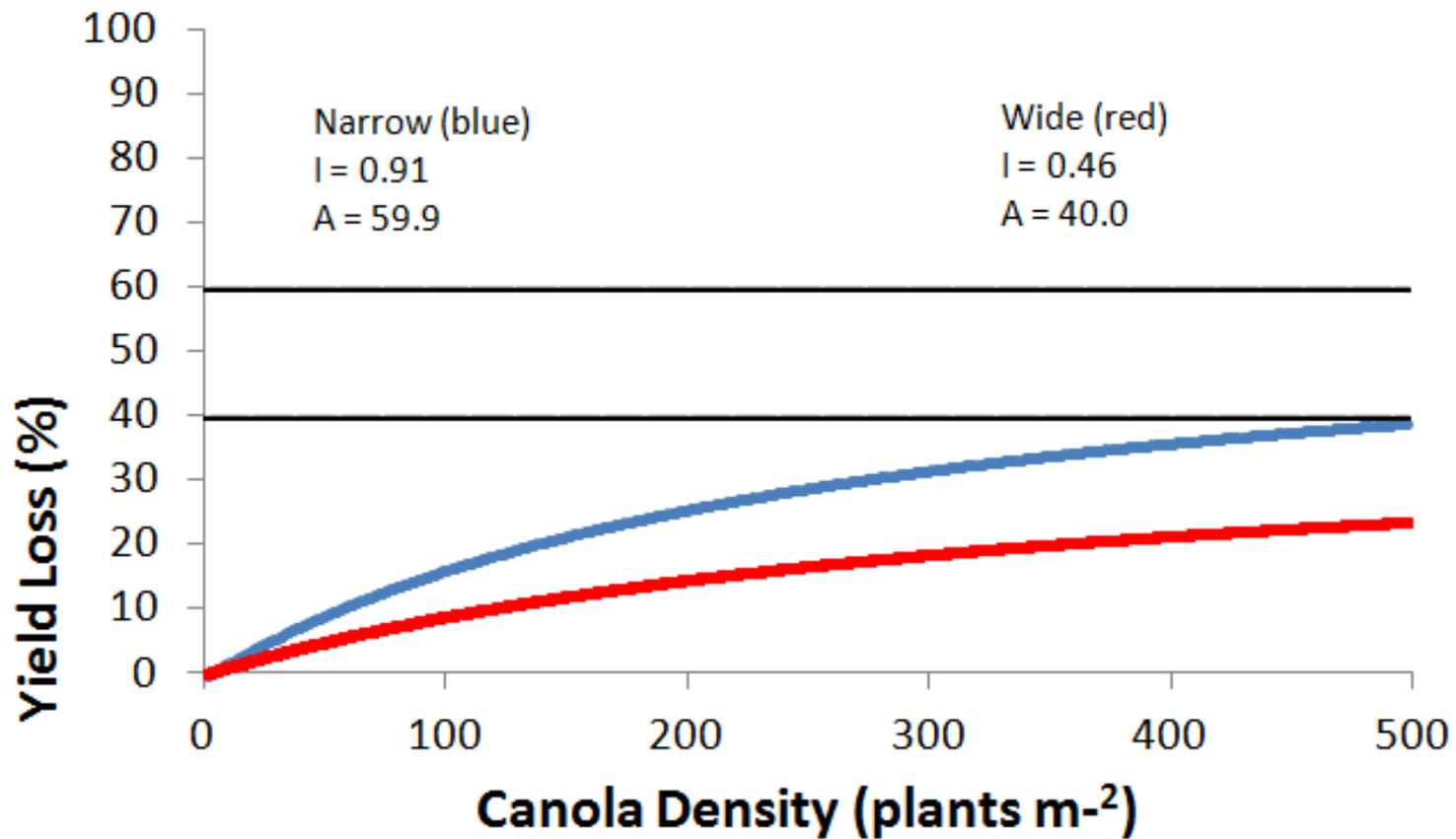
c canola development stage refers to the number of true leaves unless otherwise noted. For correlation analysis, the cotyledon stage was designated as 0.5.



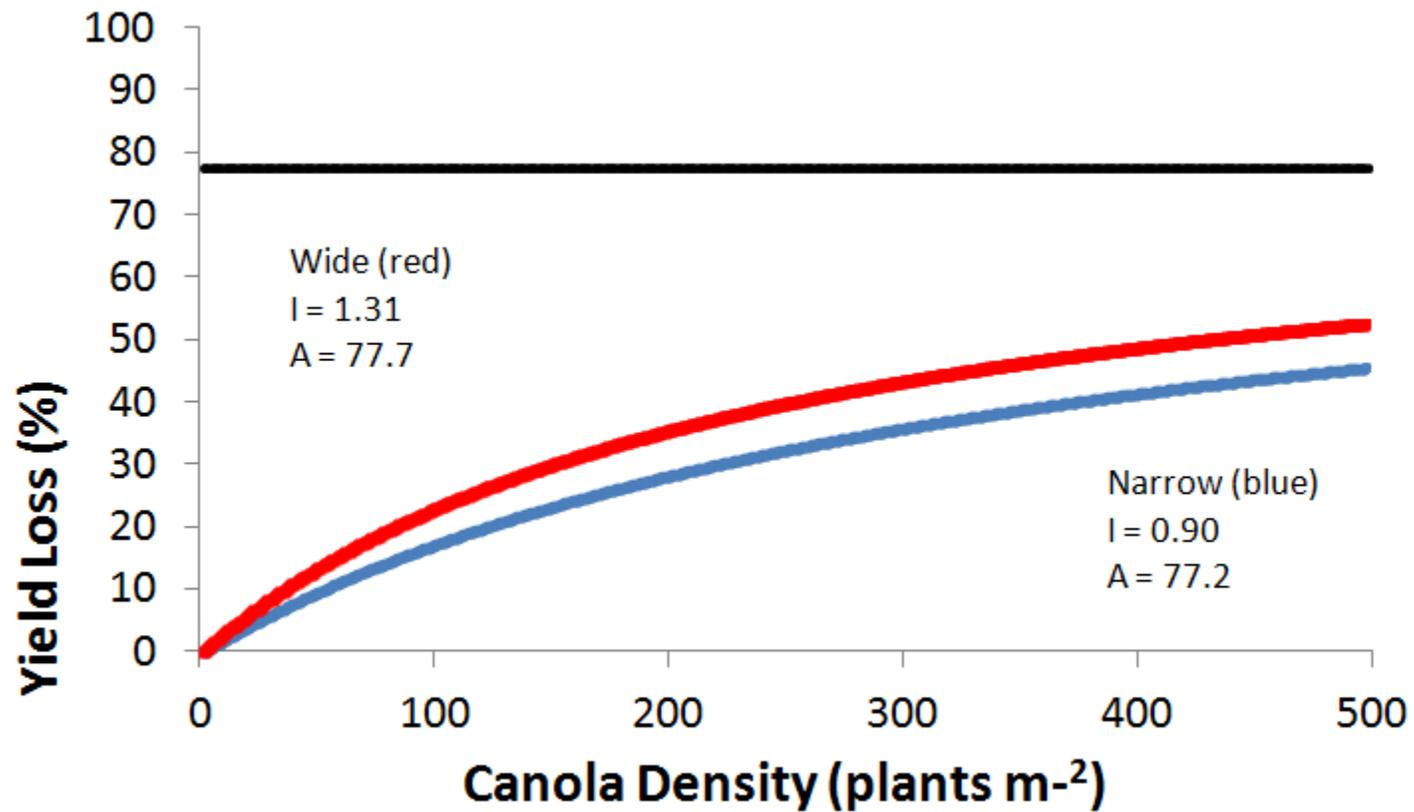
**Figure 8.1** Farmers field near Carman, MB with three densities of volunteer canola determined in a natural population in a soybean field.



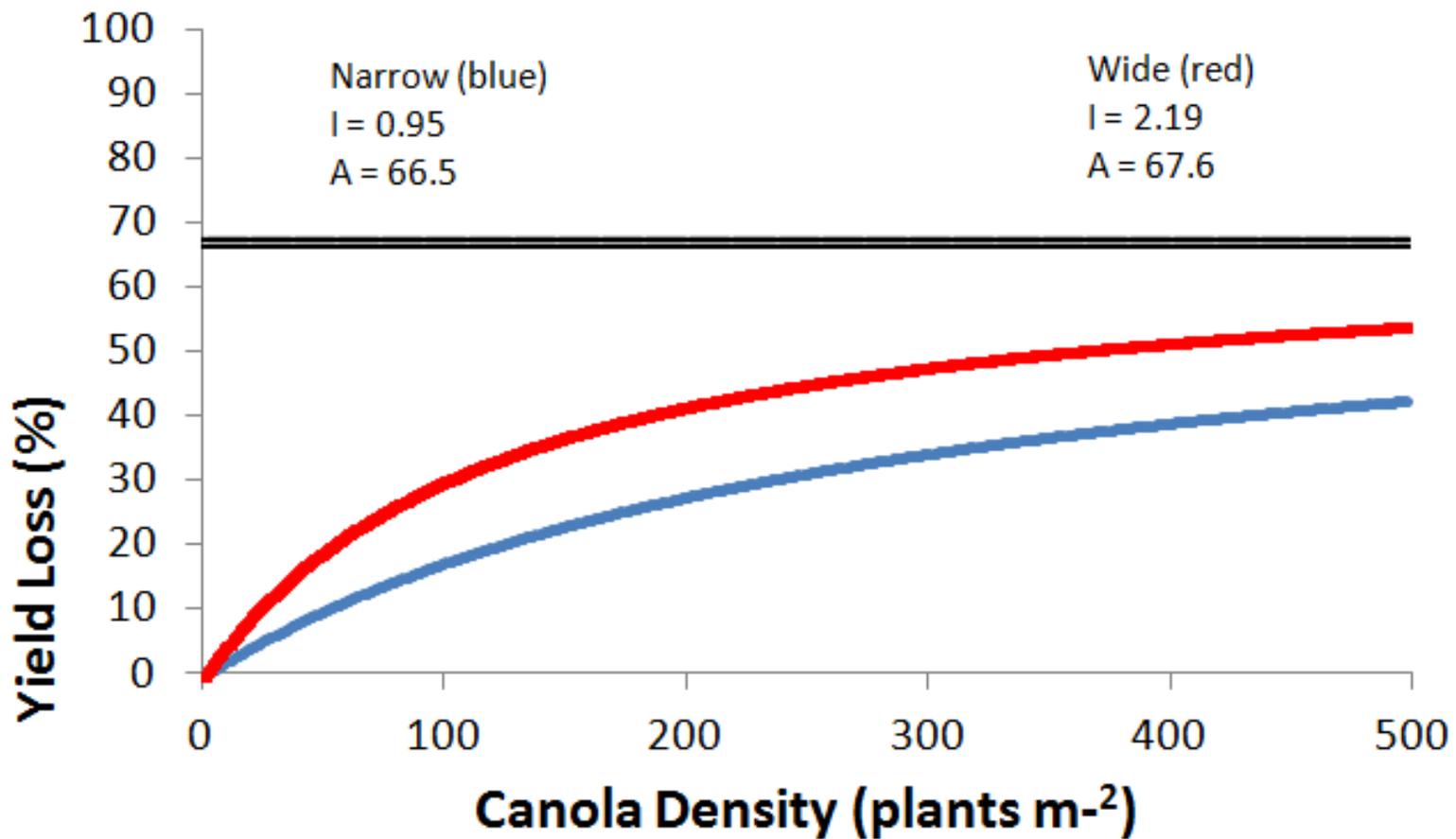
**Figure 8.2** Graphical representation of the rectangular hyperbola model for the Carman site in 2012 demonstrate the difference between narrow and wide row-spaced seeded soybean.



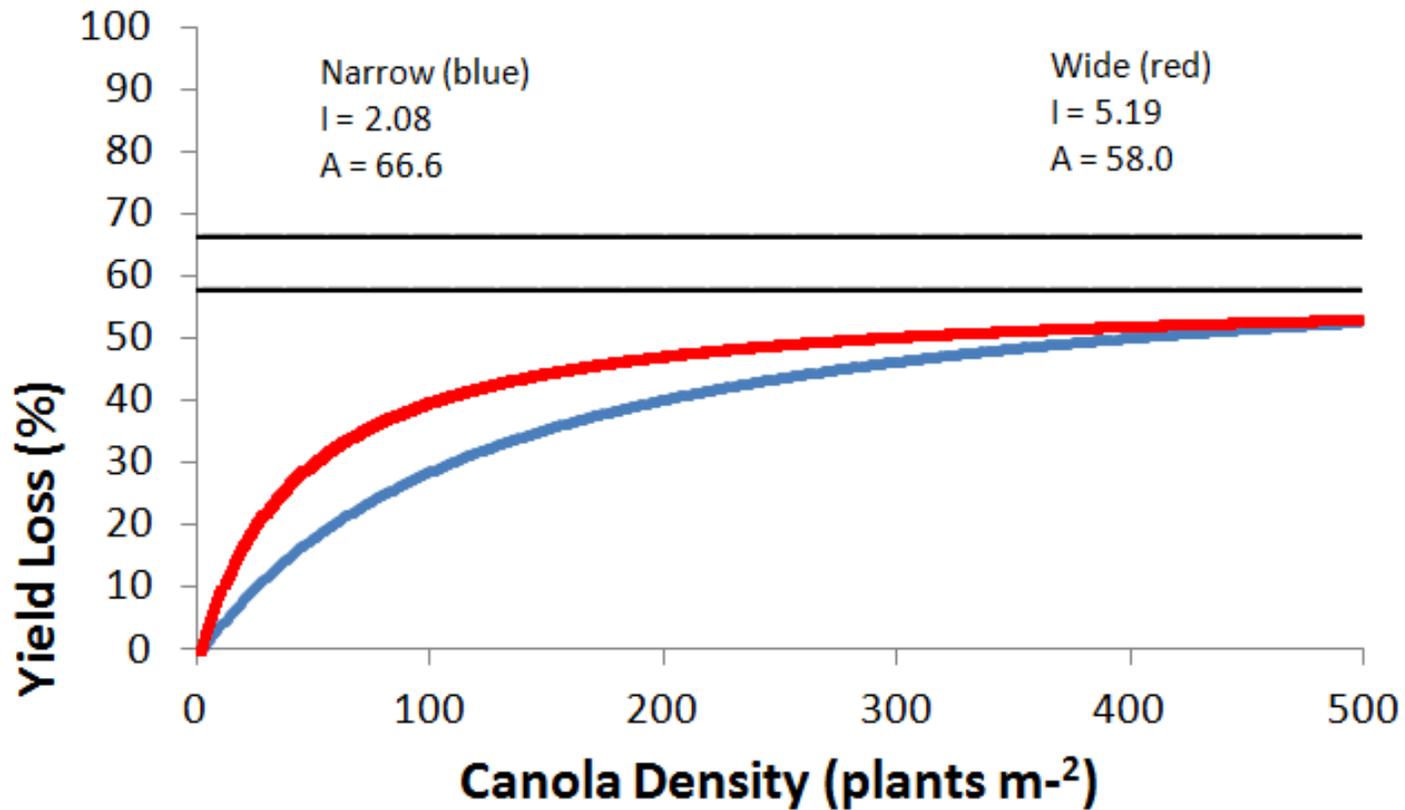
**Figure 8.3** Graphical representation of the rectangular hyperbola model for the Carman site in 2013 demonstrate the difference between narrow and wide row-spaced seeded soybean.



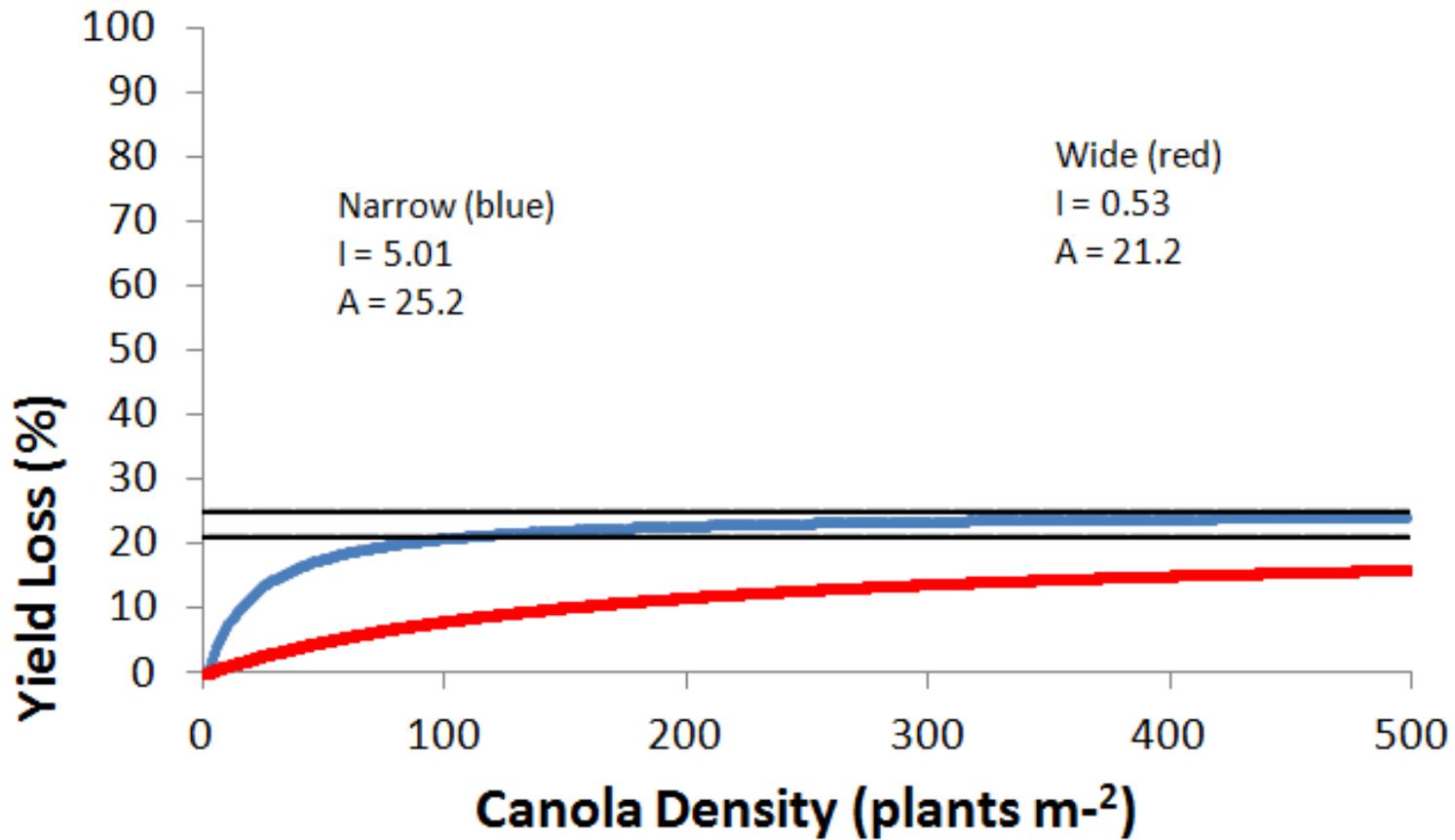
**Figure 8.4** Graphical representation of the rectangular hyperbola model for the Kelburn site in 2012 demonstrate the difference between narrow and wide row-spaced seeded soybean.



**Figure 8.5** Graphical representation of the rectangular hyperbola model for the Kelburn site in 2013 demonstrate the difference between narrow and wide row-spaced seeded soybean.



**Figure 8.6** Graphical representation of the rectangular hyperbola model for the Melita site in 2012 demonstrate the difference between narrow and wide row-spaced seeded soybean.



**Figure 8.7** Graphical representation of the rectangular hyperbola model for the Melita site in 2013 demonstrate the difference between narrow and wide row-spaced seeded soybean.