Evaluating Cultural Weed Management Techniques in Soybean in Manitoba.

BY

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg, MB

ACKNOWLEDGEMENTS

To my advisor Dr. Rob Gulden – Thank you for helping me realize my potential and achieve greater heights than I thought possible. You have been a wonderful mentor throughout my undergraduate and graduate degrees. I cherish the weird and funny friendship we've developed during this time period. Truly, I am sorry for the grey hairs I may have caused over the years!

To my advisory committee, Dr. Doug Cattani and Dr. John Markham – Thank you for the wonderful conversations and learning opportunities. You've enriched my graduate student experience in ways I cannot describe.

To our research technician, Rebecca (Becky) Dueck – Your assistance during my field seasons and the friendly banter was invaluable to both the project and to me. It was a pleasure to work with you all these years.

To the summer students, Luc Fournier, Samantha Curtis, Robyn Unrau, Spiro Veras, Sarah Glowa, Kiet Dang, and Rufaro Chiminya – Thank you for the help and the laughs during the field seasons. I truly enjoyed getting to know you and the company you provided.

To my fellow graduate students in the Weed Ecology lab, Charles Geddes, Leila Kamino, Deanna McLennan, Laura Schmitt, Leanne Koroscil, and Shuxiang Lei – Thank you for making our work environment fun and enjoyable. I look forward to our paths crossing again in the future.

To the organizations that provided funding for this project, Manitoba Pulse and Soybean Growers, Richardson International and Kelburn Farm, and Monsanto Canada Inc. – This research would not have been possible without your assistance.

To Albert and Christian Hinrichs – Thank you for stepping in at the last minute to provide me with a third location for these experiments. I hope this research brings increased visibility to your area of the province. Christian, I want to thank you for your friendship over the years, I wish you continued success with the dairy farm!

To my mother, Jeannette and my sister Genevieve – Thank you for the continued support throughout this process. You provided me a childhood that formed the basis for my success in this endeavor. Jeannette, votre dévouement à la psychologie et votre poursuite à aider les personnes souffrante me donne de l'inspiration comment vivre ma vie.

To my Rosset family, Denis, Donald, Marie & Lucien, and the rest of the extended family – Thank you for allowing me to play and learn of your farms during my youth. Your willingness to teach a city kid about farm life helped me develop my current love of agriculture and nature. I will never forget you, and the fun learning opportunities you offered me.

DEDICATION

This thesis is dedicated to the memory of my father, Gilbert Rosset and my grand-mother, Berthe Filion.

Gilbert, thank you for the love and inspiration you showed me during the first 18 years of my life. You always believed in what I could do, even when I didn't believe in myself. Your passion for nature and food has passed on to me. Your knowledge and understanding that education is the absolute way to improve our humanity and our own socio-economic situation led me to pursue this degree. I will spread that lesson until my own passing. Je t'aime énormément.

Berthe, thank you for showing me how to be strong and resilient. Your strength in the face of continued adversity was admirable and inspirational. Tu as mon cœur et mon amour pour tout le temps.

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ABSTRACT

Rosset, Jonathan D. D. J., M.Sc., The University of Manitoba, March 2020. Evaluating Cultural Weed Management Techniques on the Critical Weed Free Period in Soybean and the Weed Community in Manitoba. Major Professor: Robert H. Gulden

Soybean's popularity as a rotational crop has increased in Manitoba and the Canadian Prairies due to its nitrogen-fixing capabilities and development of short-season cultivars genetically engineered to resist glyphosate. Appropriately designed cultural weed management techniques help mitigate the development of glyphosate resistant weed biotypes caused by excessive glyphosate applications. The cultural weed management techniques of row spacing, target density, and cultivar were evaluated to determine their effects on the critical weed free period in soybean and provide a framework for future regional recommendations. Three field experiments were implemented during the summers of 2016 and 2017 at three locations in southern Manitoba to examine the effects of (1) two row spacings, (2) three target densities, and (3) three soybean cultivars on the critical weed free period in soybean (chapter 3) and the structure of the associated resident weed community (chapter 4). Narrow row soybean shortened the critical weed free period by up to three development stages compared to wide rows. Soybean grown at a target density of 333,000 plants ha^{-1} lengthened the critical weed free period by up to two development stages compared to soybean grown at 666,000 plants ha^{-1}. The effect of soybean cultivar was location dependent, however the cultivar with the shortest maturity and stature generally had the longest critical weed free period. A total of fourteen weed species were identified among field experiments, eight or less weed species occurred at individual site-years and these were dominated by fewer than three species. The structure and trajectories of the weed communities was affected overwhelmingly by the duration of the soybean weed free period. Soybean row spacing and target densities affected the trajectories of the weed community at the untreated and initial herbicide application stages. Further herbicide applications fully obscured the effects of the cultural techniques. Soybean cultivar did not influence the weed community. This research showed that soybean competitive ability can be improved by implementing specific cultural techniques that reduce the duration of time the crop must be kept weed free to minimize yield loss. Specific cultural techniques were also shown to influence the structure of the weed community.

FOREWORD

This thesis includes an introduction, literature review, and two research chapters followed by a general discussion. The research chapters contain work conducted at the University of Manitoba from May 2016 to October 2017. Chapters are written in the format of the journal Weed Science and follow the style defined by the Department of Plant Science, University of Manitoba, Winnipeg, MB, CA.

1.0 INTRODUCTION

Production of soybean [*Glycine max* (L.) Merr.] for seed is a relatively recent addition to crop rotations in the Northern Great Plains. Since 2009, a rapid increase in the Manitoba soybean seeded area has occurred (Anonymous 2009, 2018a, Statistics Canada 2017). This increased production can be attributed in part to the development of short season soybean cultivars (Akyuz et al. 2017, Anonymous 2017a, Morrison et al. 1999, Voldeng et al. 1997). Weed management in soybean was conducted traditionally with an application of a selective herbicide combined with at least one inter-row tillage operation (Bradley 2006). Glyphosate is a non-selective, broad-spectrum herbicide that has simplified traditional weed management in soybean and other crops commercialized to resist its application (Duke 2005). In traditional soybean growing regions of southern Ontario and the USA elevated use of glyphosate resistant (GR) soybean has led to the development of many GR weeds (Heap 2020). For example, glyphosate resistant biotypes of giant ragweed (*Ambrosia trifida* L.), horseweed [*Erigeron canadensis* (L.) Cron.], and tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] have been identified in GR crop rotations in southern Ontario (Schryver et al. 2017, Vink et al. 2012) and throughout the US mid-western states (Beres et al. 2018, Kruger et al. 2009). On the Northern Great Plains, GR kochia [*Bassia scoparia* (L.) Scott] has been identified recently (Beckie et al. 2014, 2019). Heavy reliance on herbicides, particularly glyphosate, in cropping systems will continue the selection for herbicide resistant weed biotypes (Shaner 2000). Crop production systems must therefore adopt agronomic practices that reduce the selection pressure for herbicide resistant weeds.

Integrated weed management strategies combine the use of biological, chemical, cultural, and mechanical techniques to improve crop competition against weed pressure

(Swanton and Weise 1991). Cultural weed management techniques encompass a diverse suite of decisions aimed at improving the crop competitive ability and are taken either prior to or at seeding. The depth and range of research conducted on the effects of various cultural weed management techniques in soybean dates back over 50 years. Cultural techniques such as row spacing and target density have generally dominated the search to improve soybean competitive ability with weeds. For early examples see Lehman and Lambert (1960), Wax and Pendleton (1968), Wax et al. (1977) and for recent examples see Arce et al. (2009), De Bruin and Peterson (2008). Cultivar selection is also considered a cultural weed management technique however, research emphasis on the effects of soybean cultivar competitiveness with weeds has been modest (Burnside 1978, Bussan et al. 1997, Jordan 1992, Norsworthy and Shipe 2006). Recent works have identified competitive soybean traits (Horneburg et al. 2017, Place et al. 2011c) may have renewed research interest. While evaluation of the effects of cultural weed management techniques generally have examined soybean seed yield under weedy compared to weed-free conditions, this may not be the most appropriate methodology to determine whether the technique was successful at improving soybean competitive ability.

The Critical Weed Free Period (CWFP) was a concept proposed initially by Nieto et al. (1968) and may provide another methodology to evaluate a crop's competitive ability. The CWFP has been defined for soybean (Halford et al. 2001, Van Acker et al. 1993) and other crops (Chaudhari et al. 2016, Fedoruk et al. 2011, Hall et al. 1992, Martin et al. 2001). No previous research has evaluated the effects cultural weed management techniques on the competitive ability of soybean or other crops by using the CWFP concept. This research gap was addressed by the design and implementation of three specific field experiments conducted in Manitoba, Canada, during the summers of 2016 and 2017. Using the CWFP concept, Chapter 3 of this thesis addressed the following objectives:

(1) To determine whether the competitive ability of soybean with weeds is improved when grown on narrow (19-cm) compared to wide (76-cm) rows.
(2) To determine whether the competitive ability of soybean with weeds was improved by increasing target densities from 333,000 plants ha^{-1} to 666,000 plants ha^{-1}.
(3) To determine if three soybean cultivars from the same development program with different time to maturities and statures differed in competitive ability with weeds.

In addition, observations were taken to explore the effects of these cultural techniques in soybean on the resident 'natural' weed community. Chapter 4 of this thesis examines the effects of (1) row spacing, (2) soybean target stand density, and (3) soybean cultivars on the composition and structure of the weed community in response to different weed free durations.

2.0 LITERATURE REVIEW

2.1 Soybean Production on the Northern Great Plains

Soybean [*Glycine max* (L.) Merr] originated in Asia (Hymowitz 1970) and was domesticated in China during the late 17th century before being imported to North America in the late 18th century (Shurtleff and Aoyagi 2010). In Canada, soybean cultivation and research began in 1881 at the Ontario Agricultural College in southern Ontario, where the first commercial soybean seed production began in 1922. Expansion of the soybean seeded area north and east of southern Ontario along the St-Lawrence watershed began in 1976 (Shurtleff and Aoyagi 2010), driven primarily by the development of short-season cultivars (Morrison et al. 1999, Voldeng et al. 1997), improvements in weed management options, and increased market demand (Shurtleff and Aoyagi 2010). As a nitrogen-fixing legume, soybean is also well suited as a rotational crop when combined with cereal and non-leguminous oilseeds. In Manitoba, the area dedicated to soybean production prior to 2008 remained limited due to cultivar choices (Anonymous 2009). A steady, yet rapid increase in the Manitoba soybean seeded area has occurred since 2011, where soybean is now the third most common crop grown in the province based on seeded area (Statistics Canada 2017), putting the province at the northern-most edge of the North American soybean growing region.

2.2 Herbicide Use in Soybean Production Systems

Prior to 1960, weed management in soybean was largely accomplished using non-chemical techniques. Use of traditional weed management strategies (i.e. delayed planting, tillage, increased plant densities, competitive cultivars) decreased with the development and adoption of herbicide formulations that were safe to use on soybean plants (reviewed in

Bradley 2006). Traditional soybean herbicide active ingredients required high application rates for effective weed management (Fernandez-Cornejo et al. 2014). Weed management in soybean became more efficient during the 1980s as new herbicide active ingredients that required lower application rates and were safer on soybean became available (Fernandez-Cornejo et al. 2014, USDA 2017). Heavy reliance on herbicides for weed management has led to the development of many herbicide resistant (HR) weed biotypes (Heap 2020). On the Northern Great Plains, many weed species quickly became resistant to the herbicide mechanisms of action commonly used in small cereal crops (e.g. acetolactate synthase-, acetyl-CoA carboxylase-, and microtubule assembly- inhibitors) (Morrison and Devine 1994). These commonly used herbicides have similar mechanisms of action as those applied regularly to soybean in other regions.

Genetically engineered (GE) soybean cultivars resistant to various herbicide active ingredients have been developed and since 1995, have been commercially available in Canada (Duke 2005). Genetically engineered soybean cultivars resistant to the active ingredient glyphosate were released first (Padgette et al. 1995), followed closely by the release of glufosinate-ammonium resistant cultivars (Zhang et al. 1999). These two initial GE herbicide resistance traits, inserted into soybean and other crops (Duke 2005), were developed primarily as a solution to the increasing cases of HR weed biotypes (Davis and Frisvold 2017). In the USA, where the number of HR weed biotype cases are the greatest (Heap 2020), adoption of GE soybean cultivars increased rapidly, and by 2008 GE cultivars were grown over 90% of the soybean seeded area (Fernandez-Cornejo et al. 2014). In Canada, adoption of GE crops is estimated through seed sales and seeding reports however no official survey has been conducted. In 2017, approximately 99% of the soybean seeded area in Manitoba was seeded to GE soybean cultivars (Anonymous 2018a). Adoption of GE soybean cultivars (primarily glyphosate-resistant) in Manitoba has been rapid due to the ease and effectiveness of weed management from the use of the respective herbicides (Duke and Powles 2008), vertical integration between seed and chemical manufacturers (Duke 2005), and previous familiarization with GE canola (*Brassica napus* L.). Similar scenarios have likely occurred in other Canadian soybean growing regions. Unfortunately, due to glyphosate's ease of use and effectiveness, diverse weed management practices have been lost in GR crops as producers increasingly rely on this single active ingredient. Consequently, the increased reliance on glyphosate for in-crop weed management has led to the rapid evolution of GR weed biotypes (Green and Owen 2011, Heap 2020, Mortensen et al. 2012, Owen 2016, Schütte et al. 2017, Waltz 2010).

In order to address the rapidly growing concern over GR weed biotypes, the industrial solution was to create new GE soybean cultivars. Commercialization of GE soybean cultivars resistant to the active ingredients 2,4-dichlorophenoxyacetic acid (2,4-D) (Li et al. 2013) and dicamba (Behrens et al. 2007) has occurred recently. These new herbicide-tolerance traits have been combined with glyphosate- and/or glufosinate-ammonium for greater weed spectrum management (Behrens et al. 2007, Li et al. 2013). Concurrent application of multiple herbicide active ingredients with different mechanisms of action has been shown to delay the development of weed biotypes resistant to the applied active ingredients (Beckie 2006, Beckie and Reboud 2009). No new herbicide mechanism of action have been discovered in the past 30 years (Heap 2020). Extending the life-span of available herbicides is important if we wish to retain their current effectiveness while delaying evolution of new HR weed biotypes.

2.3 Integrated Weed Management for Soybean Production

Integrated weed management (IWM) has been defined as the integration of biological, chemical, cultural, and mechanical techniques to improve crop competition against weeds (Beckie and Harker 2017, Swanton and Weise 1991). For a thorough review of IWM, see Buhler (2002). Briefly, biological weed management techniques refer to the use of biological organisms to control weeds; chemical weed management techniques refer to the use of xenobiotics developed specifically to control weeds; mechanical weed management techniques refer to the use of machinery to control weeds; and cultural weed management techniques refer to the decisions taken by the agriculturalist at the crop planning stage. For example, extended crop rotations, increased crop competitive ability, time of seeding, and improved fertility management are easily accessible cultural weed management techniques (Owen et al. 2015). Implementation of cultural weed management techniques work to enhance the performance of direct (i.e. tillage and chemical) weed management strategies (Stoller et al. 1987) and may extend the current usefulness of available herbicides. Effective and sustainable weed management can be achieved only through the judicious use of all weed management methods (Swanton and Murphy 1996).

2.3.1 Critical Period of Weed Control

Development of local and regional IWM strategies requires information on the ecological behaviors and interactions between crop and weed species (Zimdahl 2004). Good IWM decisions are based on knowledge of the time of weed emergence relative to the crop, and the crop's critical period of weed control (CPWC) (Harker 2013, Sanyal et al. 2008, Swanton and Weise 1991). In general, weeds that emerge earlier than the crop cause increased yield loss over weeds emerging after the crop (Harker 2013) however, this is not true for all weeds (Storkey and Neve 2018). Knowledge of the relative time of weed emergence has enhanced crop management by improving the timing of weed management techniques (i.e. tillage and sowing operations) (see for example Cousens et al. 1987, O'Donovan et al. 1985). Yet, the information gathered from time of emergence studies do not address how to minimize yield loss caused by weeds emerging after field operations. The concept of the CPWC was proposed by Nieto et al. (1968), and the definition and structure of the CPWC was improved by Zimdahl (1988). The CPWC is comprised of two parts (Nieto et al. 1968, Zimdahl 1988). The beginning of the CPWC is defined by the critical timing of weed removal (CTWR), while its end is defined by the critical weed free period (CWFP). Briefly, the CTWR determines the optimal timing to initiate weed removal and the CWFP determines the length of time a crop must be kept weed-free to minimize crop yield loss to an acceptable level (Knezevic et al. 2002). See Figure 2.1 for a visual representation of the CPWC.

Many of the assumptions and limitations of the CPWC were discussed extensively by Knezevic et al. (2002). Briefly, when determining the CPWC the assumptions are (1) the CPWC for a crop is generally only relevant to the geographical region where it was determined and (2) local crop production practices influence the duration of the CPWC. The limitations of the CPWC are (1) the inherent variability in species-specific morphology, physiology, and development that results in a unique CPWC for each crop-weed complex, and (2) variability in the CPWC experiment may be greater due to compounding errors from the two independent CTWR and CWFP experiments. Studies looking to determine a crop's CPWC should not use individual weed species since they occur in fields as part of communities and may not affect the crop in a similar manner (Storkey and Neve 2018).

Weed community dynamics also likely influence the CPWC as community composition and seedling recruitment differ among locations and years (see for example Hall et al. 1992, Martin et al. 2001). Efforts must be made to reduce the effects from these assumptions and limitations when evaluating the CPWC by explaining the causes of variations within the experiments thoroughly (Knezevic et al. 2002, Knezevic and Datta 2015).

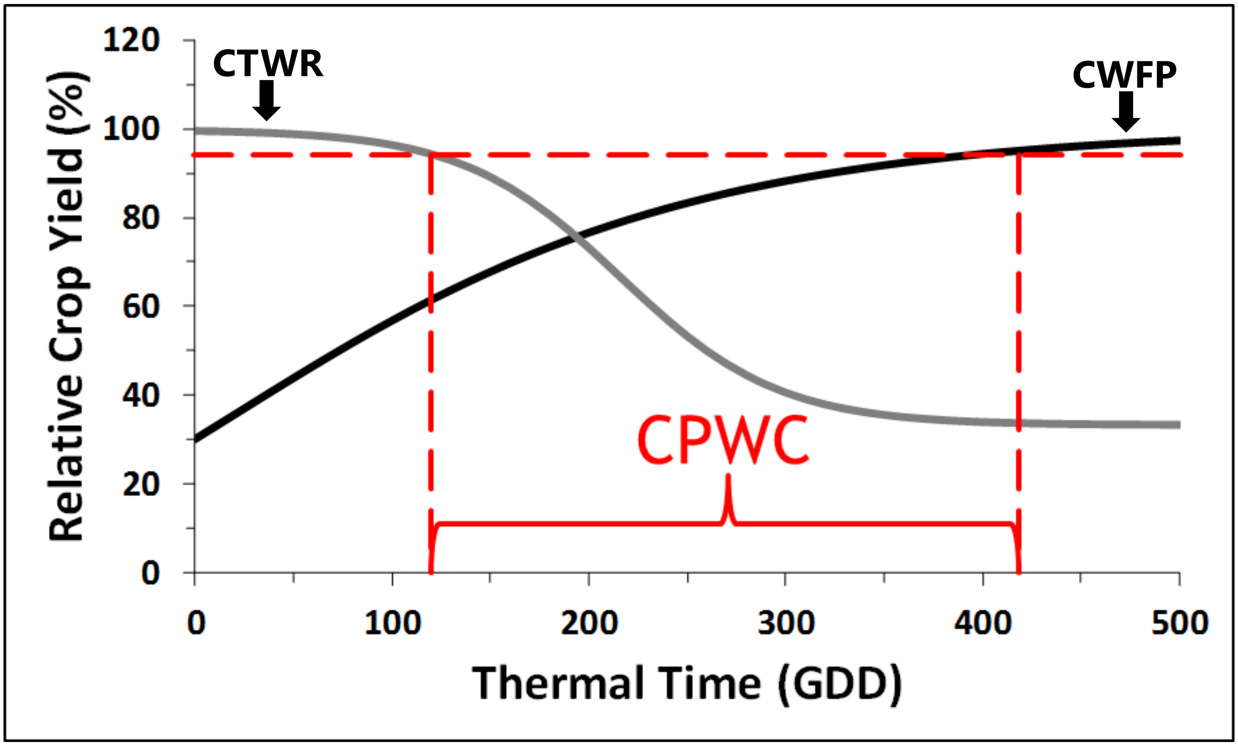

Figure 2.1 Visual representation of the critical period of weed control. Red horizontal dashed line represents a 5% yield loss. Red vertical dashed lines define the CPWC.

The CPWC has not been studied extensively in the Northern Great Plains. So far in this region, the CPWC for canola (Martin et al. 2001) and lentil (*Lens culinaris* Med.) (Fedoruk et al. 2011) have been determined. As soybean is a relatively new seed crop to the Northern Great Plains, work is currently underway to determine the duration of the CPWC for this species (E. Simpson, unpublished). In southern Ontario, the CPWC in soybean ranges from the V1 to V3 (= first to third trifoliate) development stages (Van Acker et al. 1993). It is unknown if the CPWC determined in southern Ontario is valid for the Northern

Great Plains due to climatic differences between regions and technological changes in soybean production.

2.3.2 Modelling the Critical Period of Weed Control

Determination of the CPWC requires the modelling of two different, yet complementary datasets using non-linear regression (Zimdahl 1988). These models generally fit datasets with similar characteristics and have been updated by Knezevic et al. (2002) to reflect these similarities and have become standard in CPWC studies (see for example Fedoruk et al. 2011, Norsworthy and Oliveira 2004, Singh et al. 2015). The CTWR model follows a three-parameter inverted logistic equation that describes crop yield in response to increasing duration of weed interference on crop yield:

$$Y_{CTWR} = 100 \times \left[\frac{1}{exp[c \times (T - d)] + f} + \frac{f - 1}{f}\right] \qquad [1]$$

where Y_{CTWR} is the crop yield expressed as a % of season-long weed-free yield, T represents time (expressed in thermal time), parameter c represents the maximum slope found at the inflection point, parameter d indicates the inflection point, and parameter f indicates the bottom asymptote relative to the position of the upper asymptote.

The standard model used to describe the CWFP is a three-parameter Gompertz equation that best fits the response of crop yield to increasing weed-free duration:

$$Y_{CWFP} = A \times exp[-b \times exp(-k \times T)] \qquad [2]$$

where T is the same as mentioned in eq. [1], Y_{CWFP} represents the crop yield expressed as a % of the season-long weed-free yield, parameter A is the value of the upper asymptote and represents the maximum crop yield when expressed as a percentage, parameter b indicates the inflection point, and parameter k indicates the maximum slope at the inflection point.

The inflection point represents a specific point along the abscissa, in this case thermal time. For each equation, the parameters *d* (eq. 1) and *b* (eq. 2) (i.e. the inflection point) can be used to compare timing of weed removal or weed-free duration among two or more experimental treatments. Parameters describing the slope at the inflection point [i.e. parameters *c* (eq. 1) and *k* (eq. 2)] represent the maximum growth or decay of crop yield under weed interference specific to the dataset (Tjørve and Tjørve 2010).

When modelling biological systems, it is desireable that each model parameter describe a biological function without interference from another parameter (Ratkowsky 1990). Previous CPWC studies have had difficulties identifying the biological significance of all modelled parameters and many authors have simply referred to those as constants that describe a mathematical function (for example see Chaudhari et al. 2016, Hall et al. 1992, Knezevic et al. 2002, Van Acker et al. 1993). In the CTWR and CWFP models, the estimated values of parameter *d* (eq. 1) and *b* (eq. 2) do not have **direct** biological significance. In the Gompertz equation (eq. 2), parameter *b* interacts with parameter *k* (Tjørve and Tjørve 2017). As such, to elucidate the biological significance of *b*, it must be transformed by natural-log then divided by *k*. The resulting number represents the actual position of the inflection point along the abscissa. A similar interaction between parameter *d* and parameter *f* occur in eq. [1] (Tjørve and Tjørve 2010).

Datasets collected for the purpose of modelling the CPWC are unique to the conditions where they were collected, and from a statistical perspective, models that best fit the data should be chosen (Ratkowsky 1990). Yet, when the CPWC is modelled using different equations, comparaisons with previous works become cumbersome and problematic as parameters differ in meaning. Models describing the CPWC should be reported based on their goodness-of-fit to datasets with similar characteristics (Zimdahl 1988). As such, models used to study the CTWR and CWFP must be standardized across the research area.

2.3.3 Critical Weed Free Period in Soybean

The CWFP is influenced by geographical location (Knezevic et al. 2002). Early work conducted in Nebraska determined that soybean required a minimum 4-week weed-free period after planting to minimize yield loss (Burnside 1979). Similarly, in Ontario, the CWFP for soybean ended 30 days after emergence (Van Acker et al. 1993). However, soybean grown in no-till conditions in Ontario required 44 weed-free days after emergence to minimize seed yield loss (Halford et al. 2001). In central India, soybean required up to 45 weed-free days from seeding to minimize yield loss (Chhokar and Balyan 1999). Finally, in Argentina, a CWFP of approximately 60 days after emergence was required to avoid greater than 2.5% seed yield loss (Eyherabide and Cendoya 2002). A common observation among these studies has been that soybean generally require weed free conditions until their late vegetative or early reproductive stages, regardless of the geographic production location. Row spacing and target stand density were not standardized among these experiments. Locally recommended seeding density and cultivar were used. To my knowledge, no previous study has examined the effects of different cultural weed management techniques on the CWFP for soybean.

2.4 Select Factors Influencing the Critical Period of Weed Control in Soybean

Herbicide use in soybean production has led to the reduced reliance on tillage, crop rotation, and crop competitiveness as major weed management strategies (Parish 1990).

Increased incidence of HR weed biotypes in most of the global crop production areas (Heap 2020) are renewing interests in reviving non-chemical weed management strategies (Beckie 2006, Beckie and Harker 2017, Davis and Frisvold 2017, Mortensen et al. 2012). Tillage is an effective weed management technique (Cardina et al. 2002, Derksen et al. 1993). The focus of this is project however, is on the use of cultural weed management techniques to increase crop competitive ability, therefore other techniques will not be addressed in this literature review.

Increasing crop competitive ability is effective at improving crop interference with weeds (Jordan 1993) and is essential to sustainable weed management (Blackshaw et al. 2007, Harker 2013). Cultural weed management strategies such as crop rotation, early planting dates, soil fertility management, narrow row spacing, increased stand densities, and locally adapted cultivars (Blackshaw et al. 2007) have seen various degrees of adoption by Manitoba soybean producers. Due to increasing soybean seed costs, there is interest from producers to reduce seeding rates by adopting lower stand densities, or less-than-optimal cultivars (De Bruin and Pedersen 2008, Hammer et al. 2018), and possibly wider row spacing if the equipment is readily available (Oriade et al. 1997). The following section provides background on why choosing a narrow row spacing, increased stand density, or competitive cultivars matters to soybean production.

2.4.1 Soybean Row Spacing

Prior to 1960, soybean was planted primarily in wide rows (76-cm or above) to facilitate inter-row tillage as no effective in-crop herbicides had been commercialized (Bradley 2006). Inter-row spaces have since progressively narrowed because of the potential for greater soybean seed yield and technological advancements in weed management.

Increased seed yield, up to 510 kg ha^{-1}, occurs when soybean is grown in narrow rows (50 cm or less) compared to wide rows (greater than 50 cm) (De Bruin and Pedersen 2008, Cox and Cherney 2011, Lehman and Lambert 1960, Walker et al. 2010, Walker and Buchanan 1982, Wax et al. 1977, Wax and Pendleton 1968). The increase in soybean seed yield observed in narrow rows are ultimately tied to the potential for improved stand uniformity and therefore greater plant densities (Weiner et al. 2010). Non-uniform soybean spatial arrangement, such as those found with soybean grown at high densities in wide rows, increase the potential for self-thinning and therefore lower seed yield compared to soybean with more uniform spatial arrangement (van der Werf et al. 1995a, 1995b).

Narrow row widths enhance in-crop weed management through increased light interception. Canopy closure in soybean occurs earlier in narrow rows compared to wide rows (Burnside and Colville 1964, Légère and Schreiber 1989, Wax and Pendleton 1968, Yelverton and Coble 1991), shading the soil surface earlier in the season. Puricelli et al. (2003) and Steckel and Sprague (2004) both measured greater light penetration within the canopy of wide row soybean compared to those grown in narrow rows. Shading from rapid canopy closure in narrow row soybean reduces weed seedling recruitment by inhibiting seed germination of later emerging weeds (Batlla and Benech-Arnold 2014, Mickelson and Renner 1997, Norsworthy 2004). Earlier shading from narrow row soybean also disrupts weed development. For example, narrow row soybean decreased aboveground biomass, leaf area, and seed production for velvetleaf (*Abutilon theophrasti* Medik.) (Hock et al. 2005), spurred anoda [*Anoda cristata* (L.) Schltdl.] (Puricelli et al. 2003), rhodes grass (*Chloris gayana* Kunth) (Rasool et al. 2017), sicklepod [*Senna obtusifolia* (L.) Irwin & Barn.] (Nice et al.

2001), and aboveground biomass of a mixed weed stand (Hock et al. 2006) compared with those grown in wide rows.

Narrow row spacing improves the tolerance of soybean to early-season weed interference. Knezevic et al. (2003) modelled the effects of 19-cm and 76-cm soybean rows on the CTWR and observed that the 19-cm row soybean withstood weed interference three soybean development stages longer than those grown on 76-cm rows. Similar results were observed by Mulugeta and Boerboom (2000). To my knowledge, no previous study directly examined the effects of row spacing on the CPWC or the CWFP in soybean. Halford et al. (2001) however, observed that the CWFP in soybean was different between experimental locations and commented that narrow row spacing (35-cm) at one location was a possible reason for the differences in the CWFP between locations.

2.4.2 Soybean Stand Density

Elevated crop population densities distributed uniformly over an area increases the crop's ability to capture resources (Weiner et al. 2010). Uniform crop spatial distribution, created by high crop populations seeded in narrow rows, effectively suppress weeds through increased light interception (Evers and Bastiaans 2016) and accumulation of crop biomass (Li et al. 2018, Weiner et al. 2001). Soybean leaf area index is increased by greater plant densities that results in greater light interception (Bertram and Pedersen 2004, DeWerff et al. 2014, Harder et al. 2007). Yet, with the current effectiveness of in-crop herbicides combined with increasing seed costs, producer interests are towards reducing soybean seeding rates to increase economic returns (DeWerff et al. 2014, Harder et al. 2007, Norsworthy and Frederick 2002, Oriade et al. 1997). Lower plant population densities reduce the competitive ability of soybean with weeds and lead to increased reliance on herbicides for weed management (Arce et al. 2009, Nice et al. 2001). As such, low soybean populations may diminish herbicide effectiveness and hasten development of herbicide resistant weed biotypes.

The effects of increased soybean population density on weed density and biomass production tend to be more noticeable at wider row spacings. For example, Rich and Renner (2007) observed that increasing soybean density from 185,000 to 432,000 seeds ha^{-1} reduced eastern black nightshade (*Solanum ptycanthum* Dun.) densities and aboveground biomass by 57% and 68% respectively, with soybean grown in 76-cm rows. No differences in *S. ptycanthum* densities or aboveground biomass were observed by increasing soybean densities grown on 19-cm rows. In contrast, Geddes and Gulden (2018) did not observe an effect on the aboveground biomass of volunteer *B. napus* when soybean was grown at either 19-cm or 76-cm row spacing at 420,000 plants ha^{-1}, nor when soybean density was increased to 630,000 plants ha^{-1} in the narrow rows. Differences between these two studies are likely due to the weed evaluated. Volunteer *B. napus* is a cool-season plant known for its rapid early season growth (Gulden et al. 2008) which is in contrast to the warm-season *S. ptycanthum* that is comparable to soybean in rates of development. Yet, additional work from the Canadian prairies has shown that increased soybean densities using narrow row spacings could possibly reduce volunteer *B. napus* interference in soybean (Mierau et al. 2019).

Within the same row spacing, increased soybean stand density reduces aboveground weed biomass when weeds are not otherwise managed. Control of pitted morning glory (*Ipomoea lacunosa* L.) and hemp sesbania [*Sesbania herbacea* (Mill.) McVaugh (syn. *Sesbania exaltata*)] was increased by 87% in untreated soybean plots when soybean densities were doubled to 475,000 plants ha^{-1} (Norsworthy and Oliver 2002). Similarly, up to a 31% decrease in aboveground weed biomass from mixed weed stands occurred when soybean populations were doubled to 420,000 plants ha^{-1} (Arce et al. 2009) and to 445,000 plants ha^{-1} (Harder et al. 2007). Under high weed interference, weed density and ground cover by weeds were reduced significantly when soybean seeding rates were tripled to 556,000 seeds ha^{-1} (Place et al. 2009).

2.4.3 Soybean Cultivar

Crop-weed and crop-crop interactions are ultimately about the asymmetric ability of individuals to acquire resources (Weiner 1990). Efficient and rapid resource acquisition and assimilation generally improves the competitive ability of an individual plant (Schwinning and Weiner 1998). Strong competition by a group of closely-related individuals can result in fitness penalties as the cost of competition increases (Gersani et al. 2001), or as resource over-consumption takes place (de Mazancourt and Schwartz 2012). For example, an individual plant increases in height to outcompete neighbouring plants through rapid acquisition of resources (Morrison et al. 1994). In a crowded group of closely-related species however, the benefit of being tall is lost as stems weaken and plants lodge (stem kinking at the base of the plant), resulting in decreased fecundity (Pierik and De Wit 2014). Generally, soybean breeding has overcome this type of fitness penalty through development of shorter cultivars with stronger stems, without compromising seed yield (Morrison et al. 1999). While soybean is generally not grown at densities high enough to cause stem weakening, uneven distribution of plants or nutrients may cause isolated pockets of plants with weaker stems in soybean fields.

The competitive ability of soybean with weeds varies among cultivars in conjunction with cultural weed management techniques (Callaway 1992, Zimdahl 2004). Traditionally, soybean breeding programs have focused on yield and disease resistance improvements under relatively weed-free conditions (Baenziger et al. 2006, Egli 2008), largely dismissing traits that improve competition with weeds (Hammer et al. 2018). This is likely due to the difficulty of breeding for weed competitive traits. Place et al. (2011c) had difficulties identifying weed-suppressive traits across food- (Natto & tofu), forage-, and feed-type soybean cultivars. From their analysis however, seed size was identified to be a good indicator of a soybean cultivar's weed-suppressive ability (Place et al. 2011b). Leaflet width and plant height were positively correlated with seed size, possibly indicating their potential use as an indicator of weed-suppressive capacity in soybean (Place et al. 2011c). Greater seed size, leaflet width, and plant height have been associated genetically with the absence of the narrow leaflet allele (*ln*) (Place et al. 2011c). A soybean cultivars' competitive ability with weeds is more complex than seed size and plant height. Soybean competitive ability likely involves synergy among traits such as rates of biomass accumulation (Norsworthy and Shipe 2006) and increasingly difficult to measure traits such as leaf photosynthetic conversion efficiency (Horneburg et al. 2017). Other factors influencing the competitive ability of soybean include, but may not be limited to: (1) rapid emergence (Burnside 1979, Jordan 1992, Zimdahl 2004), (2) rate of canopy closure (Bussan et al. 1997, Jordan 1992, Place et al. 2011a), (3) canopy width (Nordby et al. 2007), (4) competitive root growth (Scott and Oliver 1976), (5) time to maturation (Bussan et al. 1997, Nordby et al. 2007), and (6) possible allelopathic effects (Rose et al. 1984). Breeding soybean cultivars with these traits may help reduce reliance on herbicides for weed management.

Genetic engineering may have the potential to increase the competitive ability of soybean. Glufosinate- and glyphosate-resistant soybean cultivars had greater seed yield compared with conventional cultivars in untreated controls however, a very small sample (n=4) of modern cultivars were tested (Owen et al. 2010). This contrasts the findings which showed the competitive ability of soybean cultivars with weeds since 1930 has either stagnated or decreased (Cober and Morrison 2011, Hammer et al. 2018, Morrison et al. 1999). Multiple soybean cultivar lines were examined in these studies and found that modern cultivars had either similar or lower canopy growth rates (Cober and Morrison 2011), overall canopy area (Cober and Morrison 2011, Hammer et al. 2018), or leaf area index (Morrison et al. 1999) than cultivars developed earlier. Modern soybean cultivars however, have increased seed yield under weedy conditions (Cober and Morrison 2011, Hammer et al. 2018) compared to their predecessors, likely compensating for the loss in competitive ability.

2.5 Agronomic Factors that Influence the Weed Community

Weed species occur in communities that respond to the myriad of filters imposed on them during agricultural production (Booth and Swanton 2002, Clements et al. 1994). A filter is a broad term that incorporates everything (e.g. climatic, edaphic, anthropogenic, etc.) that influences the weed community. For the purpose of this review, filters reflect the types of weed management techniques implemented as part of a cropping plan and include activities such as in-crop herbicide applications (Doucet et al. 1999, Légère et al. 2005), tillage (Derksen et al. 1993, Smith 2006), crop rotations (Andrade et al. 2017, Thomas et al. 2010), and management intensity (Ryan et al. 2010). This section will summarize previous research that examined how these agronomic filters influence the weed community.

2.5.1 Herbicides and Crop Rotation

In-crop weed management is one of the most influential filters on the structure of the mid-season weed community (Doucet et al. 1999). Field scale studies completed in France (Fried et al. 2008) and Saskatchewan (Leeson et al. 2000) confirmed that anthropogenic sources of weed management, including the timing of herbicide application and herbicide mechanism of action (Fried et al. 2009), were primary determinants of the weed community structure. Weed community assemblage is also affected by crop rotation (Swanton et al. 2006) which drives the usage of different herbicide mechanisms of action. In Manitoba, soybean is grown principally in rotation with wheat (*Triticum aestivum* L.) and canola (Anonymous 2018a). Different morphological characteristics and herbicide selection between wheat and canola are known to result in distinct weed communities in Manitoba (Van Acker et al. 2000). Similar results were observed in Ontario over three crop cycles of a 'corn – soybean – winter wheat' rotation, where in-crop herbicide applications were responsible primarily for changing the structure of the weed community during the early years while the effects of the crop rotation became noticeable during later years (Swanton et al. 2006). Glyphosate resistant corn and soybean are grown in Manitoba, whereas producers in this province prefer to grow canola with resistance to the active ingredient glufosinate-ammonium (Anonymous 2018a).

During the early adoption phase of GR crops, Shaner (2000) predicted that intensive use of GR cropping systems would result in weed communities shifting dominance towards weed species with natural tolerance to glyphosate. This prediction was foreseen by the work of Jasieniuk et al. (1994, 1996) and Jasieniuk and Maxwell (1994) which modelled the evolution of herbicide resistance in weed species that face strong selection pressures from acetolactate synthase-, acetyl-CoA carboxylase-, and microtubule assembly-inhibiting herbicides. Research has since confirmed Shaner's (2000) prediction. Harker et al. (2005) observed shifts in the weed community attributed to different intensities of GR canola and GR wheat in rotation at different locations across western Canada. Canonical discriminant analysis identified common lambsquarter (*Chenopodium album* L.), field pennycress (*Thlapsis arvense* L.), and GR wheat were associated with increased glyphosate applications whereas wild oat (*Avena fatua* L.), yellow foxtail (*Setaria viridis* L.), redroot pigweed (*Amaranthus retroflexus* L.), *Sonchus* spp., and wild buckwheat [*Fallopia convolvulus* (L.) Löve] were associated closely with conventional herbicides. Westra et al. (2008) also observed *C. album* and *F. convolvulus* associated with increased glyphosate applications on the High Plains of the USA. In southern Ontario, Gulden et al. (2010) observed greater similarities in the structure of weed communities between GR corn and GR soybean compared when conventional herbicides were applied on these crops. A field scale study across multiple US states by Young et al. (2013) observed significant differences in the composition and structure of weed communities in cropping systems that only grew single GR crops compared to those that rotated either one GR crop with a non-GR crop or two different GR crops. The inclusion of GR soybean in Manitoba cropping systems already is shifting the composition and structure of mid-season weed communities (Beckie and Leeson 2017).

Weed communities associated with GR cropping systems shift dominance towards species tolerant to this herbicide (Owen 2008, Shaner 2000). Weed species naturally tolerant to glyphosate include common waterhemp (*Amaranthus rudis* Sauer.) (Patzoldt et al. 2002), *A. theophrasti*, *C. album*, tropical spiderwort (*Commelina benghalensis* L.), common dayflower (*Commelina communis* L.), Chinese foldwing [*Dicliptera chinensis* (L.) Jussieu], birdsfoot trefoil

(*Lotus corniculatus* L.) (Nandula et al. 2005, Owen and Zelaya 2005, Yerka et al. 2013), field bindweed (*Convolvulus arvensis* L.) (Degennaro and Weller 1984, Duncan and Weller 1987), *Ipomoea* spp. (Baucom and Mauricio 2004), and common dandelion (*Taraxacum officinale* Webber ex F.H. Wigg.) (Moyer et al. 2009). Resistance to glyphosate has evolved in many weed species (Heap 2020). For the purpose of this review, weed species with evolved glyphosate resistance relevant to western Canada crop production are *A. retroflexus* (Krga et al. 2013), *A. tuberculatus* (Schryver et al. 2017, Zelaya and Owen 2005), *Ambrosia* spp. (Nandula et al. 2017, Vink et al. 2012, Van Wely et al. 2014), wild oat (*Avena fatua* L.) (Heap 2020), horseweed [*Erigeron canadensis* (L.) Cron.] (VanGessel 2001), and kochia [*Bassia scoparia* (L.) Scott] (Beckie et al. 2014, 2019). Additional information on these weed species and other weeds of concern for Manitoba cropping systems can be found in Appendix A. As producers increasingly adopt GR cropping systems, crop rotations are being simplified (i.e. crop diversity loss followed by increased cultivation of GR crops), increasing the risk for development of GR weed biotypes (Fausti et al. 2014). In Manitoba, the inclusion of soybean in crop rotations has replaced much of the area previously dedicated to small cereal grains other than wheat (Anonymous 2009, 2018a).

2.5.2 Management Systems and Tillage Systems

Organic production systems rely on cover crops, crop diversity, cultural techniques, mulches, and tillage to manage weeds (Costanzo and Bàrberi 2014) and though they are outside the scope of this project, they utilize the same cultural weed management techniques evaluated for this project. Since herbicides are not used in organic systems, weed communities in organic systems are different than in conventional systems. Long-term experiments comparing crop management systems in Pennsylvania have shown increased total and germinable weed seed bank densities in organic systems with significantly different structure of the weed community compared to conventional systems (Ryan et al. 2010). Similar long-term experiments in Switzerland also found that organic systems had greater weed seed densities and weed species richness than in conventional systems (Rotchés-Ribalta et al. 2017). Application of fertilizer in the Swiss long-term experiments significantly affected the composition and density of the weed communities similar to the research conducted by De Cauwer et al. (2010). Weed species abundance and richness was lowest with the addition of synthetic nitrogen and phosphorous fertilizers, whereas the addition of manure increased weed seedbank abundance compared to the synthetic fertilizer. No fertilizer addition always resulted in the most abundant and richest weed seed bank.

Tillage and herbicide applications are effective at shifting the structure of the weed community (Leeson et al. 2000). However, the structural change in the weed community is different due to differences in the timing of each weed management technique (Derksen et al. 1995). For instance, differences in the composition and structure of the weed community are apparent when tillage is conducted in the fall compared to the spring (Smith 2006). Also, the weed seed bank is affected by tillage intensity, with greater abundance and a more diverse composition of weed species observed when no-till practices were used (Cardina et al. 2002). Derksen et al. (1993) observed significant differences in the structure of the aboveground weed community associated with no-till and conventional tillage systems by using canonical discriminant analysis. Weed species closely associated with the no-till system had perennial life cycles compared to the conventional and minimum till systems where weed species mostly followed annual life cycles. The effect of cultural techniques on the composition and structure of the weed community are not well documented.

2.6 Remaining Questions

In summary, a large body of research on soybean production has been produced over the past 50 years. Rarely has the concept of the CWFP been used to evaluate the efficacy of the cultural weed management techniques of row spacing, stand density, or cultivar in soybean or other crops. Knowledge of weed communities is essential to improve weed management yet, a basic understanding how weed communities interact with crops as they are filtered by cultural management techniques is lacking. No studies have examined the effects of the cultural weed management techniques on the structure of weed communities in western Canada, and possibly globally. The overall goal of this M.Sc. thesis is to use the CWFP to evaluate how the cultural weed management techniques of row spacing, stand density, and soybean cultivar can improve soybean competitive ability (chapter 3) and to provide evidence of the effects of these cultural weed management practices on the composition and structure of the weed community in soybean (chapter 4).

3.0 CULTURAL WEED MANAGEMENT PRACTICES SHORTEN THE CRITICAL WEED FREE PERIOD FOR SOYBEAN GROWN ON THE NORTHERN GREAT PLAINS.

This chapter was published in January 2020. Cultural weed management practices shorten the critical weed free period for soybean grown on the northern great plains. Jonathan D. Rosset, Robert H. Gulden. Weed Science 68:79-91. © Weed Science Society of America. Cambridge University Press. DOI: 10.1017/wsc.2019.60

3.1 Abstract

Soybean [*Glycine max* (L.) Merr.] has recently become a popular rotational crop in the Canadian Northern Great Plains where herbicide-resistant (HR) soybean cultivars have been widely adopted. Intense reliance on herbicides has contributed to the development of HR weeds in soybean and other crops. Cultural weed management practices reduce the need for herbicides and lower the selection pressure for HR weed biotypes by improving the competitiveness of the crop. The effects of two row spacings, three target densities, and three cultivars on the critical weed-free period (CWFP) in soybean were evaluated as three separate experiments in southern Manitoba. In the row-spacing experiment, soybean grown in narrow rows shortened the CWFP by up to three soybean developmental stages at site-years with increased weed pressure. In the target density experiment, low-density soybean stands lengthened the CWFP by one soybean developmental stage compared with higher-density soybean stands. The effect of soybean cultivar varied among locations, yet tended to be consistent within location over the 2-yr study, suggesting that competitive ability in these soybean cultivars was linked to edaphic and/or environmental factors. Generally, the

cultivar with the shortest days to maturity, which also had the shortest stature, consistently had a longer CWFP. Each of these cultural practices were effective at reducing the need for in-crop herbicide applications.

3.2 Introduction

Soybean [*Glycine max* (L.) Merr.] is now the third most important crop, based on seeded area (Statistics Canada 2017), in Manitoba and currently comprises the northern edge of the soybean production area in the Northern Great Plains (NGP) of North America. The development of short-season soybean cultivars (maturity groups 000, 00, and 0) has enabled primary producers in this part of the NGP to adopt this crop (Morrison et al. 1999), leading to the recent north-westward expansion of the North American soybean-growing area into Manitoba. The inclusion of soybean in the rotation has provided an additional functional crop type that fixes nitrogen in sequence with crops dominated by cereal and nonleguminous oilseed crops in this region (Morrison et al. 2006). Production recommendations are being adopted from more experienced soybean-growing regions, including southern Ontario and the U.S. midwestern states, as soybean production is relatively new to Manitoba. These practices, however, need critical evaluation to ensure that they are suitable and contribute to sustainable soybean production in the NGP.

Soybean has a relatively poor ability to compete and interfere with weeds (Hammer et al. 2018). This has resulted in the extensive use of herbicides in soybean production and, often, the need for multiple in-crop herbicide applications within a growing season, which have contributed to the selection for many herbicide-resistant (HR) weed biotypes in soybean production regions (Owen et al. 2015). Glyphosate-resistant (GR) waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), horseweed (*Erigeron canadensis* L.), and other weed species are often also resistant to multiple herbicide mechanisms of action (Heap 2019). Many of these weeds are present in the U.S. soybean-growing states bordering the Canadian NGP (Jussaume and Ervin 2016). While herbicide resistance is not new to the Canadian NGP (Heap 2019), where HR weed biotypes of kochia [*Bassia scoparia* (L.) A. J. Scott] (Beckie et al. 2019) and volunteer canola (*Brassica napus* L.) (Gulden et al. 2011) can be found, many producers in this region use zero-tillage production systems that depend on the effectiveness of glyphosate. Selection for GR weeds can occur quickly. In Delaware, as few as three successive years of multiple in-crop and out-of-crop glyphosate applications resulted in the selection of GR *E. canadensis* (VanGessel 2001).

Minimizing the selection for or the impact of HR weed biotypes requires a multifaceted, integrated approach to weed management (Harker 2013; Swanton et al. 2008). In the Canadian NGP, cultural weed management practices have been shown to reduce the need for in-crop herbicides in a number of crops (Blackshaw et al. 2007) and thereby form an important part of an integrated weed management program. Increased seeding rates (Weiner et al. 2001), cultivar selection (Fradgley et al. 2017), and narrower row spacing (Kutcher et al. 2013) all contribute to reduced weed seedling recruitment (i.e., the number of weed seedlings that emerge from the soil surface and compete with the crop), midseason weed biomass, and increased crop yield. In soybean, more uniform spatial arrangement contributes to more rapid canopy closure (Légère and Schreiber 1989) and greater light interception (Taylor et al. 1982), which results in reduced weed population densities (Nice et al. 2001), late-season weed recruitment (Harder et al. 2007), weed biomass accumulation (Arce et al. 2009), and weed seed production (Butts et al. 2016; Nice et al. 2001). In the

NGP, weed species composition and recruitment periodicity differ from those in more experienced soybean- growing regions where those studies have been conducted. Weed communities in the NGP are dominated by early-recruiting, cool-season weeds (Leeson et al. 2005) such as wild oat (*Avena fatua* L.) and volunteer *B. napus*; both highly competitive in soybean (Geddes and Gulden 2018; Rathmann and Miller 1981).

Another key component of effective integrated weed management systems that aim to reduce the reliance on herbicides is adherence to the critical period of weed control (Swanton et al. 2008). The critical period of weed control is inferred from the results of a time of weed addition experiment and a time of weed removal experiment (Nieto et al. 1968; Zimdahl 1988). Using non-linear regression analysis, the critical weed-free period (CWFP) is inferred from the latter set of experiments and describes the duration of time the crop must be kept weed-free to minimize yield loss. These experiments tend to be large and labor-intensive (Knezevic et al. 2002). Understanding the duration of this period and how to shorten it using cultural weed management practices is key to reducing the selection pressure for the development of HR weed biotypes. In Ontario and the U.S. Midwest, the average CWFP for soybean extends until the V3 developmental stage (Van Acker et al. 1993a), but may be much longer and not end until the reproductive stages in certain instances (Eyherabide and Cendoya 2002). In the short growing season of the NGP, weed recruitment periodicity tends to be short compared with warmer regions, and producers generally rely on at least one early in-crop herbicide application as their main method to manage weeds, with additional in-crop applications when deemed necessary. Within this context, it is important to understand the role of cultural weed management tools on the duration of the CWFP to determine whether they contribute to reduced need for in-crop herbicide applications to reduce the selection pressure for HR weeds.

Soybean breeding efforts included selection for the competitive ability with weeds before the availability of highly efficacious herbicides for use in soybean (Egli 2008). Greater leaflet width and plant height (Place et al. 2011a), increased seed size (Place et al. 2011b), prolific root growth (Scott and Oliver 1976), rate of biomass accumulation (Norsworthy and Shipe 2006), time to maturity (Nordby et al. 2007), and leaf photosynthetic conversion efficiency (Horneburg et al. 2017) have been associated with soybean's ability to compete with weeds. Improved herbicides and reliance on them for weed management have resulted in reduced emphasis on traits that enhance soybean's ability to interfere with weeds (Baenziger et al. 2006; Egli 2008). Consequently, the competitive ability of modern soybean cultivars tends to be lower compared with older cultivars (Cober and Morrison 2011; Hammer et al. 2018). Only modern, short-season soybean cultivars are available for production in the Canadian NGP, as soybeans have only recently been included in Manitoba cropping systems. The competitive effect and response of these cultivars, particularly within the context of time to maturity, is not known. The following three experiments evaluated the effects of row spacing, plant stand densities, and cultivar on the CWFP in soybean. We hypothesize that the CWFP for soybean can be shortened through (1) narrowed row spacing, (2) increased plant stand densities, and (3) using longer- season cultivars that have a tall stature.

3.3 Materials and Methods

3.3.1 Experimental description

The effects of soybean row spacing, target densities, and cultivar on the CWFP were evaluated at 6 site-years in southern Manitoba. Field experiments were conducted during the 2016 and 2017 growing seasons at research farms near Carman (49.490348°N, 98.038191°W) and St-Adolphe (49.691525°N, 97.127181°W) and a producer's field in the rural municipality of Whitemouth (2016: 50.024792°N, 96.036137°W; 2017: 50.001285°N, 96.050290°W). Fields were managed using fall and/or spring conventional tillage practices before establishment of the experiments. Soils at Carman were a fine loamy clay (Rignold series) and at St-Adolphe, experiments were conducted on a heavy clay soil (Scanterbury series). Experiments at Whitemouth were conducted in 2016 on a moderately fine loamy sand (Kiplin series), and in 2017 on the B horizon of a Baynham:Katimik:Stead (5:3:2) soil series, from which the organic A horizon had previously been removed (ca. 1970s). Other soil characteristics and the previous crop at each site year are listed in Table 3.1. At Carman and St-Adolphe, 40 kg ha^{-1} of actual phosphate was applied before seeding to meet the fertility requirements for soybean in each year. At the Whitemouth locations, an unknown rate of liquid dairy manure was applied in 2013 where the 2016 field experiments were located and in both years preceding the 2017 field experiments. No additional fertilizer was applied to the soybean at the Whitemouth site-years. To facilitate symbiotic nitrogen fixation in soybean, a commercial peat-based granular inoculant containing 2×10^9 viable cells of *Bradyrhizobium japonicum* (Kirch.) Jordan g^{-1} (Cell-Tech, Monsanto BioAg, Winnipeg, MB, R3T 6E3, Canada) was applied in the seed row at a rate of 3.6 kg ha^{-1} at the time of seeding.

Table 3.1 Important field dates and soil characteristics for three locations in Manitoba over two years.

		Important dates [a]				Soil Chemistry [b]						
		Seeding	Emergence	Harvest	Previous Crop	NO_3^-	Olsen -P	K_2O	SO_4^{2-}	Soil pH	O. M.	Sol. Salts
		May	May / * June	October		kg ha^{-1}	ppm	ppm	kg ha^{-1}		%	mmho cm^{-1}
2016	Carman	11	27	3	Alfalfa	9.0	7	223	9.0	6.3	4.4	0.15
	St-Adolphe	25	* 2	19	Wheat	30.3	25	408	9.0	6.5	7.1	0.61
	Whitemouth	24	* 1	15	Soybean	16.8	17	214	n.a.	8.0	3.8	0.48
2017	Carman [c]	18	27	4	Oat	15.7	7	150	13.5	5.2	2.8	0.11
	St-Adolphe	31	21	12	No Crop	17.9	22	397	7.9	6.6	7.0	0.81
	Whitemouth	30	* 8	19	No Crop	11.2	33	157	74.1	8.1	5.9	0.43

[a] If emergence occurred in June, a * was place in front of the date. Previous crop refers to the preceding stubble at the site-year.

[b] Residual nutrient status and soil characteristics reported for top 15cm of the soil profile and were completed by AgVise Laboratories, Northwood, ND. Abbreviations used in this table reflect residual nitrates (NO_3^-), residual phosphorous as determined by the Olsen test (Olsen-P), residual potassium (K_20), residual sulphur (SO_4^{2-}), organic matter (O.M.), and soluble salts (sol. salts).

[c] Row spacing experiment was re-seeded on June 12 and its emergence occurred on June 26.

Three different experiments were established at each location in each year of the study. The effects of row spacing were investigated in the first experiment, a second experiment investigated the effect of plant target density, and a third experiment evaluated the effect of soybean cultivar on the duration of the CWFP. The large size of these experiments and unknown spatial variability of resident weed populations precluded combining all treatments into a single experiment. Each of these three experiments was established as a randomized complete block design with a split-plot treatment arrangement. Experiments were composed of four replicated blocks with either two (row-spacing experiment) or three (soybean density and variety experiments) main plots with nine subplots (time of weed management) within each main plot. Subplot sizes were 2.5-m wide by 6-m long. In the row-spacing experiment, main plot treatments included narrow (19-cm) and wide (76-cm) soybean row spacing. In the soybean density experiment, target densities of 333,500, 444,600, and 666,900 plants ha^{-1} (0.75X, 1.0X, and 1.5X, respectively, of locally recommended standard target densities) were compared, while the variety experiment compared three soybean cultivars from the same commercial breeding program with different maturity ratings and plant architecture/stature. GR soybean cultivars used for the variety experiment were 'DeKalb® 22-60' (DKB2260), 'DeKalb® 23-60' (DKB2360), and 'DeKalb® 24-10' (DKB2410) (Monsanto Canada, Winnipeg, MB, R3T 6E3, Canada). These soybean cultivars were chosen from one breeding program that differed in time to maturation and plant height. The cultivar DKB2260 is a short-statured plant that matures at about 2,275 CHU (corn heat unit); DKB2360 is a tall-statured plant that matures at 2,350 CHU; and DKB2410 is a medium- to tall-statured plant that matures at about 2,425 CHU. In the row-spacing experiment, DKB2360 was seeded at a density 444,600 plants ha^{-1}, and the same cultivar was used in the target density experiment. In the target density and variety experiments, all soybeans were seeded at an intermediate row spacing of 37.5 cm.

Five subplots were kept weed-free until a specific soybean developmental stage, with one season-long weedy and three season-long weed-free controls included. Developmental stages at which treatments were applied in these studies included soybean with expanded unifoliate leaves (VC), the specific number of fully expanded trifoliate leaves (V1, V2, or V4), and the beginning of the flowering period (R1) (Fehr et al. 1971). Soybean development was recorded weekly until the R1 stage to approximate soybean development based on accumulated GDD_5 (growing degree days with a base of 5C). In each subplot treatment, weed removal ceased at the specific soybean developmental stage, and the resident "natural" weed community was allowed to recruit and interfere with soybean for the remainder of the growing season. Weed removal until the designated soybean developmental stage was achieved by applying glyphosate (358 g ae ha^{-1}; Monsanto Canada, Winnipeg, MB R3T6E3, Canada) and bentazon (889 g ai ha^{-1}; BASF Canada, Mississauga, ON L5R 4H1, Canada) in mixture. These active ingredients were chosen for their efficacious weed control and safety on soybean. Herbicides were applied using a bicycle-wheel push-type sprayer equipped with a 2-m boom set at 50 cm above the crop canopy. Four AirMix 110-01 (Greenleaf Technologies, Covington, LA 70433) nozzles were spaced at 50 cm along the boom and calibrated to apply 100 L water volume ha^{-1} at 276 kPa.

Explanatory measurements of actual soybean density and soybean plant heights were taken at the V3 and R4 developmental stages, respectively. In each subplot, actual soybean density was determined by counting soybean along two 1-m lengths of row, while soybean heights were determined by measuring five individual plants at the highest point along the main stem. Aboveground weed shoot biomass was collected in lieu of weed density counts from two 0.09-m2 quadrats within each subplot at the R5/R6 soybean developmental stage and oven-dried at 65 C until equilibrium. Weeds were sorted by species at time of collection and processed individually.

Table 3.2 Mean monthly air temperature during the growing season at three locations in Manitoba over two years.

		May	June	July	August	Sept.
		------------------------ °C -------------------------				
2016	Carman	13.6	17.1	19.4	18.4	14.1
	St-Adolphe	14.7	17.9	20.2	19.2	14.8
	Whitemouth	12.6	16.2	19.0	18.4	13.9
2017	Carman	12.1	17.1	19.4	17.7	13.7
	St-Adolphe	11.9	17.1	19.7	18.2	14.3
	Whitemouth	10.8	16.6	20.0	17.9	13.8
30-Year Average [a]		11.7	16.9	19.4	18.5	12.7

[a] Calculated between 1981 and 2010 from data compiled by Environment Canada

Air temperature and precipitation data were obtained from the Manitoba Ag-Weather program database (Manitoba Agriculture 2017) for all but the Whitemouth 2017 site-years (Table 3.2; Figure 3.1). Manitoba Agriculture recorded temperatures and precipitation at Carman and St-Adolphe in 2016 and 2017. Temperatures used for analysis at Whitemouth 2016 were obtained from the Environment Canada Pinawa weather station. At Whitemouth 2017, a weather station manufactured by ONSET Computer Corporation (Bourne, MA 02532) was installed directly in the field, adjacent to the experiments. Temperature was captured using a S-THB-M002 temperature and relative humidity sensor connected to a H21-001 HOBO field data logger, while a RG2-M rain gauge recorded precipitation. Long-term 30-yr weather averages were retrieved from Environment Canada.

For determination of the CWFP, air temperatures were converted to GDD using a base temperature of 5 C beginning the day soybeans were seeded (Knezevic et al. 2002).

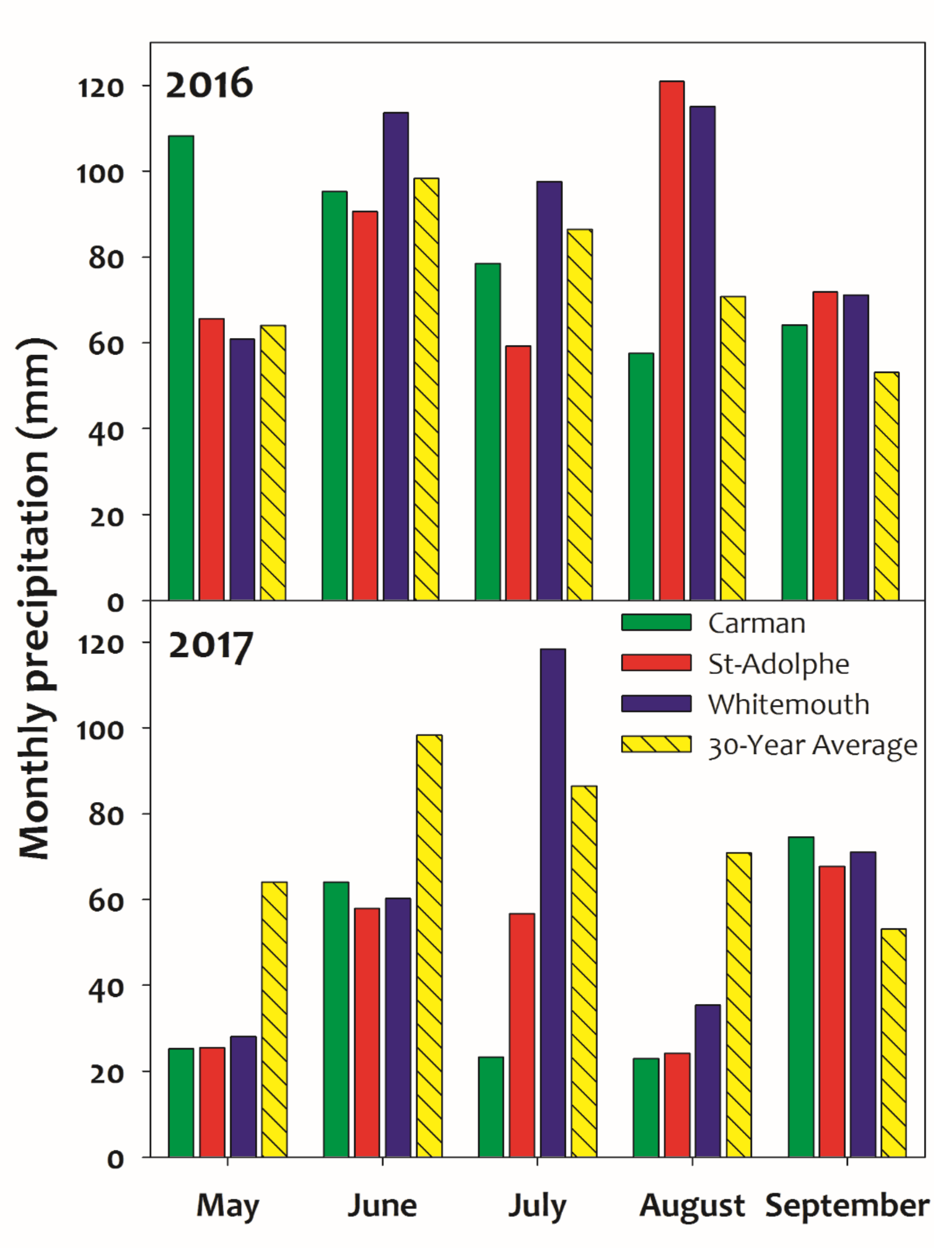

Figure 3.1 Total monthly precipitation during the growing season at three locations in Manitoba over two years. The 30-year precipitation normal (yellow slashed bar) was determined from data collected by Environment Canada between 1981 and 2010.

3.3.2 Statistical analysis

Statistical analysis was performed using SAS Studio v. 14.1 (SAS Institute, Cary, NC 27513). To determine differences in weed-free soybean yields, actual soybean plant densities, soybean plant heights, and total weed biomass from weedy subplots, data were subjected to ANOVA using a linear mixed model (PROC MIXED) approach (Littell et al. 2006). For each experiment and variable, fixed effects used in the linear mixed models were the main experimental treatment (row spacing, target density, or cultivar) and site-year. Location and year effects were also analyzed as fixed effects to determine the significance of their interaction and were combined when the interaction was significant. The duration of the weed-free period was included as a fixed effect when soybean heights and weed-free yields were analyzed to determine whether differences existed between the weed-free and R1 sub- treatments. The experimental block was included as a fixed effect when analyzing the total weed biomass to determine whether weed biomass was uniform across each experiment within site-year. Random effects were the experimental block nested within site-year and the main treatment plot nested within the interaction between the experimental block and site-year. Conformation of the residuals to the Gaussian “normal” distribution was determined using the Shapiro-Wilk statistic (Littell et al. 2006). Lund’s test (Lund 1975) was used to determine whether extreme outliers were present and required further examination. Heteroscedasticity was tested by visual inspection of residual versus predicted values (Kozak and Piepho 2018) and was corrected using the group option in the repeated statement to minimize the Akaike information criterion (Littell et al. 2006). Using the PDMIX800 macro (Saxton 1998), Fisher’s protected LSD at a significance level of 5% ($\alpha = 0.05$) was used to separate the means.

Soybean yield was determined for each subplot treatment as its proportion of the mean weed-free yield in each main plot treatment within each block. To determine differences among main plot treatments within experiments within site-years, relative soybean yield was modeled to GDD_5 for each subplot treatment (developmental stage at which weed management was terminated) with the Gompertz function (Equation 3) using PROC NLMIXED in SAS described by and adapted from Knezevic et al. (2002). The procedure was repeated to determine differences among site-years within main plot treatments, and among main plot treatments with site-years combined. To determine differences between the intermediate and narrow or wide row spacings, an *ex-post* analysis was performed using the same procedure on the data from the row-spacing experiment and the intermediate row-spacing treatments of the same cultivar and densities from the density and variety experiments.

$$Soybean\ Yield_{GDD}(\%) = A \times e^{-B \times e^{-k \times GDD}} \qquad [3]$$

In Equation 3, parameter *A* describes the upper asymptote of the curve or, biologically, the maximum relative potential yield of the crop. The *B* parameter infers the curve's inflection point. To determine the location of the inflection point on the abscissa (i.e., GDD_5), the value of the *B* parameter must be transformed to the natural-log scale and divided by *k*. This yields the inflection point of the Gompertz function in thermal time, which is of biological significance (Tjørve and Tjørve 2017). Finally, the *k* parameter is the maximum slope of the curve, which is located at the inflection point, and refers to how quickly soybean seed yield reaches its potential maximum. All three parameters for main plot models were compared using single-degree-of-freedom estimates (Knezevic et al. 2002).

Within experiment, random effects used in the nonlinear mixed model were experimental block and the main plot treatment nested within the experimental block, and for the combined analysis, site-year, the experimental block nested within site-year, and the main plot nested within the interaction between experimental block and site-year. Initial parameters were optimized by choosing a set of values minimizing the negative log likelihood, following the sample code used by Coffey (2016). In brief, a range of probable values was specified for each parameter and set for stepwise increases within that range. To achieve convergence of the procedure, three strategies were employed as necessary: (1) a bounds statement was invoked to keep variance estimates greater than or equal to zero, (2) the relative gradient convergence criterion was set to zero (Kiernan et al. 2012), and (3) the optimization algorithm was set to either quasi-Newton, double-dogleg, Newton-Raphson, or conjugate-gradient (SAS Institute 2017). GDD values corresponding to 95% and 97.5% of potential maximum soybean yield were determined for each model by rearranging Equation 3 once models were built. These values reflect a 5% and 2.5% acceptable yield loss (AYL) and were then associated with the nearest soybean developmental stage at individual site-years.

3.4 Results and Discussion

3.4.1 Critical Weed Free Period

Row spacing, target density, and cultivar were all effective at reducing the duration of the CWFP in soybean. Within experiments and site-years, differences among treatments were due to differences in the *B* parameters of the Gompertz function only (Table 3.3), while no differences were observed among the asymptote *A* or the slope *k* parameters. Parameter *B*, particularly in the absence of differences in the *k* parameter, reflects the

Table 3.3 Gompertz parameter estimates and contrast p-values for three cultural weed management experiments in soybean.

		Parameter Estimates [a]									'B' Parameter Contrast P-values [b]		
			A			B			k				
Row Spacing Experiment			19 cm	76 cm		19 cm	76 cm		19 cm	76 cm		19 vs 76 cm	
2016	Carman		109±1.9	111±2.6		0.801±0.066	1.299±0.108		0.003±0.0002	0.003±0.0001		**0.0001**	
	St-Adolphe		100±5.2	100±4.2		0.024±0.094	0.076±0.081		0.002±0.0049	0.003±0.0024		0.6739	
	Whitemouth		100±1.7	102±1.7		0.095±0.002	0.299±0.048		0.005±0.0019	0.005±0.0008		**0.0005**	
2017	Carman		100±1.9	100±2.0		0.800±0.101	1.300±0.149		0.012±0.0018	0.010±0.0011		**0.0064**	
	St-Adolphe		100±3.9	100±4.6		0.459±0.102	0.435±0.105		0.008±0.0084	0.004±0.0015		0.9740	
	Whitemouth		104±6.4	104±6.6		0.463±0.085	0.476±0.086		0.004±0.0017	0.004±0.0016		0.9136	
	Combined analysis		102±2.1	105±2.3		0.381±0.038	0.517±0.043		0.006±0.0008	0.004±0.0003		**0.0158**	
Target Density Experiment [c]		0.75X	1.0X	1.5X	0.75X	1.0X	1.5X	0.75X	1.0X	1.5X	0.75X vs 1.0X	0.75X vs 1.5X	1.0X vs 1.5X
2016	Carman	104±2.4	104±2.2	102±1.9	0.578±0.065	0.448±0.065	0.436±0.058	0.005±0.0007	0.004±0.0005	0.004±0.0005	0.1698	0.1000	0.8959
	St-Adolphe	101±1.2	100±1.1	101±1.0	0.193±0.031	0.119±0.030	0.085±0.029	0.006±0.0011	0.007±0.0028	0.016±0.0189	0.0829	**0.0092**	0.4138
	Whitemouth	102±4.4	103±6.4	101±0.9	0.124±0.047	0.046±0.072	0.012±0.021	0.004±0.0036	0.002±0.0030	0.067±31.71	0.3687	**0.0312**	0.6451
2017	Carman	104±2.1	104±3.3	102±2.3	1.204±0.141	0.901±0.156	0.801±0.137	0.007±0.0009	0.008±0.0016	0.008±0.0013	0.1460	**0.0411**	0.6300
	St-Adolphe	100±1.6	102±1.6	102±1.6	0.118±0.052	0.138±0.052	0.086±0.050	0.056±14.33	0.061±24.85	0.060±28.80	0.7834	0.6571	0.4687
	Whitemouth	106±9.6	101±2.1	102±1.1	0.384±0.105	0.225±0.095	0.226±0.017	0.003±0.0016	0.005±0.0022	0.005±0.0012	0.2627	0.1366	0.9978
	Combined analysis	100±1.6	100±1.6	99.9±1.5	0.411±0.036	0.292±0.032	0.254±0.031	0.006±0.0006	0.007±0.0011	0.007±0.0013	**0.0118**	**0.0008**	0.3797
Variety Experiment		DKB2260	DKB2360	DKB2410	DKB2260	DKB2360	DKB2410	DKB2260	DKB2360	DKB2410	2260 vs 2360	2260 vs 2410	2360 vs 2410
2016	Carman	101±2.2	104±3.0	103±2.7	0.935±0.089	0.533±0.061	0.589±0.073	0.006±0.0012	0.005±0.0014	0.005±0.0013	**0.0003**	**0.0030**	0.5582
	St-Adolphe	100±3.1	100±3.0	100±3.0	0.265±0.089	0.655±0.112	0.144±0.081	0.007±0.0043	0.009±0.0028	0.006±0.0048	**0.0070**	0.3170	**0.0003**
	Whitemouth	100±2.1	100±3.1	100±2.0	0.288±0.054	0.340±0.068	0.260±0.053	0.005±0.0010	0.003±0.0004	0.006±0.0014	0.5476	0.7161	0.3548
2017	Carman	106±3.7	102±4.2	103±2.7	2.337±0.394	0.910±0.105	1.194±0.144	0.008±0.0014	0.007±0.0019	0.010±0.0017	**0.0006**	**0.0070**	0.1121
	St-Adolphe	99.2±2.3	101±2.1	101±3.2	0.370±0.080	0.059±0.058	0.318±0.077	0.012±0.0157	0.057±24.400	0.006±0.0041	**0.0022**	0.6471	**0.0081**
	Whitemouth	100±3.2	100±3.1	100±3.2	0.478±0.093	0.384±0.084	0.312±0.086	0.005±0.0008	0.005±0.0010	0.005±0.0010	0.4456	0.2161	0.5532
	Combined analysis	100±1.5	100±1.6	100±1.4	0.698±0.055	0.472±0.044	0.463±0.044	0.006±0.0005	0.005±0.0006	0.006±0.0007	**0.0012**	**0.0008**	0.8814

[a] Model parameters (see equation [3]) were estimated using maximum likelihood approximation

[b] No statistical differences were observed between 'A' or 'k' parameters within experiment within site-year

[c] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

inflection point of the Gompertz function (Tjørve and Tjørve 2017) and thereby infers differences in the duration of the CWFP. Using these parameters, the CWFP was determined at the 5% AYL level, which is typical (Knezevic et al. 2002), and at the 2.5% AYL level, which may be more pragmatic, as it more closely approaches the visual threshold used by practitioners and also accounts for the generally low cost of glyphosate (Stewart et al. 2011).

3.4.1.1 Row spacing experiment

Seeding soybean in narrow rows reduced the duration of the CWFP compared with soybean seeded in wide rows (Table 3.3). This was observed in the combined analysis and at 3 of 6 site-years (Carman 2016 and 2017 and Whitemouth 2016). A similar trend also was observed at both St-Adolphe site-years, although these differences were not statistically significant. When all site-years were combined, the CWFP in soybean grown in narrow rows was shortened by 104 GDD at the 5% AYL level (Table 3.4; Figure 3.2A), which equated to about 1.5 soybean developmental stages. At the individual site-years, the narrow-row treatment shortened the CWFP by 85 to 94 GDD at the 2.5% AYL level and 77 to 156 GDD at the 5% AYL level compared with wide rows (Table 3.4). This range of GDDs corresponded to between one (Carman 2016) and three (Carman 2017) soybean developmental stages at both AYL levels. Studies in other regions have found similar results (Hock et al. 2006; Nice et al. 2001; Rasool et al. 2017), as wide-row spacing extends the period of light penetration into the canopy (Puricelli et al. 2003; Steckel and Sprague 2004). In this experiment, the canopy did not close completely in the wide-row treatments at any of the site-years (data not shown). An open canopy facilitates weed seedling recruitment by lengthening the seedling recruitment period (Batlla and Benech-Arnold 2014), and increases

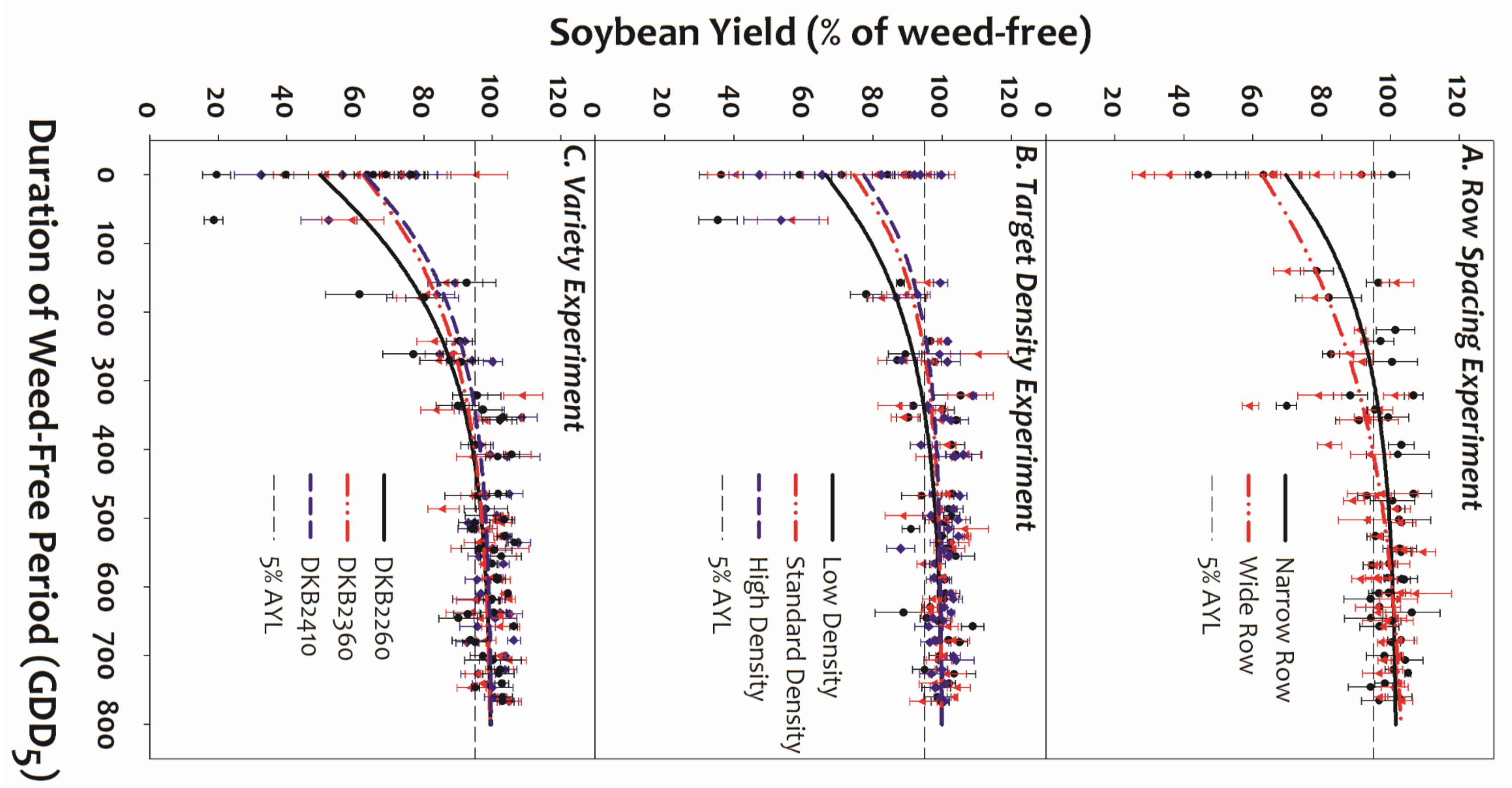

Figure 3.2 Graphical representation of the critical weed free period of soybean for three cultural weed management experiments in Manitoba. Site years were combined and equation [3] ($Soybean\ Yield_{GDD}(\%) = A \times e^{-B \times e^{-k \times GDD}}$) was fitted to treatments within experiments. Parameter estimates and contrasts can be found in Table 3.3. Standard target density (1.0 X) was 444,600 soybean plants ha^{-1}. Abbreviation: A.Y.L. = acceptable yield loss.

weed interference with the crop (Légère and Schreiber 1989). In the narrow-row soybean treatments, canopy closure occurred between the third (V3) and fourth (V4) trifoliate leaf stages (data not shown).

A combination of site-specific factors appeared to contribute to the detection of differences in the CWFP in response to soybean row spacing. At the 3 site-years at which the CWFP was affected by row spacing, peak-season weed biomass was greatest (Figure 3.3), indicating the necessity of a competitive weed community for observing significant effects on the CWFP. The 3 site-years at which the wide-row spacing increased the duration of the CWFP (Table 3.3) also had a number of similarities among their soil parameters, including the lowest soil organic matter (OM) content, the lowest spring Olsen-P levels, and among the lowest spring mineral nitrogen content, and soybean were seeded earlier and emerged earlier at these sites compared with others (Table 3.1). Low soil OM and low nutrient status are often associated with poor crop productivity; however, low soil nitrogen (Geddes and Gulden 2018) and early planting (Lenssen 2008) would be expected to shift the competitive balance toward the nitrogen-fixing soybean crop. This did not happen, and therefore other factors clearly also influenced our observations. In Manitoba, soybean do not respond to soil Olsen-P levels (Bardella 2016).

In addition, these 3 site-years and St-Adolphe 2016 had significantly lower soybean plant stand densities at V4 in the wide-row treatments (Table 3.5). However, while it appears that this may have influenced the results, using actual stand density as a covariate when modeling the CWFP did not affect the interpretation of the results. At the 3 significant site-years, soybean densities in the wide-row-spacing treatments were 62% to 75% of those observed in the narrow-row treatments. At St-Adolphe 2016, where row spacing did not affect the CWFP, soybean stand density in the wide-row treatment was only 57% that in the narrow-row treatment. Seedling or plant attrition is commonly observed in wide-row production systems (De Bruin and Pedersen 2008; Weiner and Freckleton 2010). At St-Adolphe and Whitemouth 2017, soybean stand densities did not differ between row-spacing treatments (Table 3.3). Taken together, a combination of edaphic factors, site-specific self-thinning, and site-specific weed community characteristics appeared to contribute to the efficacy of the row-spacing treatments on the CWFP. The importance of edaphic factors, including soil nutrient status (Geddes and Gulden 2018; Mohammadi and Amiri 2011) and weed biomass (Martin et al. 2001; Van Acker et al. 1993b), to the outcome of weed–crop interference is well established.

The intermediate row–spacing (37.5-cm) treatment from both the density and the variety comparison experiments with the same target density and cultivar were compared with the narrow (19-cm) and wide (76-cm) row–spacing treatments to better understand the effect of row spacing on the duration of the CWFP. In this *ex-post* analysis, intermediate soybean row spacing shortened the CWFP by up to 90 GDD_5 (5% AYL) compared with wide-row spacing (*B* parameter P = 0.0493). No differences were observed between the 19-cm and 37.5-cm soybean row spacing. These results must be interpreted with caution, however, as these experiments were not designed for direct comparison among these treatments. The large size of the individual experiments led to spatial variability in the resident weed communities among experiments at some of the site-years (Figure 3.3), which may have influenced these results.

Table 3.4 End of the critical weed free period for soybean in growing degree days and their corresponding development stages for three cultural weed management experiments. [a]

		5.0 % A.Y.L.			2.5 % A.Y.L.		
Row Spacing Experiment		---------- GDD$_5$ (SDS) ----------					
			19 cm	76 cm		19 cm	76 cm
2016	Carman		**523 (V4)**	**622 (V5)**		**585 (V5)**	**675 (R1)**
	St-Adolphe		0 (PRE)	131 (VE)		0 (PRE)	365 (V2)
	Whitemouth		**135 (V1)**	**291 (V3)**		**289 (V3)**	**383 (V5)**
2017	Carman		**234 (V1)**	**311 (V4)**		**294 (V2)**	**379 (V5)**
	St-Adolphe		284 (VC)	539 (R1)		376 (V2)	717 (R2)
	Whitemouth		434 (V3)	435 (V3)		525 (V4)	521 (V4)
Site-Years Combined			**298**	**402**		**378**	**475**
Target Density Experiment [b]		0.75X	1.0X	1.5X	0.75X	1.0X	1.5X
2016	Carman	350 (V2)	380 (V2)	452 (V3)	414 (V3)	461 (V3)	566 (V4)
	St-Adolphe	**203 (VC)**	116 (VE)	**24 (PRE)**	**300 (V1)**	212 (VC)	**60 (VE)**
	Whitemouth	**128 (VC)**	0 (PRE)	**0 (PRE)**	**236 (V2)**	0 (PRE)	**0 (PRE)**
2017	Carman	**362 (V2)**	273 (V1)	**298 (V1)**	**410 (V3)**	313 (V2)	**353 (V2)**
	St-Adolphe	14 (PRE)	11 (PRE)	3 (PRE)	26 (PRE)	19 (PRE)	10 (PRE)
	Whitemouth	443 (V2)	290 (VC)	210 (VE)	539 (V4)	418 (V1)	291 (VC)
Site-Years Combined		**346**	**261**	**228**	**455**	**366**	**330**
Variety Experiment		DKB2260	DKB2360	DKB2410	DKB2260	DKB2360	DKB2410
2016	Carman	**441 (V3)**	**381 (V2)**	**410 (V2)**	**529 (V5)**	**455 (V3)**	**492 (V4)**
	St-Adolphe	**231 (VC)**	**298 (V1)**	**164 (VE)**	**330 (V1)**	**380 (V2)**	**275 (V1)**
	Whitemouth	332 (V2)	580 (R1)	285 (V1)	467 (V4)	796 (R3)	409 (V3)
2017	Carman	**372 (V3)**	**367 (V2)**	**282 (V1)**	**405 (V3)**	**432 (V4)**	**324 (V2)**
	St-Adolphe	**176 (VE)**	**0 (PRE)**	**272 (VC)**	**251 (VC)**	**0 (PRE)**	**323 (V2)**
	Whitemouth	453 (V2)	420 (V2)	401 (V1)	597 (V5)	564 (V4)	558 (V4)
Site-Years Combined		**419**	**405**	**342**	**533**	**535**	**451**

[a] Abbreviations: A.Y.L. = acceptable yield loss, GDD = growing degree days, SDS = Soybean development stage, PRE = pre-emergence, VC = unifoliate development stage, V1 to V5 = first to fifth trifoliate development stage, R1 = beginning of flowering stage. Bold values indicate significant differences among treatments within experiment based on 'B' parameter contrast analysis found in Table 3.3.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

Table 3.5 Actual soybean plant densities for three cultural weed management experiments in Manitoba. [a]

		Soybean Density			
Row Spacing Experiment		---------- '000 plants ha^{-1} --------			Site-Year LSD
		19 cm	76 cm		
2016	Carman	448.0 b	274.4 e		
	St-Adolphe	508.9 a	290.4 de		
	Whitemouth	537.8 a	372.8 c		
2017	Carman	484.3 ab	366.8 c		
	St-Adolphe	385.2 c	334.8 cd		
	Whitemouth	349.4 c	391.1 c		
Target Density Experiment [b]		0.75X	1.0X	1.5X	
2016	Carman	352.5	438.5	611.4	c
	St-Adolphe	402.0	487.0	689.1	b
	Whitemouth	378.7	489.6	716.7	b
2017	Carman	453.2	534.9	755.1	a
	St-Adolphe	317.5	357.6	524.9	d
	Whitemouth	442.9	525.6	698.5	ab
	Main effect LSD	c	b	a	
Variety Experiment		DKB2260	DKB2360	DKB2410	
2016	Carman	430.1	426.4	425.4	bc
	St-Adolphe	454.6	460.8	447.6	b
	Whitemouth	411.9	422.8	408.3	bc
2017	Carman	501.6	564.7	567.6	a
	St-Adolphe	396.3	391.2	393.3	c
	Whitemouth	430.5	411.5	518.4	bc

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation. Letters are presented beside the means if the treatment by site-year interaction was significant.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

3.4.1.2 Target density experiment

The combined analysis revealed that the low (0.75X) soybean plant densities lengthened the CWFP compared with standard (1.0X) or increased (1.5X) soybean plant densities (Table 3.3; Figure 3.2B). No differences in the CWFP were observed between the 1.0X and 1.5X plant densities in the combined analysis or in the individual experiments (Table 3.3). Overall, the CWFP was extended by 85 and 118 GDD in low-density soybean stands compared with the standard and increased stand densities, respectively, at 5% AYL (Table 3.4; Figure 3.2B), which corresponded to roughly two soybean developmental stages. At the individual site-years, low soybean plant densities extended the CWFP from 57 to 240 GDD at the 2.5% AYL level and 64 to 179 GDD at the 5% AYL level in the lowest- compared with the highest-density treatments (Table 3.4). This corresponded to between one and two soybean developmental stages. An extended CWFP between the low and standard soybean density treatments was found only in the combined analysis, but not in any of the individual experiments. It is unclear why this occurred. In the individual density experiments, the CWFP was affected by soybean density at 3 of 6 site-years (Table 3.3). Two of these 3 site-years also showed a significant row-spacing effect on the CWFP. The importance of plant densities to crop productivity, particularly under weed interference, is well known in soybean (Arce et al. 2009; Nice et al. 2001) and other crops (Ball et al. 1997; Fradgley et al. 2017; O'Donovan et al. 1999). The dose-response work by Redlick et al. (2017a) eloquently showed the trade-off between plant densities and herbicides for effective weed management and the importance of plant stand densities for improving herbicide efficacy and reducing the necessity for herbicides for weed management in lentil (*Lens culinaris* Medik.) (Redlick et al. 2017b). Increasing soybean plant densities by 50% above the standard density did not translate into a shortening of the CWFP. This was surprising, as

Table 3.6 Mean soybean plant heights for three cultural weed management experiments in Manitoba. [a]

		Soybean Height			
		------------------- cm -----------------			
Row Spacing Experiment		19 cm	76 cm		Site-Year LSD
2016	Carman	89.9 c	83.1 d		
	St-Adolphe	102.9 a	98.0 ab		
	Whitemouth	92.0 bc	90.8 c		
2017	Carman	97.3 ab	98.6 a		
	St-Adolphe	77.2 d	77.8 d		
	Whitemouth	83.1 d	92.5 bc		
Target Density Experiment [b]		0.75X	1.0X	1.5X	
2016	Carman	90.8	91.9	91.0	b
	St-Adolphe	94.5	97.6	99.8	a
	Whitemouth	92.6	93.1	94.5	ab
2017	Carman	92.7	98.4	86.5	ab
	St-Adolphe	81.1	82.5	81.5	c
	Whitemouth	79.4	83.4	85.4	c
Variety Experiment		DKB2260	DKB2360	DKB2410	
2016	Carman	62.1 ghi	88.4 bc	76.3 def	
	St-Adolphe	98.5 ab	101.2 a	93.0 ab	
	Whitemouth	53.5 i	96.0 ab	71.7 defg	
2017	Carman	61.4 hi	81.7 cd	67.4 fgh	
	St-Adolphe	62.7 ghi	78.1 de	69.5 efgh	
	Whitemouth	54.2 i	77.0 def	62.3 ghi	

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation. Letters are presented beside the means if the treatment by site-year interaction was significant.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

increasing soybean densities several-fold above standard densities resulted in continued improvements to the competitive effect and competitive response in soybean when subjected to interference from volunteer *B. napus*, the dominant weed in soybean in western Canada (Mierau et al. 2019). The findings here suggest an upper limit to the effect of soybean density on the CWFP in soybean; it must be noted that at most site-years, the CWFP at the standard soybean plant densities was short (V1–V2) with little room for further improvement (Table 3.4). While increasing soybean target densities above the standard treatment did not result in a more competitive crop, when weeds were not controlled, seed yields in the highest soybean plant densities were 11% and 24% greater than the standard and lowest target densities (ANOVA $P = 0.0002$), respectively. Therefore, high soybean densities contributed to the competitive response in soybean without contributing to the competitive effect.

Unlike in the row-spacing experiments, the 3 site-years where the CWFP was affected by soybean density do not share similar edaphic characteristics. The soil characteristics at St-Adolphe 2016 were quite different from those of the 2 other site-years where an effect was observed (Table 3.1). In contrast to the row-spacing experiments, significant differences in the CWFP were observed at site-years with the lowest inherent midseason weed biomass (Figure 3.3), where soybean in the weed-free treatments at the R4 stage were tallest (Table 3.6) and where differences in stand densities between the greatest and lowest soybean densities at the V4 developmental stage were largest (287,000 to 340,000 plants ha^{-1} vs. 210,000 to 260,000 plants ha^{-1}) (Table 3.5). Soybean plant height under weed-free conditions was not different among plant density treatments, which in conjunction with low weed biomass suggests limited shade avoidance response (Green-Tracewicz et al. 2012). Therefore, it seems that differences in the CWFP in response to soybean density were associated with site-years at which resource limitations and weed interference were lowest (Table 3.7).

3.4.1.3 Variety experiment

The effect of soybean cultivar on the CWFP was unique to each location and consistent over the 2 study years at Carman and at Whitemouth (Table 3.3). At Carman, the duration of the CWFP was lengthened in the most rapidly maturing soybean cultivar, DKB2260, compared with the other two cultivars, DKB2360 and DKB2410. No differences in the duration of the CWFP were observed between DKB2360 and DKB2410 at Carman in both years. Similar results were observed in the combined analysis, in which the CWFP was 14 and 77 GDD longer for DKB2260, the quickest-maturing cultivar tested, compared with DKB2360 and DKB2410, respectively (Table 3.4; Figure 3.2C). This equated to about one soybean developmental stage. Shorter-season cultivars develop more rapidly than longer-season cultivars (Cober and Morrison 2011; Morrison et al. 1999; Place et al. 2011c). In these experiments, DKB2260 began anthesis up to 7 d earlier and matured up to 10 d before the other two cultivars. In addition, row closure occurred at a later developmental stage in DKB2260 (R1/R2 vs. V4 for the other two cultivars), and when different, DKB2260 plants were shorter in maximum height (Table 3.6) than DKB2360 or DKB2410 plants. These characteristics, in combination with the high weed biomass at Carman (Figure 3.3), likely contributed to these results at this location and overall. At Whitemouth, soybean cultivar had no effect on the CWFP in either year (Table 3.3). The differences in soybean plant height among the three soybean cultivars at Whitemouth were greater than at the other locations (Table 3.6), and therefore, plant height did not appear to be a key factor contributing to the duration of the CWFP. Reasons for the lack of a cultivar effect on the CWFP on soybean at Whitemouth are not clear.

Inconsistent results were observed with the CWFP of DKB2360 at the St-Adolphe location. In 2016, the CWFP in DKB2360 was up to 67 GDD longer than in the other two cultivars. The opposite was observed in 2017, when the CWFP in DKB2360 was 176 and 272 GDD shorter than in DKB2260 and DKB2410, respectively (Table 3.4). DKB2360 was taller at R4 than both other cultivars at all site-years except at St-Adolphe 2016, where it grew to the same height as the other cultivars (Table 3.6). None of the other measured morphological characteristics showed an obvious link to the observed difference in the CWFP in this cultivar at this site-year. Favorable environmental conditions at St-Adolphe in 2016 triggered a large weed seedling recruitment event 6 d before soybean emergence. Earlier weed emergence may have intensified weed interference (Korres et al. 2019; Van Acker et al. 1993b) with soybean at this site-year. It is possible that DKB2360 is more sensitive to more intense early weed interference than the other two cultivars, which consequently would have led to a longer CWFP under these circumstances in 2016. In 2017, uncharacteristically dry spring soil conditions delayed soybean and weed emergence at this site-year until June 17 and June 22, respectively.

Thousand-seed weights among the soybean cultivars were not the same, and seed lots could not be standardized for this difference. DKB2260 consistently had a smaller average seed size (157.4 g 1,000-kernel weight [TKW]) than DKB2360 (185.9 g TKW) and DKB2410 (187.4 g TKW). Large seed size has been directly associated with increased seedling vigor (Fatichin et al. 2013; Place et al. 2011b, 2011c) and the competitive response to weed interference in soybean (Jannink et al. 2000; Jordan 1993; Place et al. 2011c) and wheat (*Triticum aestivum* L.) (Stougaard and Xue 2004). Seed size may have contributed to the results of this study, although the results at some of the individual locations indicate that other factors were more influential in determining the CWFP among soybean cultivars.

3.4.2 Weed effects

Among site-years, the greatest accumulation of midseason weed biomass was generally observed at Carman (Figure 3.3), and this contributed to the greatest soybean yield loss in weedy treatments. At site-years with very low midseason weed biomass, minimal soybean yield loss was observed. Among experiments within site-years, weed biomass differed between treatments in the target density experiment only. High soybean density decreased weed shoot biomass by 44% compared with the low soybean density ($P = 0.0398$). Reductions in weed biomass are known to occur as a result of increased densities of soybean (Liebert and Ryan 2017; Place et al. 2009). The lack of differences among treatments in the variety and row-spacing experiments was likely due to differences in the range in midseason weed biomass observed among experiments within site-years. In the variety experiment, about a 4-fold difference in the midseason weed biomass was observed among treatments within site-years, while in the row-spacing and plant density experiments, this range expanded to about 7- and 10-fold, respectively. Total weed shoot biomass differed among experiments at Whitemouth 2016 and Carman 2017 (data not shown). At each of these site-years, weed biomass was greatest in the row-spacing experiment followed by the variety experiment and then the density experiment. At Whitemouth 2016, a heavy rainstorm during the spring weed seedling recruitment period influenced weed species recruitment and contributed to spatial variation among these experiments, with decreased weed seedling recruitment in the density experiment due to extended waterlogged soil conditions. Soybean performance appeared not to be affected by this event. At Carman

2017, poor soybean emergence in the row-spacing experiment resulted in the experiment being reseeded a month later. A shallow-tillage pass was used to terminate the initial experiment, which, combined with timely precipitation and hot temperatures, increased weed recruitment compared with the other established experiments.

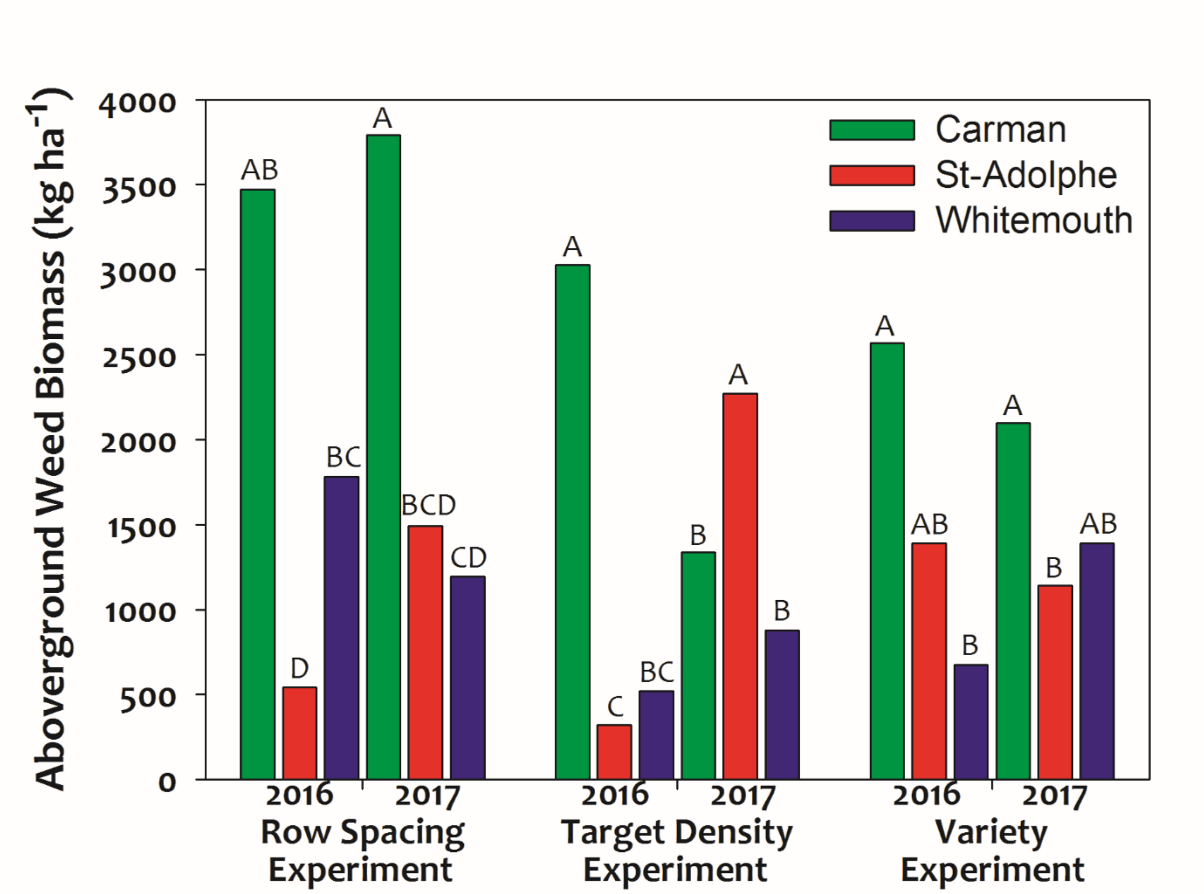

Figure 3.3 Aboveground weed shoot biomass for three soybean cultural weed management experiments in Manitoba. Within experiment, least square mean separation is indicated by different letters above columns according to Fischer's protected LSD (p<0.05)

In addition to midseason weed biomass, differences in the mid- season weed communities among these experiments may have also contributed to the observed differences. Weed species richness among site-years ranged from 7 species at Carman 2016 to 14 species at Whitemouth 2017, and dominant weed species were not the same among the site-years. Warm-season grasses such as barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], and green foxtail [*Setaria viridis* (L.) P. Beauv.] were dominant and ubiquitous at Carman and St-Adolphe in both years, but not at Whitemouth. *Avena fatua*, a highly competitive cool-season grass (Beckie et al. 2012; Rathmann and Miller 1981), was a dominant grassy weed species at St-Adolphe and Whitemouth 2016. This was not unexpected, as *S. viridis* and *A. fatua* have been the dominant grassy midseason weeds in the Canadian NGP for decades (Leeson et al. 2005). Due to a greater array of broadleaf weed species, dominant broadleaf weeds varied more among site-years than grasses. In 2016, the dominant broadleaf weeds at Carman and Whitemouth were redroot pigweed (*Amaranthus retroflexus* L.) and wild buckwheat [*Fallopia convolvulus* (L.) Á. Löve], while at St-Adolphe, the primary broadleaf weeds were pale smartweed [*Persicaria lapathifolia* (L.) Delarbre] and ladysthumb (*Persicaria maculosa* Gray). In 2017, common lambsquarters (*Chenopodium album* L.) was observed at all locations, albeit sparsely. At Whitemouth, *A. retroflexus* and shepherd's-purse [*Capsella bursa-pastoris* (L.) Medik.] were the prominent broadleaf weeds. *Amaranthus retroflexus* and other members of the genus have been found to be highly competitive with soybean (Butts et al. 2018; Légère and Schreiber 1989; Van Acker et al. 1993b), which likely influenced the results in 2016. At St-Adolphe in 2017, few broadleaf weed species were observed; nevertheless, low densities of volunteer *B. napus* were present in the row-spacing experiment. *Brassica napus* can be highly competitive with soybean (Geddes and Gulden 2018; P Gregoire, personal communication).

3.4.3 Soybean weed-free yield

Weed-free yields were different among site-years and reflect differences in the carrying capacities, which may also have contributed to the sensitivity of the CWFP in soybean. Overall, greater soybean yields were observed in 2016 than 2017 in all experiments (Table 3.7), as 2017 was an uncharacteristically dry growing season (Table 3.2). In 2016,

Table 3.7 Mean weed-free soybean yields for three cultural weed management experiments in Manitoba. [a]

		Soybean Yield			
Row Spacing Experiment		---------------- kg ha^{-1} ---------------- 19 cm	76 cm		Site-Year LSD
2016	Carman	3315 a	2673 b		
	St-Adolphe	3238 a	2273 c		
	Whitemouth	3358 a	2561 b		
2017	Carman	1847 def	1900 def		
	St-Adolphe	1715 f	1780 ef		
	Whitemouth	2063 cd	2000 cde		
Target Density Experiment [b]		0.75X	1.0X	1.5X	
2016	Carman	3204	3698	3840	a
	St-Adolphe	3324	3338	3557	b
	Whitemouth	3132	3113	3139	c
2017	Carman	2144	2210	2099	e
	St-Adolphe	1434	1502	1593	f
	Whitemouth	2205	2442	2512	d
	Main effect LSD	b	a	a	
Variety Experiment		DKB2260	DKB2360	DKB2410	
2016	Carman	3546 ab	3574 ab	3799 a	
	St-Adolphe	3180 c	3198 c	3134 c	
	Whitemouth	2393 d	3576 a	3258 bc	
2017	Carman	2099 defg	2217 de	2156 def	
	St-Adolphe	1675 h	1620 h	1794 gh	
	Whitemouth	1844 fgh	2074 efg	2188 de	

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation. Letters are presented beside the means if the treatment by site-year interaction was significant.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

narrow-row soybean produced between 19% and 29% more seed yield than wide-row soybean. Lodging occurred in the wide-row soybean treatment during seed fill at St-Adolphe in 2016 and may have influenced weed-free yields in this experiment (Cooper 1971). No differences in soybean yield were observed between the row-spacing treatments in 2017. In the target density experiment, soybean yield in the low-density treatment was 5% and 8% lower than the standard- and high-density treatments. These results agree with other studies that observed lower soybean seed yield at decreased population densities (Cox and Cherney 2011; De Bruin and Pedersen 2008). No yield differences were observed between the standard and high-density treatments, suggesting the law of constant final yield was operative in the weed-free treatments in these experiments (Weiner and Freckleton 2010). In the variety experiment, differences in soybean seed yield among cultivars were only observed at Whitemouth, the northernmost location of these studies. In 2016 at this location, DKB2360 produced 9% and 33% greater yield than DKB2410 and DKB2260, respectively, and DKB2410 produced 26% and 16% greater yield than DKB2260 in 2016 and 2017, respectively.

Determination of the CWFP using nonlinear mixed models proved highly effective at elucidating the effects of cultural practices on the competitive ability of soybean and the potential selection pressure for HR weeds. Overall, the range of soybean row-spacing treatments in these experiments appeared to be more effective at reducing the CWFP (up to three soybean developmental stages) than the range of soybean densities (up to two soybean developmental stages). The duration of the CWFP, however, was more extended (V3 to V5) in the row-spacing experiments than in any of the density treatments (maximum V2). Compared with row spacing and plant density, the effect of cultivar on the CWFP was relatively small (fewer than two soybean developmental stages). While potentially interesting, comparisons of results among experiments should be interpreted with caution. All three experiments were adjacent to each other at each site-year, yet these were large experiments and inherent spatial variation in midseason weed biomass (Figure 3.3) and community composition were observed among site-years and experiments within site-year. Implementation of these cultural practices was shown to reduce the CWFP in soybean by up to several developmental stages in the NGP. While the cultural weed management practices were evaluated separately in these experiments, they are generally more effective when used in combination (Swanton et al. 2008). Economic (i.e., equipment and seed costs), social (i.e., "my neighbor does it that way"), and technical (i.e., lack of information on weed competitiveness of specific cultivars) factors play a role in why primary producers have not more readily adopted these practices in this region or elsewhere. Combining these techniques (narrow-row spacing and standard or increased plant densities with a regionally appropriate cultivar choice) may further shorten the CWFP in soybean grown in the NGP, thereby potentially further reducing the need for herbicides and the selection pressure for HR weed biotypes.

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4.0 DURATION OF WEED-FREE PERIOD AND CULTURAL WEED MANAGEMENT TECHNIQUES INFLUENCE THE ASSEMBLAGE OF WEED COMMUNITIES IN SOYBEAN.

4.1 Abstract

Weeds occur in communities that are generally dominated by only a few species and their competition with soybean present a continuing challenge to soybean production. Glyphosate use in glyphosate-resistant soybean cultivars acts as a strong filter to weed communities. Cultural weed management techniques improve the competitiveness of soybean with weeds, however, less is known about how they filter weed communities. Experiments were designed to examine the effects of two row spacings, three target densities, and three soybean cultivars with increasing weed-free duration on the assemblage of weed communities over six site-years in southern Manitoba. Assemblage of the resident weed community was evaluated based on the accumulated mid-season aboveground biomass. Weed community data were subjected to principal component analysis to elucidate the response in the structure of the weed community to the timing of herbicide application and the cultural weed management technique. In total, eighteen weed species were observed among all site-years, and generally less than five weeds occurred at any one site-year within an experiment. Weed communities were affected primarily by the increasing weed-free duration. Weed communities followed unique trajectories that started with varying degrees of separation among the weedy treatments and converged as they resembled the weed-free treatments. Generally, weedy treatments were associated with dominant weed species. Trajectories of the weed community differed either in weedy treatments or after a

single herbicide treatment in the row spacing and target density experiments. Soybean cultivar had little effect on the structure of the weed community.

4.2 Introduction

Organisms occur and interact in communities (Grime 2006, Harper 1977). In agricultural fields, weed communities are ubiquitous and present a continuing challenge to crop production (Booth and Swanton 2002, Van Acker et al. 2000). Many filters affect the assemblage of weed communities including edaphic factors (Fried et al. 2008), the environment (Altieri and Liebman 1988, Radosevich et al. 1997), and anthropogenic management practices such as crop rotation (Andrade et al. 2017, Weisberger et al. 2019), fertilizer use (Hume 1982, Mohammaddoust Chamanabad et al. 2009), herbicide use (Harker et al. 2005, Johnson et al. 2009, Storkey et al. 2012), or tillage system (Derksen et al. 1993, Nichols et al. 2015). For example, Leeson et al. (2000) examined the effects of various agronomic management practices on weed communities on Saskatchewan farms and concluded that herbicide usage and tillage intensity had the greatest effects on weed community assembly followed by crop rotation. These results are supported by the work of Doucet et al. (1999) which found that in-season weed management accounted for 38% of the variability in weed density, whereas crop rotation only accounted for 5.5% of the total variability. Implementation of in-crop weed management efforts, combined with crop rotations that use more than two crops (Fausti et al. 2014, Weisberger et al. 2019, Young et al. 2013), appear to be primary determinants of the structure of weed communities (Gulden et al. 2011, Thomas et al. 2010).

Glyphosate-resistant (GR) crops have been adopted widely in Canadian cropping systems and represent a unique filter of the weed community. The inclusion of GR crops in rotation with non-GR crops have a large effect on weed communities (Owen 2008, Schütte et al. 2017). Harker et al. (2005) studied changes in the weed community of crop rotations with different use intensities of GR canola (*Brassica napus* L.) and GR wheat (*Triticum aestivum* L.) at six locations in western Canada. After four years, canonical discriminant analysis revealed that common lambsquarters (*Chenopodium album* L.), field pennycress (*Thlaspi arvense* L.), and volunteer GR wheat were associated with crop rotations that included at least one GR crop, whereas wild oat (*Avena fatua* L.), green foxtail (*Setaria viridis* L.), redroot pigweed (*Amaranthus retroflexus* L.), *Sonchus* spp., and wild buckwheat [*Fallopia convolvulus* (L.) Löve] were more closely associated with conventional herbicide applications. On the High Plains of the USA, Westra et al. (2008) observed large increases in populations of *C. album* and *F. convolvulus* in GR cropping systems compared with conventional herbicide systems. These results were echoed in southern Ontario by Gulden et al. (2010) where strong differences in the structure of weed communities (specific weed species varied among the five locations) between GR and conventional herbicide cropping systems were observed using redundancy analysis. These previous studies have provided good evidence of the effects of crop rotations and herbicide use on the structure of the weed community.

Cultural weed management techniques, such as row spacing and stand density, improve a crop's competitiveness with weeds (Chapter 3, Liebman and Gallandt 1997, Weiner et al. 2010, Zimdahl 2004) and as such, apply filters to the weed community (Leeson et al. 2000, Menalled et al. 2001, Rotchés-Ribalta et al. 2017, Ryan et al. 2010). In soybean, narrow row spacing is known to reduce the accumulation of weed shoot biomass from *A. retroflexus* (Légère and Schreiber 1989), smooth pigweed (*Amaranthus hybridus* L.), *Digitaria* spp., giant foxtail (*Setaria faberii* Herrm.), prickly sida (*Sida spinosa* L.) (Wax and Pendleton 1968), common ragweed (*Ambrosia artemisiifolia* L.), yellow rocket (*Barbarea vulgaris* Brown), *C. album*, wild mustard (*Sinapis arvensis* L.), *Setaria* spp. (Harder et al. 2007), and sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby] (Nice et al. 2001), and weed seedling recruitment of pitted morning glory (*Ipomoea lacunosa* L.) and common cocklebur (*Xanthium strumarium* L.) (Norsworthy 2004) compared to wide row soybean. Similar results were observed in chickpea (*Cicer arietinum* L.) (Whish et al. 2002), corn (*Zea mays* L.) (Bradley 2006), cotton (*Gossypium* spp.) (Wilson Jr. et al. 2007), oat (*Avena sativa* L.) (Li et al. 2018), sorghum [*Sorghum bicolor* (L.) Mönch] (Wiese et al. 1964), and winter wheat (Teich et al. 1993). Increased crop seeding rates and population densities are known to reduce aboveground biomass of volunteer *B. napus* (Mierau et al. 2019, Weiner et al. 2001), *A. retroflexus*, *C. album*, large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Li et al. 2018), *A. artemisiifolia*, black medic (*Medicago lupulina* L.), fall panicum (*Panicum dichotomiflorum* Michx.), barnyardgrass [*Echinogloa crus-galli* (L.) Beauv.] (Liebert and Ryan 2017), and *X. strumarium* (Jordan 1992, McWhorter and Barrentine 1975). Competitive crop cultivars with weed suppressive traits, such as a larger leaf area index, greater relative growth rate, rapid canopy formation (Beckie et al. 2008a, 2008b, Hammer et al. 2018, Horneburg et al. 2017, Jordan 1993, Nordby et al. 2007), and increased seed size (Place et al. 2011a), reduce the population density and shoot biomass of many weed species (Fradgley et al. 2017, Jordan 1992, Monks and Oliver 1988). The reduction in weed shoot biomass and weed density from the effects of row spacing, crop density, and cultivar imply that these cultural weed management techniques influence the composition of the weed community. To my knowledge, no previous studies have documented the effects of these cultural weed management techniques on the structure of the weed community. The objectives of the following experiments were to explore the effects of (1) row spacing, (2) target density, and (3) soybean cultivar, alone and with specific weed-free duration periods based on soybean developmental stages, on the composition and structure of the residual weed communities.

4.3 Materials and Methods

4.3.1 Experimental description

The effects of three cultural weed management practices on soybean's critical weed free period (refer to chapter 3) and the associated field weed community were evaluated at six site-years in southern Manitoba. An in-depth description of the experiment locations, design, and management, is provided in chapter 3. In brief, in 2016 and 2017, field experiments were established at research farms near Carman and St-Adolphe, and two fields in the rural municipality of Whitemouth. At Carman, experiments were conducted on a fine loamy clay, while at St-Adolphe the soil was a heavy clay. Experiments at Whitemouth in 2016 were conducted on a moderately fine loam sand and in 2017, on a clay soil. Soil characteristics and previous crop at each site-year can be found in Chapter 3, Table 3.1. Prior to the establishment of the experiments, fields were managed with fall or spring conventional tillage practices. To meet the fertility requirements for soybean in each year, 40 kg ha^{-1} of actual phosphate was applied at Carman and St-Adolphe prior to seeding. At Whitemouth, an unknown rate of liquid dairy manure was applied within the previous three years. Peat-based granular inoculant was applied in the soybean seed row to facilitate nitrogen fixation.

Three different experiments were established at each location in 2016 and 2017 to examine the effects of (1) row spacing, (2) target density, and (3) cultivar on the CWFP for soybean. Each experiment was laid out as a split-plot randomized complete block design with two (row spacing experiment) or three (target density and variety experiments) main plots and nine sub-plots (duration of weed free period) within each main plot. Each sub-plot covered a 2.5-meter by 6-meter area. In the row spacing experiment, main plot treatments included narrow (19-cm) and wide (76-cm) soybean row spacing. In the target density experiment, soybean densities of 333,500, 444,600, and 666,900 plants ha^{-1} (0.75X, 1.0X, and 1.5X of locally recommended densities) were evaluated. For the variety experiment, three commercial soybean cultivars were studied: DeKalb® 22-60 (DKB2260), Dekalb® 23-10 (DKB2360), and Dekalb® 24-10 (DKB2410). Apart from treatments specific to each experiment, DKB2360 soybean was grown on 37.5-cm rows at a density of 444,600 plants ha^{-1}. To facilitate weed management in these experiments, soybean cultivars used for these experiments were glyphosate resistant. Sub-plots were kept weed-free until specific soybean development stages, and included season-long weedy and weed-free controls. Weed removal was achieved by applying commercial grade glyphosate (358 g a.e. ha^{-1}; Monsanto Canada, Winnipeg, MB, R3T 6E3, Canada) in mixture with bentazon (889 g a.i. ha^{-1}; BASF Canada, Mississauga, ON, L5R 4H1, Canada). The herbicides were applied with a bicycle wheel push-type sprayer equipped with a 2-meter boom set at 50-cm above the crop canopy. Four Airmix 110-01 (Greenleaf Technologies, Covington, LA, 70433) were spaced 50 cm apart along the boom applying 100 L water volume ha^{-1} @ 276 kPa. Once weed removal was completed, the resident 'natural' weed community was allowed to recruit and interfere with soybean for the remainder of the growing season. At each site-year within experiment, the composition of the weed community was determined through the collection of mid-season shoot biomass from individual weed species once soybean reached the R5 stage.

Within each sub-plot, weed shoot biomass was harvested from two 0.09 m^2 quadrats, which included at least one soybean row per quadrat, and oven-dried at 65°C until equilibrium was reached.

Table 4.1 List of predominant weed species observed at six site-years in southern Manitoba.

Taxonomic Group	Code[a]	Binomial name	Common name
Broadleaf	1LTHG	*Lathyrus spp.*	Vetchling
	1PRAG	*Persicaria spp.*	Smartweed
	1TRFG	*Trifolium spp.*	Clover, red or white
	AMARE	*Amaranthus retroflexus*	Red Root Pigweed
	ARTBI	*Artemisia biennis*	Biennial wormwood
	CAPBP	*Capsella bursa-pastoris*	Shepard's purse
	CHEAL	*Chenopodium album*	Common Lambsquarter
	MALPU	*Malva pusilla*	Round leaf mallow
	MEDSA	*Medicago sativa*	Volunteer alfalfa
	POLCO	*Fallopia convolvulus*	Wild buckwheat
	RUMPL	*Rumex palustris*	Marsh dock
	TAROF	*Taraxacum officinale*	Dandelion
	VERPG	*Veronica peregrina*	Purslane speedwell
	SINAR	*Sinapis arvensis*	Wild mustard
Grass	AVEFA	*Avena fatua*	Wild oat
	ECHCG	*Echinochloa crus-galli*	Barnyardgrass
	SETPU	*Setaria pumila*	Yellow foxtail
	SETVI	*Setaria viridis*	Green foxtail

[a] European and Mediterranean Plant Protection Organization (EPPO) codes, formerly known as BAYER codes. Available online: EPPO (2019) EPPO Global Database. https://gd.eppo.int

4.3.2 Statistical analysis

Treatment by weed species (shoot biomass) contingency matrices for each experiment at each site-year were evaluated for non-occurrences (i.e. “0” data points) prior to community analysis (Digby and Kempton 1987, McCune and Grace 2002). Weed-free sub-plots were excluded from the data matrices. Other sub-plot treatments were inspected visually and evaluated for resemblance to the weed-free controls. Sub-plot treatments were removed from further analysis if weeds occurred in less than half the blocks for that treatment. Rare species are known to strongly influence principal component analysis (Digby and Kempton 1987, Kenkel et al. 2002). Weed species that occurred in 10% or less of the remaining sub-plots were considered rare species. Weed species considered rare were removed from the matrices unless they displayed an obvious association with a specific weed-free duration or experimental treatment. To be considered associated with a specific treatment, a rare species had to be observed in more than 1 experimental unit.

Statistical analysis was performed using SAS Studio 14.3 (SAS Institute Inc., Cary, NC). Within experiment and site-year, weed shoot biomass for each species was subjected to a mixed model (PROC MIXED) approach to evaluate the conformation of the residuals to the Gaussian 'normal' distribution using the Shapiro-Wilk statistic (Littell et al. 2006) and visual inspection of residual plots (Kozak and Piepho 2018). The fixed effects used in these, and other mixed models described below were the duration of the weed-free period and the main experimental treatment (i.e., soybean row spacing, density or cultivar). Random effects used in the mixed models were the experimental block nested within site-year and the error term with which to test main plot effects (main plot treatment by block nested within site-year). Weed species were separated by taxonomic group (Table 4.1) for community analysis. Taxonomic groups were considered to dominate a weed community if their biomass was greater than 5% from parity. This level from parity was chosen based on statistical tests using a 5% significance level.

Diversity measurements were conducted on the resident weed communities observed only in the weedy (untreated) treatments to determine the differences among treatments and site-years within experiments. Formulas used to calculate diversity measurements are found in Table 4.2, where p_i is the proportion of dry shoot biomass belonging to the i^{th} weed species. Magurran (2004) explains in detail the diversity measures used in this study. In brief, species richness (*S*) is the most basic measure of diversity and indicates the total number of weed species observed in the sampling area. Shannon's evenness (*J'*) represents the degree to which shoot biomass is distributed among species, with low values indicating that shoot biomass is distributed among very few species, and high values approaching 1.0 indicate that shoot biomass is relatively equal among weed species. The Shannon-Weiner (*H'*) diversity index was chosen because it puts equal emphasis on dominant and rare species, whereas the Gini-Simpson (D_{GS}) diversity index was chosen as it weighs dominant species more prominently. Both indices were chosen for this study in order to provide a more complete understanding of the weed community (Morris et al. 2014). Diversity indices were converted to effective number of species as described by Jost (2006). In brief, the exponential of *H'* and the inverse of D_{GS} are calculated such that the original indices are converted into their respective effective number of species. The effective number of species is a "true" measure of diversity that is comparable to species richness, unlike the diversity indices from which they were determined. For a detailed definition and explanation of 'effective number of species', see Jost (2006). The conversion of diversity indices to effective number of species allows for the degree of dominance intensity of a community to be analyzed (Jost 2007, 2009). The intensity of dominance is the decrease from species richness to the effective number of species (Jost 2009).

Diversity measures were analyzed individually using a mixed model approach with the same fixed and random effects described above. Denominator degrees of freedom were approximated using the Kenward-Roger method (Kenward and Roger 1997). Conformation of the residuals to the Gaussian 'normal' distribution was tested using the Shapiro-Wilk statistic (Littell et al. 2006). Homogeneity of variance was first tested by visual inspection of residual vs predicted values (Kozak and Piepho 2018). Lund's test (Lund 1975) was used to determine whether extreme outliers influenced the means and required further examination. Heteroscedasticity was corrected using the repeated statement to minimize the Aikaike information criterion (Littell et al. 2006). Fisher's protected LSD was used for least-square means differentiation at the 5% significance level (α=0.05) using the PDMIX800 macro (Saxton 1998).

Table 4.2 Formulas used to calculate the diversity measures analyzed.

Metric	Traditional Formula [a]	Effective No. of Species
Species richness (S)	$\sum P_i^0$	S
Shannon's evenness (J')	$H'/\ln(S)$	---
Shannon-Weiner Diversity (H')	$-\sum P_i ln(P_i)$	$\exp(H')$
Gini-Simpson Diversity (D_{GS})	$1-\sum P_i^2$	$1/D_{GS}$

[a] P_i is the proportion of dry shoot biomass belonging to the i^{th} species. Formulas adapted from Jost, 2006 and Magurran, 2004.

The weed community data were subjected to principal component analysis (PROC FACTOR) within each experiment at each site-year. To reduce the skewness of the residuals caused by the effect of zeros in the data (i.e. non-occurences), a nominal number (0.0001) was added to each observation and biomass data were then natural-log ($\log_e$) transformed (Digby and Kempton 1987, Kenkel et al. 2002, McGarigal et al. 2000). Significant components were retained using a bootstrapped version (n=1000) of Horn's parallel analysis (Glorfeld 1995, Peres-Neto et al. 2005). This bootstrapped version has been shown to provide one of the best objective methods to estimate the correct number of significant components (Franklin et al. 2006). The orthogonal rotation Varimax was applied *post-hoc* to the principal components to aid interpretation (Mulaik 2009). Due to the low number of weed species present at most site-years however, only the first two components were retained unless the parallel analysis returned more than two significant components. Principal component analysis was chosen for this analysis as it is among the simplest ordination techniques available that results in an optimized representation of the original data (Kenkel 2006). To determine differences among the weed communities, retained principal components were subjected to a mixed model approach (PROC MIXED) with the same fixed and random effects described above. Denominator degrees of freedom used for the mixed model analysis of the principal components were approximated using the containment method. Least square means of principal component scores from fixed effects and their interaction were separated using Fisher's Protected LSD at the 5% significance level. Biplots were constructed using the least square means of the interaction between fixed effects as the centroids and the standardized scoring coefficients of weed species as vectors to visualize the results.

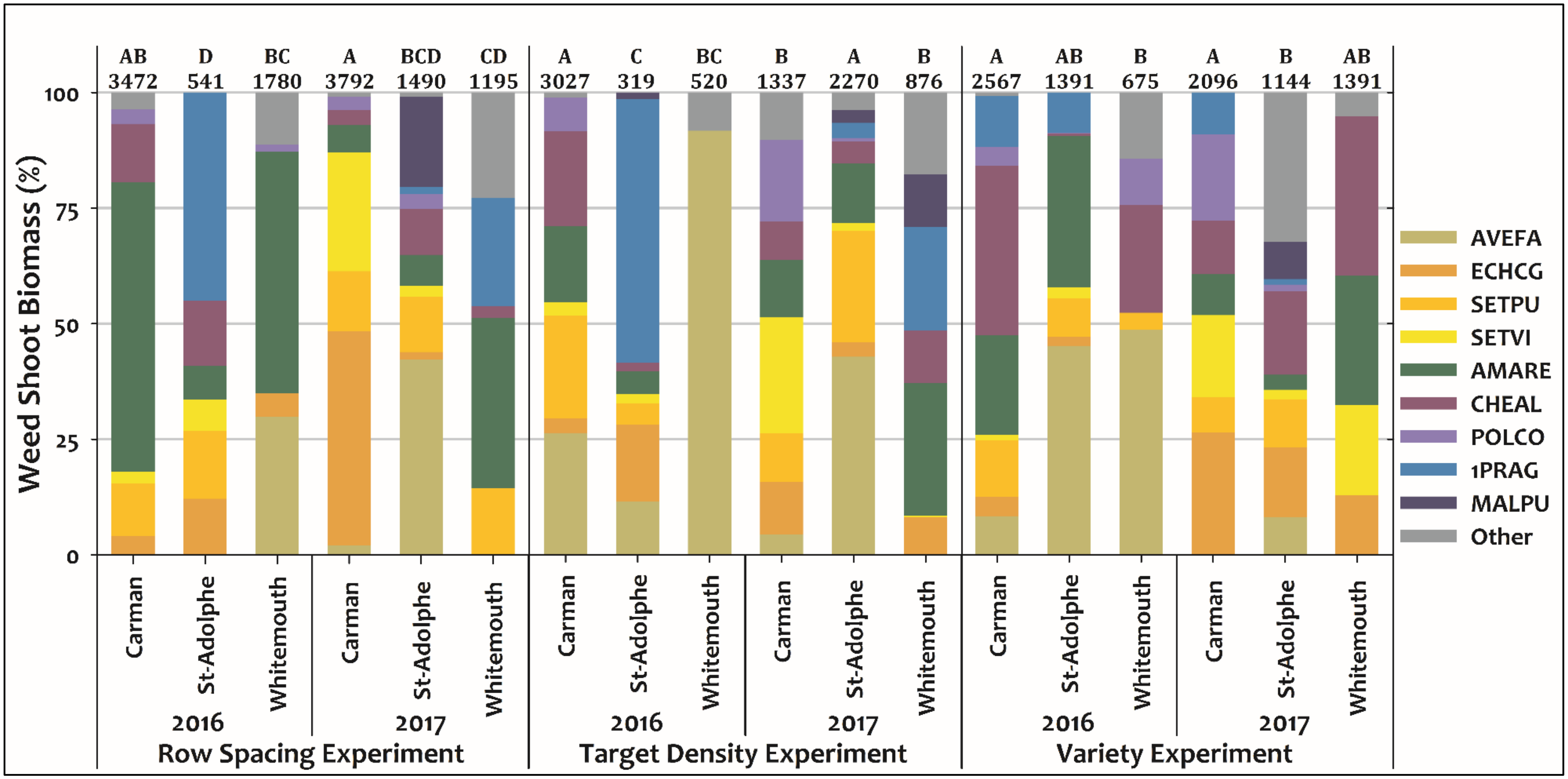

Figure 4.1 Relative aboveground biomass of primary weed species observed in weedy controls of three experiments at six site-years in southern Manitoba. Weed species codes are found in Table 4.1. Bold numbers above bars represent the LS-mean total aboveground biomass observed at each site-year. Within experiment, different bold letters above numbers separate total aboveground weed biomass means based on Fischer's protected LSD at a $p<0.05$.

4.4 Results and Discussion

4.4.1 Composition and Diversity of the Resident Weed Community

Overall, eleven broadleaved and four grassy weed species were identified to the species level and three broadleaved weeds were identified to the genus level from the weedy treatment plots within site-years and experiments (Table 4.1). Of the eighteen weed species that were identified, half of them occurred at most site-years while the others occurred more sporadically among experiments and site-years (Figure 4.1). Among experiments, the aboveground biomass of broadleaved weed species dominated the resident weed communities at nine site-years and grasses dominated at five site-years, whereas no clear dominance among broadleaves and grasses was observed at the remaining four site-years. In 2016, *A. retroflexus* and *Persicaria* spp. made up most of the broadleaf weed shoot biomass with the exception of the variety experiment where *C. album* was present in greater quantity than *Persicaria* spp. In 2017, broadleaved weed shoot biomass generally was distributed more evenly among *A. retroflexus*, *C. album*, *Persicaria* spp., and *Malva pusilla* L. The presence of *M. pusilla* as a dominant species in 2017 compared with 2016 likely can be attributed to the dry spring conditions (Blackshaw 1990, Morrison and Makowski 1989) which may have reduced the ability of other weed species to accumulate biomass effectively.

Dominance from grassy weed species was attributed largely to the presence of *A. fatua*, a cool-season C_3 species with early seedling emergence and a rapid relative growth rate (Beckie et al. 2012). These characteristics provide a competitive advantage to *A. fatua* in the Northern Great Plains (Carlson and Hill 1985, O'Donovan et al. 1985) and likely explain why it tends to dominate grassy weed communities at moderate and greater population densities. Otherwise, grassy weed communities were characterized at most site-years by the presence of the species complex consisting of *E. crus-galli*, yellow foxtail (*Setaria pumila* L.), and *S. viridis*. These warm-season C_4 grasses are not known to accumulate large amounts of shoot biomass rapidly when growing in short photoperiod days or under shaded conditions (Douglas et al. 1985, Maun and Barett 1986, Steel et al. 1983) and likely explain why this complex of species rarely dominated the overall resident weed communities. It is unclear why so few grassy weed species were present in all experiments at Whitemouth in 2017 compared to other site-years. No other grassy weed species were observed among site-years and experiments. Since the composition of the weed communities was based on weed shoot biomass, these results were not unexpected. Many broadleaved weed species tend to accumulate shoot biomass more quickly than C_4 grassy weed species over a similar period of time (Cowan et al. 1998, Pollnac et al. 2009, Toler et al. 1996) however, *A. fatua* has similar shoot biomass accumulation than many of the broadleaved weed species that were dominant in these experiments (Storkey 2004). It is unclear if these differences in rates of shoot biomass accumulation from southern Onatrio and the U.S.A. also apply to the shorter growing season of Western Canada.

Species richness of resident weed communities within experiments differed among site-years and generally had less than six weed species in the weedy treatment (Table 4.3). Whitemouth 2016 had the lowest weed species richness whereas, the richest site-year was St-Adolphe 2017. At Carman, weed species richness was similar within experiments between years. Different resident weed populations among experimental locations may be on reason for these differences. Weed species richness also was greater in 2017 than 2016, likely due to differences in environmental conditions between years. Hot and dry

Table 4.3 Mean weed community diversity metrics of three soybean competition experiments in southern Manitoba.

		Species Richness (S)[a]	Species Evenness (J')	Shannon-Weiner (H')	Effective no. species $exp(H')$	Gini-Simpson (D_{GS})	Effective no. species (D_{GS}^{-1})
Row Spacing Experiment [b]							
2016	Carman	4.00 ab	0.57	0.73 ab	2.21 ab	0.39 ab	1.81
	St-Adolphe	2.38 bc	0.57	0.62 ab	1.97 ab	0.63 a	1.54
	Whitemouth	2.13 c	0.35	0.34 b	1.48 b	0.20 b	1.37
2017	Carman	4.75 a	0.67	1.02 a	2.96 a	0.55 a	2.58
	St-Adolphe	5.50 a	0.66	1.12 a	3.53 a	0.55 a	2.92
	Whitemouth	4.38 a	0.56	0.83 ab	2.55 ab	0.43 ab	2.15
Target Density Experiment							
2016	Carman	3.92 b	0.75 a	1.02 a	2.87 a	0.57	2.51 a
	St-Adolphe	2.25 c	0.46 b	0.54 b	1.85 b	0.65	1.32 b
	Whitemouth	0.83 d	0.08 c	0.10 c	1.18 b	0.56	0.63 c
2017	Carman	3.58 b	0.76 a	1.00 a	2.92 a	0.56	2.65 a
	St-Adolphe	6.33 a	0.56 b	1.02 a	2.92 a	0.53	2.30 a
	Whitemouth	4.58 b	0.64 ab	0.98 a	2.84 a	0.53	2.44 a
Variety Experiment							
2016	Carman	4.92 b	0.66	1.03 ab	2.94 b	0.55 ab	2.46 ab
	St-Adolphe	2.75 cd	0.53	0.57 c	1.89 c	0.50 ab	1.53 c
	Whitemouth	2.50 d	0.49	0.57 c	1.96 c	0.58 ab	1.50 c
2017	Carman	3.50 bcd	0.68	0.88 bc	2.54 bc	0.49 b	2.21 bc
	St-Adolphe	6.42 a	0.72	1.32 a	3.83 a	0.65 a	3.11 a
	Whitemouth	4.17 bc	0.65	0.87 bc	2.46 bc	0.49 b	2.10 bc

[a] Formulas used to determine these six metrics are found in Table 4.2. Diversity measures were based on weed shoot biomass.

[b] Fisher's protected LSD ($p<0.05$) was used for letter means separation. When similar letters are shared between means within columns within experiment, these means are not significantly different from each other.

conditions characterized the second year of this study (Chapter 3, Figure 3.1 & Table 3.2), possibly preventing the dominant weeds observed in 2016 from dominance in 2017. *Amaranthus retroflexus* for example, was a dominant weed species among experiments and locations in 2016, yet in 2017 was dominant at Whitemouth only. July is a month of rapid relative growth for *A. retroflexus*, a weed that prefers hot temperatures with adequate moisture (Costea et al. 2003, Weaver 2003), and above average precipitation was recorded in the month of July at Whitemouth 2017 compared to other locations (Chapter 3, Figure 3.1). Differences in weed species richness among site-years however, should be interpreted with caution as the full composition of the weed seed bank at each site-year was unknown.

Weed species richness was greater in 2017 than in 2016 by 1.8 times at St-Adolphe and 4.5 times at Whitemouth (Table 4.3). Field areas where experiments were conducted at St-Adolphe and Whitemouth in 2017 were not managed (i.e. no crop was grown and no weed management was conducted) in 2016 (visual observations; Brian Hellegards, personal communication; Albert Hinrichs, personal communication). This allowed the resident weed populations to replenish the soil seed bank through increased seed rain (Baskin and Baskin 2014, Benvenuti 2007) and likely contributed to increased weed seedling recruitment in 2017 at these locations.

Species richness however, does not convey information about the dominance of a community (Magurran 2004). The degree in the difference between species richness and the effective number of species however, does describe the intensity of the dominance in a community (Jost 2006, 2007, 2009). When species were considered equal (Shannon-Wiener index), the intensity of dominance was generally low and ranged between 17% and 54% among experiments and site-years. When weighted towards dominant species (Gini-

Simpson index) however, the range in the intensity of dominance among site-years was greater in the target density and variety experiments than in the row spacing experiment. Among site-years, the intensity of dominance was less than 54% in the row spacing experiment, whereas in the variety experiment, dominance intensity ranged between 58% and 106% and in the target density experiment, this range was up to 175% . Among site-years within experiments, greater intensity of dominance meant the weed community was dominated by fewer and fewer weed species. This was more evident when determining the intensity of dominance using the effective number of species based on the Gini-Simpson index than the Shannon-Weiner index as dominant species carry more weight in the former (Magurran 2004). Nevertheless, the intensity of dominance within experiments and site-years indicate that weed communities were strongly dominated by few weed species and corroborate the works of Clements et al. (1994), Dekker (1997), Leeson et al. (2005), Menalled et al. (2001), and Thomas et al. (2010). Measurements of weed community diversity however, were not affected by soybean row spacing, population density, or cultivar treatments.

4.4.2 Weed Community Response

4.4.2.1 Duration of the weed-free period

Principal component analysis revealed that significant differences in the structure of the weed community within experiments were driven almost exclusively by the duration of the soybean weed-free period (Table 4.4; Figures 4.2-4.4). In all experiments, increasing the weed-free duration (i.e. increasing amount of herbicide applications) described a similar trajectory in the structural changes to the weed community over time. The observed trajectories began in the weedy treatments which were associated with the dominant weed

Table 4.4 Summary of p-values from ANOVA test of retained principal components.

		Weed-free duration [a]		Cultural Practice		Duration X Practice	
		PC 1	PC 2	PC 1	PC 2	PC 1	PC 2
Row Spacing Experiment [b]							
2016	Carman	**0.0010**	**0.0058**	0.7367	0.3074	0.3122	0.8158
	St-Adolphe	0.1138	0.0697	0.0731	0.8210	0.7991	0.1391
	Whitemouth	**0.0285**	0.4455	0.2504	0.5933	0.3847	0.4734
2017	Carman	**<.0001**	**<.0001**	0.6496	0.3771	0.0656	0.5174
	St-Adolphe	**0.0303**	**0.0042**	0.2694	0.6266	0.1437	0.1357
	Whitemouth	**0.0178**	**0.0307**	0.4743	0.6156	0.8446	0.3981
Target Density Experiment							
2016	Carman	**<.0001**	**<.0001**	0.2964	0.6370	**0.0274**	**0.0427**
	St-Adolphe	**0.0534**	**0.0224**	0.6157	0.2946	0.5026	**0.0249**
	Whitemouth	0.2806	0.5182	0.3021	0.6022	0.8678	0.4426
2017	Carman	**0.0376**	**<.0001**	0.3342	0.1086	0.3990	0.8707
	St-Adolphe	**<.0001**	**0.0146**	0.2530	0.4574	0.5147	0.4971
	Whitemouth	**0.0002**	0.1050	0.8628	0.0818	0.3772	0.2632
Variety Experiment							
2016	Carman	**<.0001**	0.7320	0.6965	0.5366	0.4370	0.7443
	St-Adolphe	**0.0099**	0.1341	0.8365	0.1784	0.9541	0.1060
	Whitemouth	0.1015	0.4114	0.7340	0.9711	0.6613	0.6725
2017	Carman	0.5130	0.2805	0.6123	0.9224	0.4978	0.8653
	St-Adolphe	**0.0003**	**0.0389**	0.5484	0.2747	0.5948	0.9512
	Whitemouth	**0.0009**	**0.0038**	0.7571	0.6208	0.7659	0.5008

[a] Weed-free duration refers to fixed effect of timing of last weed removal. Cultural practice refers to the weed management practice evaluated in the experiment (i.e. row spacing, target density, variety). Abbreviation: PC = principal component.

[b] Bold values highlight the factors that had a significant ANOVA test at an $\alpha=0.05$.

species in the resident weed communities. Although the trajectories described different patterns among the experiments and site years as soybean developed, the structure of the weed community in the longest weed-free period treatments was always negatively associated with the dominant weed species. The duration of the weed-free period was maintained by consecutive low-dose glyphosate applications. Repeated low-dose herbicide applications (i.e. below label recommendations) lead to improved mid-season weed control than a single herbicide application due to fewer late-emerging weeds (Harker 1995, Mathiassen et al. 2007) and was the principal cause for the observed changes of the trajectories in the structure of the weed community in these experiments.

Distances between successive weed-free periods along the trajectories were not equal (Figures 4.2–4.4). These differences in distance indicate that the soybean developmental stage at which significant changes occurred in the structure of the weed community in response to herbicide application were not the same among the site-years within experiments. To some degree, this was expected due to inherent differences in the resident weed communities among the experiments and site-years (Table 4.3, Figure 4.1). In general however, the largest impact was observed between the absence (weedy control) and the initial herbicide treatments (shortest weed-free period). The importance of herbicides in shaping weed community assembly is well recognized (Derksen et al. 1995, Hyvönen and Salonen 2002, Leeson et al. 2000, Menalled et al. 2001, Owen 2008, Rotchés-Ribalta et al. 2017, Ryan et al. 2010, Storkey et al. 2012). The large difference between the weedy control and the initial herbicide treatments suggests that at the time of initial herbicide treatment, the majority of weed seedling recruitment for the season may have occurred already. The structure of the weed community however, was based on weed biomass rather than weed densities and therefore, this effect may have been more a reflection of the importance of early season weed biomass accumulation (Blackshaw 1993b, Dieleman et al. 1995, O'Donovan et al. 1985, Weaver 2003).

The structure of the weed community became more similar between the cultural weed management treatments in each experiment as the weed-free period increased. As a result, the trajectories in the structure of the weed community among treatments converged as the weed-free period lengthened and initial differences in the structure of the weed community in untreated or short duration weed-free treatments disappeared (Figures 4.2–4.4). Increased weed-free duration results in progressively reduced weed seedling recruitment and weed shoot biomass (Burnside 1979, Chhokar and Balyan 1999, Halford et al. 2001, Van Acker et al. 1993). Any differences in the structure of the weed community that may have been present in the weedy control treatments (i.e. where the weed community and treatment had the longest time to interact) disappeared as the season progressed and were no longer significant.

4.4.2.2 Cultural Weed Management Techniques

Significant differences among target density treatments in the weedy controls or among the shortest weed-free durations (i.e. single herbicide application) were observed at some site-years (Table 4.4; Figure 4.2). No differences were observed among the structures of the weed communities among row spacing or soybean cultivar experiments (Table 4.4). In the target density experiment, the weed community in the weedy control treatment in the low and standard soybean target density treatments at Carman 2016 were associated with *A. retroflexus* and *S. pumila* while the weed community in the high soybean density was associated with *A. retroflexus* and *F. convolvulus* (Figure 4.2). At St-Adolphe 2016, *A. fatua*

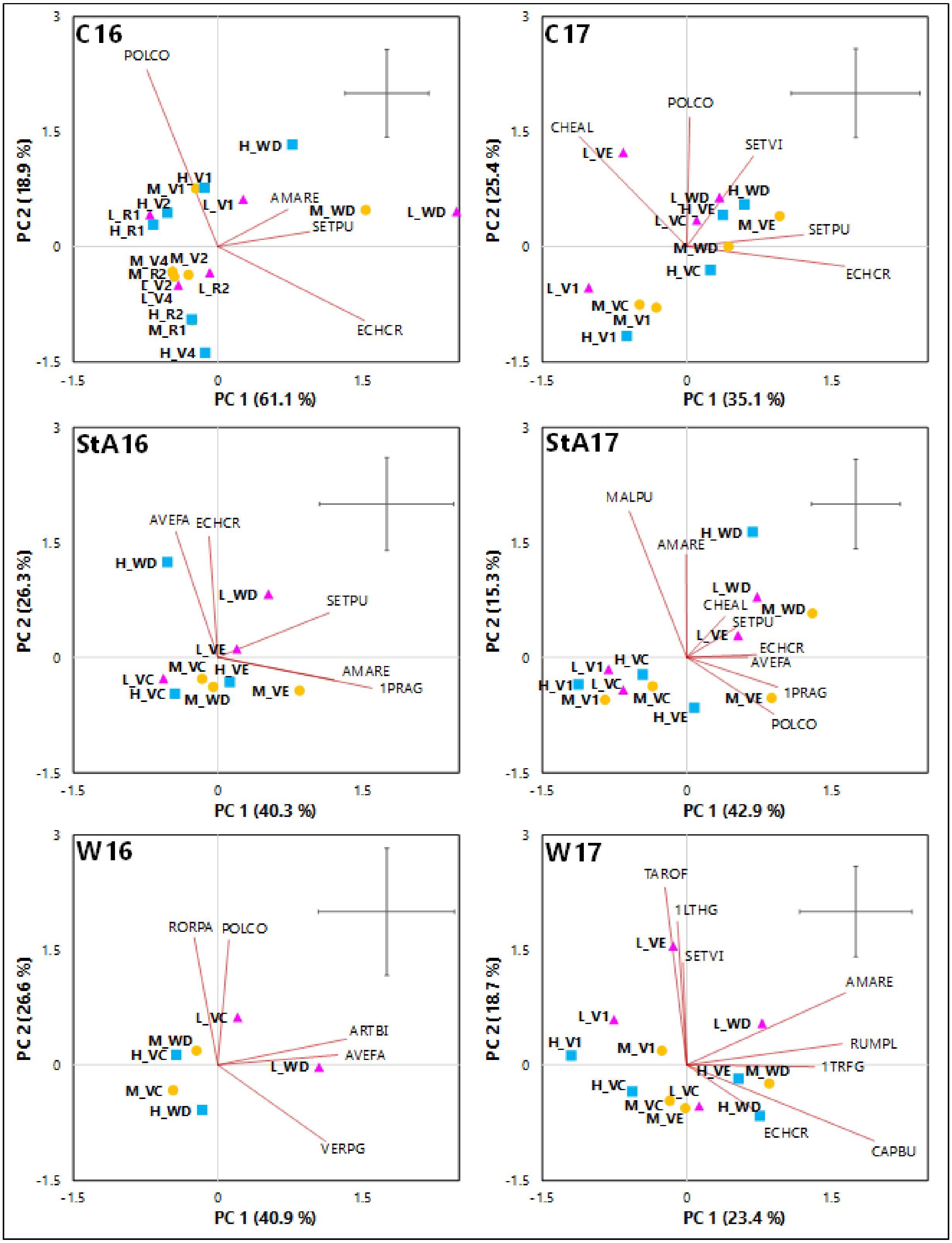

Figure 4.2 Principal component analysis of soybean target density effects on weed communities. Principal component 1 (PC1) and 2 (PC2) are represented at individual site-

years (**C**=Carman, **StA**=St-Adolphe, **W**=Whitemouth for 2016 and 2017). Centroids are the least square mean of treatments which represent 0.75X (**L_**; ▲), 1.0X (**M_**;●), or 1.5X (**H_**; ■) of a standard (444,000 plants ha^{-1}) target density kept weed-free until a specific soybean development stage (**WD**=no weed control; **VE**, **VC**, **V1**-**V4**, and **R1**, see Fehr et al. (1971)). Vectors represent original axes of weed species (codes can be found in Table 4.1) included in the analysis. Error bars in top right hand corner of biplots represent one LSD for each respective principal component.

was the only weed species associated with the weed community in the weedy control in the high density treatment, while *E. crus-galli* and *S. pumila*, and *A. retroflexus* and *Persicaria* spp. was associated with the weed communities in the low and standard density treatments, respectively. These results are similar to those observed in the row spacing experiment, where C$_4$ grasses were more closely associated with the wide row spacing, and may be explained by the earlier closure of the crop canopy in the high soybean density treatment (data not shown). Elevated crop densities generally result in earlier canopy closures when compared to lower crop densities (Arce et al. 2009, Blackshaw 1993a, De Bruin and Pedersen 2008, Rich and Renner 2007) and may have reduced C$_4$ grass seedling emergence, similar to the row spacing experiment.

The difference between the composition of the weed community in the high density treatment at Carman and St-Adolphe in 2016 (i.e. grassy weeds were more prominent at St-Adolphe) was likely due to a couple of reasons. First at St-Adolphe 2016, *A. fatua* began to emerge prior to seeding the experiments (visual observations, data not shown). In this eco-region of North America, cool-season C$_3$ grasses like *A. fatua* are prominent (Leeson et al. 2005, Van Acker et al. 2000) and emerge early in spring (Beckie et al. 2012). A delay in seeding soybean at St-Adolphe 2016 (Chapter 3, Table 3.1), combined with no spring weed management before seeding, meant that *A. fatua* likely had a competitive advantage over the soybean crop (Blackshaw 1993b, Rathmann and Miller 1981, Willenborg et al. 2005). Second, broadleaved weeds at both these site-years in this experiment were warm-season species that generally emerge later in the spring. It is possible that the combination of the early competition from *A. fatua* and the high density soybean at St-Adolphe in 2016 sufficiently suppressed other weeds in the community when compared to Carman 2016, however further testing is required.

Differences in the structure of the weed community also were observed among the shortest weed-free durations in the target density experiment (Figure 4.2). At Whitemouth in 2017, the low soybean density treatment that remained weed-free until the VE stage was associated with *Lathyrus* spp., *S. viridis*, and common dandelion (*Taraxacum officinale* Weber ex F.H. Wigg.), whereas the standard and high density treatments were associated with Shepard's purse [*Capsella bursa-pastoris* (L.) Medik.], *E. crus-galli*, and *Trifolium* species. The importance of *A. retroflexus* in the weed community of the low density treatment appeared to be decreased in the earliest weed removal treatment, which likely allowed for greater biomass accumulation of *Lathyrus* spp., *S. viridis* and *T. officinale*. The change in the structure of the weed community from the weedy control to the earliest weed-free period may be due to a combination of the intense competitive ability of *A. retroflexus* (Costea et al. 2003, Stewart-Wade et al. 2002), the natural tolerance to glyphosate of *T. officinale* (Moyer et al. 2009), and the decreased competitive ability of low density soybean (Arce et al. 2009, DeWerff et al. 2014, Mierau et al. 2019). Little is known about the competitive ability or the response of *Lathyrus* species to herbicides used in these experiments.

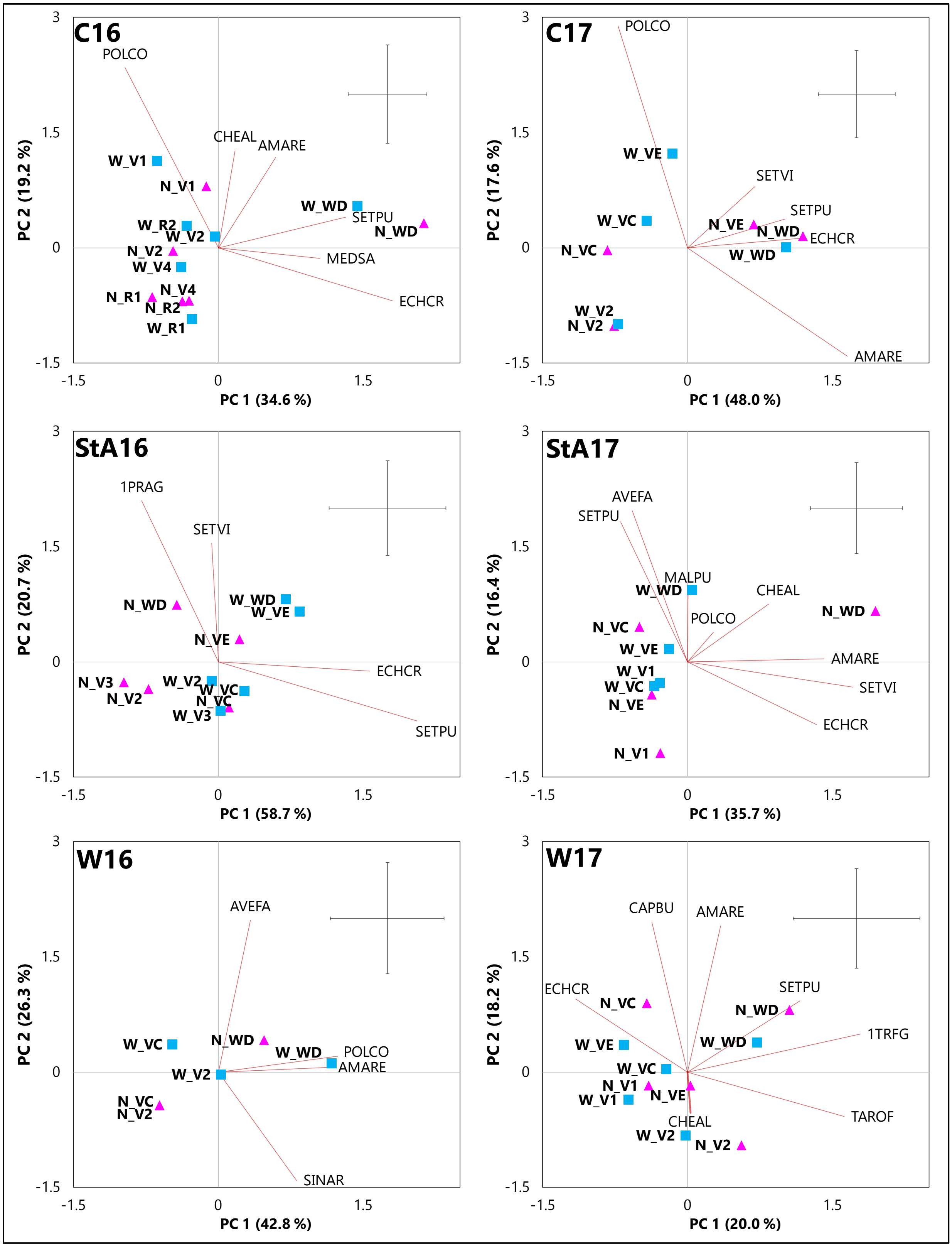

Figure 4. Principal component analysis of soybean row spacing effects on weed communities. Principal component 1 (PC1) and 2 (PC2) are represented at individual site-

years (**C**=Carman, **StA**=St-Adolphe, **W**=Whitemouth for 20**16** and 20**17**). Centroids are the least square mean of treatments which represent narrow (**N_**; ▲) or wide (**W_**; ■) row spacing kept weed-free until a specific soybean development stage (**WD**=no weed control; **VE**, **VC**, **V1-V4**, and **R1**, see Fehr et al. (1971)). Vectors represent original axes of weed species (codes found in Table 4.1) included in the analysis. Error bars in top right hand corner of biplots represent one LSD for the respective principal component.

An anomalous extreme weather event occurred 26 days after seeding at the Whitemouth location in 2016. Accumulated precipitation during this single event was greater than 150 mm (data not shown). Water from this event flooded the target density experiment for an extended period compared to the row spacing and variety experiments (personal observation, Figure C2). The extended flooding in the experiments likely contributed to the low species richness within experiments and extremely low evenness in the target density experiment at this site-year (Table 4.4). This weather event also affected the weed-free duration treatments that could be retained for principal component analysis (i.e. only those containing weeds). The reduced number of centroids presented for this site-year in Figures 4.2–4.4 reflect the effects of this weather event.

While no significant differences were observed between row spacing, at St-Adolphe 2016 (Figure 4.3) the weed community in the weedy control of the narrow row treatment appeared to be associated with *Persicaria* spp. whereas the weed community in the wide row weedy control treatment appeared to be associated with *E. crus-galli*, *S. pumila*, and *S. viridis*. The weed community in the weedy control in the narrow row treatment at St-Adolphe 2017 was associated with the broadleaved species *A. retroflexus* and *C. album* whereas the weed community in the wide row control was associated with *A. fatua*, *M. pusilla*, and *S. pumila*. Canopy closure occurred earlier in narrow row soybean compared to those grown in wide rows (data not shown). An extended duration before canopy closure in wide row soybean results in increased weed seedling recruitment (Andrade et al. 2019, De Bruin and Pedersen 2008, Bullock et al. 1998, Yelverton and Coble 1991). This is a plausible explanation why warm-season C_4 grassy weeds and low-growing weeds like *M. pusilla* were more prevalent in the wide row weedy control treatment whereas fast growing weed species like *A. retroflexus* may be more resilient to light competition in narrow row soybean (Cowan et al. 1998).

Soybean cultivar had no effect on the composition and structure of the weed community with the exception of Carman 2017 (Table 4.4; Figure 4.4). At this site-year, the weed community in the weedy control treatment in the DKB2260 treatment was associated with *A. retroflexus* and *E. crus-galli* and negatively associated to the weed community observed in DKB2360 which was dominated by *F. convolvulus*. The weed community observed in the weedy control treatment in the DKB2410 treatment was associated with *A. retroflexus*, *S. pumila*, and *S. viridis*. It is unclear what may have caused these differences among cultivars at this site-year. These observations were unique to this site-year only. The lack of differences in the structure of the weed community among soybean cultivars however, may be due to the lack of morphological and/or physiological differences that drive soybean weed-suppressive ability (Horneburg et al. 2017, Place et al. 2011b). For example, soybean cultivars with elevated rates of canopy closure and extended time to maturation had an improved competitive ability against weeds (Bussan et al. 1997). Using oat cultivars, Benaragama et al. (2014) showed an improved competitive ability with *A. fatua* based on oat leaf area and plant height. Further research on the structure of the weed community using soybean cultivars with a greater spectrum of weed-suppressive traits may elucidate why these results occurred.

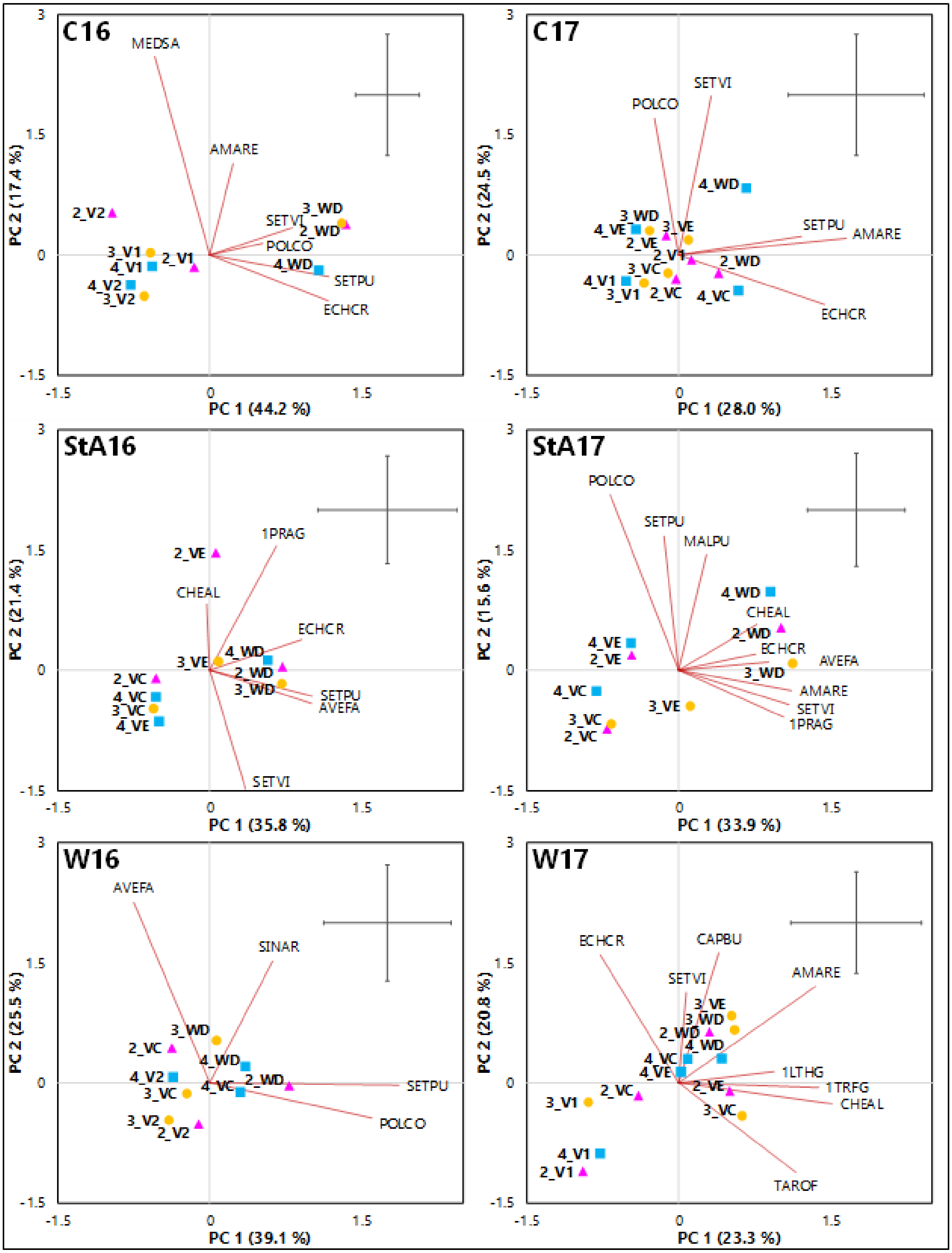

Figure 4.4 Principal component analysis of soybean cultivar effects on weed communities. Principal component 1 (PC1) and 2 (PC2) are represented at individual site-years

(**C**=Carman, **StA**=St-Adolphe, **W**=Whitemouth for 20**16** and 20**17**). Centroids are the least square mean of treatments which represent DKB2260 (**2_**; ▲), DKB2360 (**3_**;●), or DKB2410 (**4_**; ■) kept weed-free until a specific soybean development stage (**WD**=no weed control; **VE**, **VC**, **V1**-**V4**, and **R1**, see Fehr et al. (1971)). Vectors represent original axes of weed species (EPPO codes can be found in Table 4.1) included in the analysis. Error bars in top right hand corner of biplots represent one LSD for each respective principal component.

A greater number of differences in the structure of the weed communities due to cultural weed management techniques were observed in the target density experiment compared to the other two experiments (Table 4.4). This was unexpected as soybean row spacing tends to have a greater impact on weed biomass production (Harder et al. 2007, Hock et al. 2006, Légère and Schreiber 1989, Nice et al. 2001, Wax and Pendleton 1968) and weed seedling recruitment (Norsworthy 2004, Yelverton and Coble 1991) than soybean density (Rasool et al. 2017). Soybean cultivars also appear to have a larger effect on the crop competitive ability with weeds than soybean density in this region (see Chapter 3). Soybean density likely influenced the structure of the weed community through increased asymmetric competition associated with greater crop densities (Weiner 1990). For example, increased crop densities lead to elevated intra-specific crop competition, resulting in increased aboveground growth rates compared to lower crop densities (Weiner et al. 2010). This is advantageous to high density soybean as they may outgrow and quickly shade certain weed species through increased intra-specific competition as shown by Weiner et al. (2001) using wheat. Therefore, the resulting weed community is likely composed of weed species with matching or greater relative growth rates to the crop or an increased shade tolerance. This idea is supported by the works of Fried et al. (2012) and Gulden et al. (2010) where herbicide applications change the structure of the functional traits of weed communities, indicating that weed communities adapt to management techniques based on specific traits. Further experimentation examining functional traits of both soybean and the weed community in relation to soybean management practices may provide additional information to validate this observation.

In summary, differences in the structure of the weed community were observed regardless of the composition of the weed community. Timing of in-crop herbicide application (i.e. the duration of the weed-free period) had the greatest effect on the structure of the weed community. Regardless of the experiment, keeping soybean weed-free until the V1 development stage tended to change the structure of the weed community such that it was associated negatively with the weed species that defined the weedy treatments. The structure of the weed community also was influenced at the earliest weed-free duration periods by soybean target density and row spacing. The structure of the weed community tended to change among cultural weed management techniques towards weed species which likely could best adapt to these conditions. Cultural weed management techniques were shown to alter the structure of the weed community and act as a filter to the developing weed community. The intensity of change in the structure of the weed community from these techniques however, was weak compared to herbicide applications and was inconsistent among site-years. Further testing of these cultural weed management techniques on the structure of designed weed communities with variable functional traits may corroborate these results.

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5.0 GENERAL DISCUSSION AND CONCLUSION

This M.Sc. thesis furthered knowledge on the effects of cultural weed management techniques in soybean. The research filled in pertinent knowledge gaps on how row spacing, target plant density, and soybean cultivar can be used to improve the competitive ability of the crop with weeds by using the critical weed free period (CWFP) concept. The overall objectives of this project aimed to provide (1) improved weed management recommendations for soybean production in the Northern Great Plains in order to reduce the selection pressure for herbicide-resistant (chapter 3) and (2) basic knowledge of the effects of row spacing, target density, and soybean cultivar on the structure of the weed community (chapter 4). This chapter will discuss the resemblance between the research chapters and formulate conclusions based on the findings from this research.

5.1 Effect of Cultural Weed Management Techniques on the Critical Weed Free Period in Soybean

The primary objective of this experiment was to determine how cultural weed management techniques (i.e. row spacing, target density, and soybean cultivar) could be useful tools to shorten the CWFP in soybean. Significant changes in the duration of the CWFP in soybean were observed in all three cultural weed management techniques evaluated as part of this experiment. A shortened CWFP meant fewer herbicide applications were required to manage weeds and avoid yield loss, and as such may be useful at reducing the selection pressure for the development of glyphosate resistant weed biotypes. These experiments were the first to quantify the effects of these cultural weed managements techniques on the CWFP in soybean.

5.1.1 Recommendations from the CWFP Experiments

Results from these experiments have added to the more than a 50-year old body of literature that provides strong evidence that narrow row spacing (Harder et al. 2007, Hock et al. 2006, Légère and Schreiber 1989, Nice et al. 2001, Rasool et al. 2017, Wax and Pendleton 1968, Wax et al. 1977) and elevated plant densities (Arce et al. 2009, Cox and Cherney 2011, De Bruin and Pederson 2008, DeWerff et al. 2014, Liebert and Ryan 2017, McWorther and Barrentine 1975, Norsworthy and Oliver 2002, Rich and Renner 2007) result in soybean that are more competitive with weeds. Results from the soybean cultivar experiment may have varied among experimental locations, yet corroborate previous research that examined the competitive ability of soybean cultivars (Bussan et al. 1997, Jordan 1992, Monks and Oliver 1988) and research that showed increased plant height and an extended time to maturity aid soybean in suppressing weeds (Jannick et al. 2000, Hammer et al. 2018, Horneburg et al. 2017, Place et al. 2011c). Among all cultural weed management experiments however, soybean row spacing shortened the duration of the CWFP more than target density or cultivar (Figure B4).

Overall, the following production practices for soybean production in northern growing regions can be derived from the results of this experiment:

1. It is recommended that soybean be grown in narrow rows (either 19-cm or 37.5-cm), and row spacing of 76-cm or greater should be avoided.
2. It is recommended that soybean target density remain at or be increased above the current local standard of 444,600 plants ha^{-1}.
3. It is recommended that producers adopt soybean cultivars that are locally adapted.

These recommendations have been shown to shorten the CWFP in soybean individually, however the combination of these techniques is likely to enhance their effect. Future experiments should be directed to evaluate the combination of these techniques in soybean using the CWFP.

5.1.2 Caveats to the Recommendations from the CWFP Experiment

Caveats associated with these recommendations must be addressed. First, row spacing is a weed management technique that is determined by equipment that is difficult, and sometimes cost prohibitive to modify. Wide row precision planters are configured for optimal corn production (Bradley 2006) and increasingly, have become available for purchase as used equipment from southern Ontario and the USA (personal observation, agricultural equipment auction websites). Corn is slowly, yet steadily being adopted for grain production in Manitoba (Anonymous 2018a). For this reason, an increase in purchase and usage of precision planters in corn is likely and Manitoba producers may be tempted to also use this seeding equipment in soybean as seed costs continue to rise (Oriade et al. 1997). In other soybean growing regions, this practice likely led to the development of GR weed biotypes in soybean (Beckie and Harker 2017, Beckie et al. 2006, Harker 2013, Heap 2020), and perceptions that GR weeds are not a problem in an area can be detrimental to the adoption of effective weed management techniques that can delay the onset of GR weeds (Johnson et al. 2009, Kruger et al. 2009). Based on this information and the results from the row spacing experiment, I recommend also that wide row planting equipment be avoided for soybean production.

Second, in the target density experiment, no differences among the standard and high soybean densities were observed, which contradicted the work of Mierau et al. (2019).

It is difficult to determine why their results contradicted the present results, Storkey and Neve (2018) however, may provide an explanation. Their experiment showed that the detrimental effects of dominant weed species on yield loss in wheat is mediated by increasingly diverse weed communities. The weed community observed in Mierau et al. (2019) had a richness of one (volunteer *B. napus*), whereas the weed community in the current experiments had greater richness, up to seven weed species at some site-years (Chapter 4). Also, the weed used by Mierau et al. (2019), volunteer *B. napus*, is very competitive weed with soybean (Geddes and Gulden, 2017). The diverse weed community observed in these experiments (Chapter 4) also may explain why the CWFP was not shortened in the highest soybean density treatments. Nevertheless, soybean yields in the weedy treatments were greatest in the high density treatment (Table B1) suggesting that increasing soybean densities to 666,000 plants ha^{-1} may mitigate the effects from weed escapes in case of herbicide failure.

Finally, other weed suppressive traits not measured in these experiments (e.g. early season relative growth rate and specific leaf area) (Horneburg et al. 2017, Place et al. 2011c) may further explain the observed differences in the CWFP. Everything being equal, greater mid-season soybean heights may infer increased early season growth rates (Bullock et al. 1998, Vega and Sadras 2003). The consistently longer CWFP observed in the shortest statured soybean cultivar DKB2260 (Chapter 3 Table 3.6 & Figure 3.2) may be explained by decreased early-season growth rate, inferred by the shorter height and lower mid-season shoot biomass (Table B2), compared to the taller statured cultivars. Further experiments are needed to confirm this observation.

Soybean leaf area was measured and leaf area index was determined in these experiments in 2016 but, due to labour and time constraints not in 2017. Results from 2016 show significant differences in leaf area index among weedy and weed-free control treatments however, differences among soybean density or cultivar treatments were observed only under weed-free conditions (Table B3). A greater mid-season leaf area index infers that soybean captured more solar radiation therefore increasing the shade within the crop canopy (Harder et al. 2007) and the results from 2016 appear to help explain the differences in the CWFP observed in 2016. Improved soybean growth rates or leaf area may likely further shorten the CWFP in these northern growing regions and it would benefit primary producers to know the weed suppressive traits of their soybean cultivars prior to purchase. While weed-suppressive traits can be difficult to select for (Place et al. 2011c), their identification would be a useful tool for agriculturalists in selecting a soybean cultivar that is both competitive with weeds and high yielding. I would recommend identification of these weed-suppresive traits by soybean breeders and used in the commercialization of cultivars, similar to other agronomic ratings (Anonymous 2017a). In the near term however, a more reasonable recommendation from this study is, the inclusion of soybean yield grown in weedy conditions as a surrogate measure of the competitive ability of the cultivar.

5.2 Cultural Weed Management Techniques and the Weed Community

The goal of this thesis was to explore the effects of cultural weed management techniques on the composition and structure of the weed community. Results from these experiments showed that increasing weed-free duration was the main determinant of the weed community and corroborates previous findings by Doucet et al. (1999), Gulden et al. (2010), Harker et al. (2005), Johnson et al. (2009), Ryan et al. (2010), Schutte et al. (2017), Storkey et al. (2012), and Westra et al. (2008). Of the weed species observed in these experiments, natural tolerance to glyphosate is known to occur in *C. album* (Nandula et al. 2005) and *T. officinale* (Moyer et al. 2009), while *A. retroflexus* has shown signs of evolved glyphosate resistance in Serbia (Krga et al. 2013). The increased area and use of glyphosate resistant crops and the associated increase in glyphosate use in Manitoba and the Northern Great Plains is already shifting dominance towards weeds with natural tolerance to glyphosate (Van Acker et al. 2000), or towards those with a greater chance at evolving glyphosate resistance (Beckie and Leeson 2017). Continued reliance on glyphosate likely will lead to increased costs as producers are required to mix alternate herbicide mechanisms of action (Beckie and Harker 2017, Owen 2016), and at worst may result in a loss of this useful weed management tool. Judicious use of glyphosate is recommended to avoid or delay the onset of weed communities dominated by weeds naturally tolerant or resistant to glyphosate.

The effects of cultural weed management techniques on the structure of the weed community in this experiment were not as strong as those caused by increasing the duration of the CWFP. Regardless, results from these experiments on the structural changes of the weed community were observed across a range of different conditions and weed community compositions. To my knowledge, this is the first study that observed these changes in the structure of the weed community among row spacing, target density, and cultivar treatments in soybean or other crops. This experiment resulted in a novel understanding of weed communities and how cultural weed management techniques influence their structure. Future research will need to evaluate the link between the CWFP and different weed communities. Generally, a shortened CWFP was observed at site-years where total weed shoot biomass was elevated whereas, no discernable pattern could be observed between a shortened CWFP and changes in the structure of the weed community. In these experiments, it appeared the composition and structure of the weed community did not matter to the determination of the CWFP when compared to overall weed shoot biomass. A recently revived research area describing plant communities based on functional traits may elucidate this question (Shipley et al. 2016).

5.2.1 Weed Community and Functional Traits

While this thesis did not address plant functional traits, it may be an avenue for future research. In brief, functional trait analysis describes plant communities by their morphological characteristics instead of their species composition (Garnier et al. 2016) and is considered an ultimate goal in understanding ecosystem functions and services (Diaz and Cabido 2001, Funk et al. 2017, Lavorel and Garnier 2002, Shipley et al. 2016, Weiher et al. 1999). Many functional traits of plant communities were identified and discussed by Bàrberi et al. (2018), Fried et al. (2012), Funk et al. (2017), and Martin and Isaac (2015). Agricultural weed communities present a unique opportunity to develop and test hypotheses surrounding ecosystem functions and services as they readily respond to environmental conditions and management techniques (Chapter 2 & 4) (Costanzo and Bàrberi 2014, Gaba et al. 2017, Garnier and Navas 2012, Losová et al. 2006, Storkey 2006). Fried et al. (2012) for example, successfully tested hypotheses that linked weed species and their functional traits to the herbicide and tillage management intensity. Consideration of which traits to be collected is of absolute concern to test these types of hypotheses correctly since results may be misrepresented by omitting specific traits (Shipley et al. 2016). Also, the analysis of these datasets must be accomplished using high-level multivariate statistical methods, and require a greater degree of understanding and interpretation for meaningful results (Dolédec et al. 1996, Kleyer et al. 2012). This type of analysis would have been preferable in linking the cultural weed management techniques and the structure of the weed community however, I was not aware of this research area until late into the 2017 field season and could not collect the necessary trait information for analysis. Future studies should examine the link between functional traits and the structure of the weed community as they respond to these cultural weed management techniques. Results from these future studies may provide a greater understanding of weed community dynamics and ecosystem functions and services.

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7.0 APPENDICES

7.1 Appendix A: Herbicide-Resistant Weed Biotypes of Concern for Manitoba

The number of herbicide resistant weeds are increasing globally. As part of the international survey of herbicide resistant weeds, Heap (2020) compiled 287 reports of HR weeds identified in global soybean production fields. Many of the reported herbicide resistance cases are to a single mechanism of action, yet increasingly, researchers are reporting cases of multiple-resistant weed biotypes. These reports however, only cover weeds found to be resistant during the year of soybean production (Heap 2020), and do not mention previous production practices. As soybean production is global and there is an abundance of herbicide resistant weeds already identified within these production systems, this section will focus on weeds of concern either already found, or able to develop in Manitoba.

7.1 Amaranthus species

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is native to southern North America whose biology, ecology, and history was reviewed extensively by Ward et al. (2013). Though it is a native species of southern regions, it is adaptable to cooler climatic conditions, as observed by its spread into the northern growing regions of Michigan, Wisconsin, and southern Ontario (Anonymous 2017b). Populations of *A. palmeri* have developed resistance to single and multiple herbicide mechanisms of action (Heap 2020). Resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD) - (Jhala et al. 2014, Thompson et al. 2012), acetolactate synthase (ALS) - (Burgos et al. 2001, Gaeddert et al. 1997, Horak and Peterson 1995, Sprague et al. 1997, Wise et al. 2009), photosystem II

(PSII) - (Jhala et al. 2014, Kohrt et al. 2017), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) - (Butts and Davis 2014, Culpepper et al. 2006, Norsworthy et al. 2008, Steckel et al. 2008), microtubule assembly (MA)- (Gossett et al. 1992), and, protoporphyrinogen oxidase (PPO)-inhibitors (Salas et al. 2016) have all been confirmed in the USA. When faced with intense selection pressure, *A. palmeri* has shown to quickly evolve resistance to auxin transport inhibitors from sub-lethal exposure within three generations (Tehranchian et al. 2017). *Amaranthus palmeri* is a troublesome weed in southern USA soybean production (Webster and Nichols 2012) and was identified in North Dakota and Minnesota in 2013 (Stachler et al. 2013). Introduction of *A. palmeri* into the Canadian Prairies has yet to be reported.

Red root pigweed (*Amaranthus retroflexus* L.) is native to the southern regions of North America. Its biology, ecology, and history, including many cases of herbicide resistance were reviewed extensively by Costea et al. (2003). Briefly, populations of *A. retroflexus* developed resistance to PSII- and ALS-inhibiting herbicides in North America and Europe (Heap 2020). Locally, an ALS-inhibiting resistant population was confirmed in Manitoba in 2002 (Beckie et al. 2004). Resistance to PPO inhibitors were confirmed in Brazilian and Chinese *A. retroflexus* populations (Heap 2020). Glyphosate resistance has yet to be confirmed, but work in Serbia showed evolution of resistance to EPSPS inhibitors in orchard populations (Krga et al. 2013). *Amaranthus retroflexus* is well adapted to, and wide-spread in the northern soybean growing regions, and has shown potential for developing herbicide resistance across climatic zones.

Tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] and its close relative common waterhemp (*Amaranthus rudis* Sauer) are species native to North America that are found from Ohio westward to Nebraska and south into Texas (Costea et al. 2005). Both waterhemp species however, have spread far beyond their native boundaries, invading southern Ontario, Quebec and most of the eastern United States (Anonymous 2017b). Both species were reviewed extensively by Costea et al. (2005). Of special interest, *A. tuberculatus* is a dioecious plant (i.e. an obligate outcrosser), which leads to larger genetic variability within populations therefore, a greater potential for development of herbicide resistance. Of concern, resistance to multiple mechanisms of action within localized populations of *A. tuberculatus* have been reported in Illinois (Bell et al. 2013, Patzoldt et al. 2005), Iowa (McMullan and Green 2011), Missouri (Schultz et al. 2015), and other regions of the USA and Canada (Heap 2020). In Manitoba, a glyphosate resistant *A. tuberculatus* population was reported (Robert Gulden, personal communication). The spread of *A. tuberculatus* into the Northern Great Plains and the western Canadian prairies appears to be a matter of time and, with its propensity to develop herbicide resistance, will be problematic to control if not caught early.

7.2 Ambrosia species

Common and giant ragweed (*Ambrosia artemisiifolia* L. and *Ambrosia trifida* L.) are native to North America and are found in Canada from southern Ontario into the Maritime provinces and portions of south eastern Manitoba (Bassett and Crompton 1975, 1982). Both *Ambrosia* species were found historically in ditches and along rivers and wetlands, but have since moved into agricultural fields. *Ambrosia trifida* is capable of causing severe soybean yield loss due to its competitive nature (Baysinger and Sims 1991). Both *Ambrosia* species are known to contribute to pollinosis, a seasonal human allergy that can lead to the development of asthma in certain individuals (Déchamp 2013), therefore is also of concern to the general human population. These species are difficult to control with mowing or grazing as plants re-grow and flower within 10 days of the mowing activity (Bassett and Crompton 1975, 1982). Herbicide resistance was identified initially to PSII inhibitors in a population of *A. artemisiifolia* in southern Ontario in 1976 (Stephenson et al. 1990). Since then, populations of *A. artemisiifolia* and *A. trifida* across the USA and southern Ontario have developed resistance alone and in combinations to ALS-, EPSPS-, PPO-, and substituted ureas- inhibitors (Heap 2020, Nandula et al. 2017, Rousonelos et al. 2012, Simard et al. 2017, Van Wely et al. 2014). The nearest ALS- and EPSPS-resistant *A. artemisiifolia* population to Manitoba were identified in northern Minnesota (Heap 2020). A glyphosate resistant *A. trifida* population was reported in Manitoba where GR soybean was grown intensely (Robert Gulden, personal communication). The spread of herbicide resistant *A. artemisiifolia* and *A. trifida* into the Northern Great Plains likely is inevitable with continued herbicide, and in particular glyphosate use. Vigilance from growers and field personnel should be emphasized in order to delay their spread.

7.3 Wild oats

Wild oats (*Avena fatua* L.) are ubiquitous to the Northern Great Plains, and a problematic weed of temperate regions. For an in-depth review of the biology, ecology, and management of wild oats, see Beckie et al. (2012). The emergence periodicity in wild oat begins early and can last up to three months. This in conjunction with vigorous growth allows wild oat to compete strongly with surrounding plants (O'Donovan et al. 1985, Rathmann and Miller 1981, Willenborg et al. 2005). *Avena fatua* has developed resistance to multiple mechanisms of action due to their long emergence periodicity and genetic variability (Beckie et al. 2012). On the Northern Great Plains, confirmed resistance in *A. fatua* to acetyl CoA carboxylase (ACCase), ALS-, Lipid biosynthesis (LB)-, PPO-, and long chain fatty acid (LCFA) inhibitors (Beckie et al. 2012b, Heap 2020, O'Donovan et al. 1994). Recently, the first global case of glyphosate resistant *A. fatua* was reported in a chickpea field near Queensland, Australia (Heap 2020).

7.4 Common lambsquarter

Common lambsquarter (*Chenopodium album* L.) is prolific across disturbed sites of temperate regions. The biology and ecology of *C. album* was reviewed by Bassett and Crompton (1978), however this work has not been updated since its publication to better reflect new knowledge of herbicide resistance in this species. Continuous application of the herbicide atrazine led to the first confirmed case of PSII resistant *C. album* confirmed in Ontario (Bandeen and McLaren 1976) and has since developed resistance to this and other PSII inhibitors in other countries (Heap 2020). Resistance to ALS inhibitors has been reported globally (Konstantinović et al. 2015, Heap 2020, Nandula et al. 2005). The concern is that ALS inhibiting herbicides are used extensively in most crops and resistance can develop rapidly (Jasieniuk et al. 1996). Recently in New Zealand, synthetic auxin resistance has been confirmed in populations of *C. album* (Ghanizadeh and Harrington 2017, Rahman et al. 2014). Glyphosate resistance has yet to be confirmed however, *C. album* is known to have natural tolerance to glyphosate (Hite et al. 2008, Kniss et al. 2007, Nandula et al. 2005, Yerka et al. 2013).

7.5 Kochia

Kochia [*Bassia scoparia* (L.) Scott] is common and widespread throughout the Great Plains of North America, especially in arid and semi-arid conditions (Friesen et al. 2009). *Bassia scoparia* is a problematic weed due to its early seedling recruitment, rapid growth, and extended emergence periodicity (Dille et al. 2017, Schwinghamer and Van Acker 2008). Populations of *B. scoparia* are genetically diverse. The degree of genetic variability within *B. scoparia* populations collected at five locations in the USA (three from Montana and one each from Kansas and North Dakota) was as great as that among populations (Dyer et al. 1993). *Bassia scoparia* primarily self-pollinates, however a substantial amount of outcrossing (13% within 1.5m; Friesen et al. 2009) is responsible for the degree of genetic diversity observed by Dyer et al. (1993). Beckie et al. (2016) determined that pollen mediated gene flow likely does not spread further than 30 meters from the *B. scoparia* mother plant however, the rolling tumbleweed mediates gene flow from seeds up to one km away. It is unknown how exactly herbicide resistance from one local population of *B. scoparia* spreads to another within a regional setting.

The initial case of herbicide resistant *B. scoparia* occurred in Kansas, 1976 to PSII inhibitors and spread throughout the US Midwest over the following decade (Heap 2020). The intense use of ALS inhibiting herbicides in North America led to the evolution of ALS resistant *B. scoparia* populations from Kansas (Primiani et al. 1990) northward to Manitoba (Friesen et al. 1993). The majority of *B. scoparia* populations in North America are now assumed to be resistant to ALS inhibiting herbicides (Beckie and Leeson 2017). Since, resistance to synthetic auxin herbicides (Cranston et al. 2001) and EPSPS inhibitors (Beckie et al. 2014, 2019, Waite et al. 2013) have been confirmed throughout the Great Plains of North America. In Kansas, *B. scoparia* has evolved resistance to four herbicide mechanisms of action (PSII-, ALS-, EPSPS- inhibitor, and synthetic auxin) (Varanasi et al. 2015), signaling a loss of effective chemical control options in that population.

7.6 Setaria species

Green foxtail [*Setaria viridis* (L.) Beauv.] is a weedy annual grassy plant of cultivated fields and disturbed sites across the Northern Great Plains (Douglas et al. 1985). For over three decades, *S. viridis* has bee the most abundant weed species found in Manitoba (Beckie and Leeson 2017, Beckie et al. 2004). Yield loss in small grain cereal crops can be great when *S. viridis* is present in high densities (Hume 1989, O'Donovan 1994), however less is known about its competitive effects with soybean. Across western Canada, *S. viridis* populations developed resistance to ACCase-, ALS- and MA- inhibiting herbicides (Heap and Morrison 1996, Morrison et al. 1989, Volenberg et al. 2002) and in Europe, resistance has evolved to PSII inhibiting herbicides (Heap 2020).

Yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult. syn. *S. glauca*] grows in full sunlight in cultivated fields and disturbed sites across the southern reaches of Canada into the USA (Steel et al. 1983). Occurrence of this species in Manitoba has increased radically since 2002 (Beckie and Leeson 2017), likely due to changes in cropping systems and a warming climate (Crossman et al. 2011). So far, herbicide resistance in this species has not been problematic however, four cases of PSII- and one case each of ACCase- and ALS-inhibitor resistance have been reported globally (Beckie and Leeson 2017, Heap 2020).

7.7 Other weeds of concern

Of concern to Manitoba agriculture are barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], wild buckwheat [*Fallopia convolvulus* (L.) Löve], pale smartweed [*Persicaria* *lapathifolium* (L.) Delarbre], spotted lady's thumb (*Persicaria maculosa* Gray), and wild mustard (*Sinapis arvensis* L.) (Heap 2020). The aforementioned weed species are detrimental to crop production for their ability to reduce grain yields, hamper harvest efforts, and contaminate seed lots. Each of these species have developed resistance to ALS inhibiting herbicides, the largest chemical weed control category registered for use in soybean (Anonymous 2018b), in Canadian crop production systems (Heap 2020). In November 2019, a glyphosate resistant biotype of *E. crus-galli* was found in an Argentinian corn field (Heap 2020) however, none of the other species mentioned in this section have yet to develop resistance to glyphosate.

7.2 Appendix B: Critical Weed Free Period in Soybean

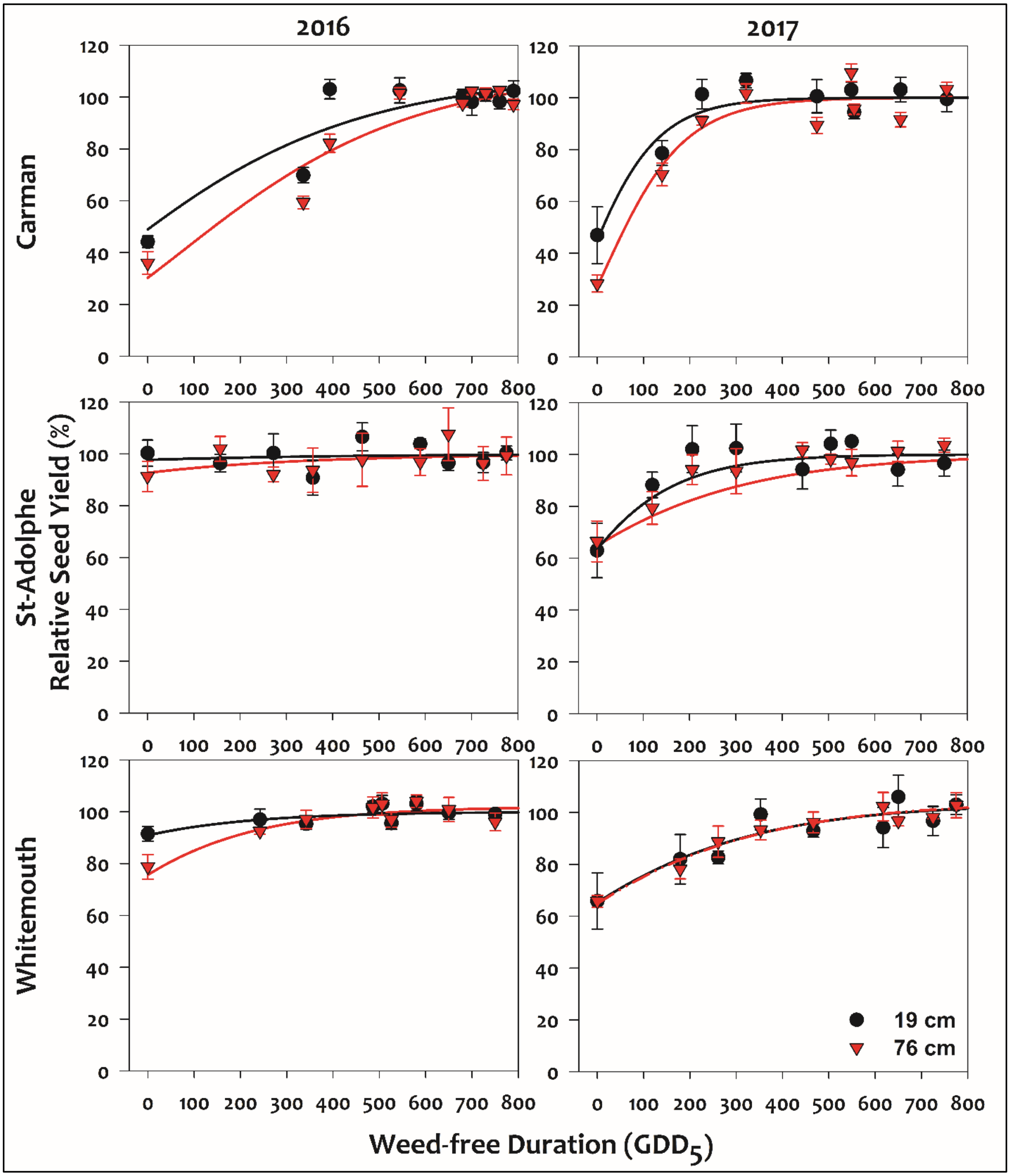


Figure B1 Graphical representation of the critical weed free period of soybean for two row spacing at six site-years in Manitoba. The Gompertz equation, eq. [3], was fitted to treatments within site-years. Parameter estimates and contrasts can be found in Table 3.3.

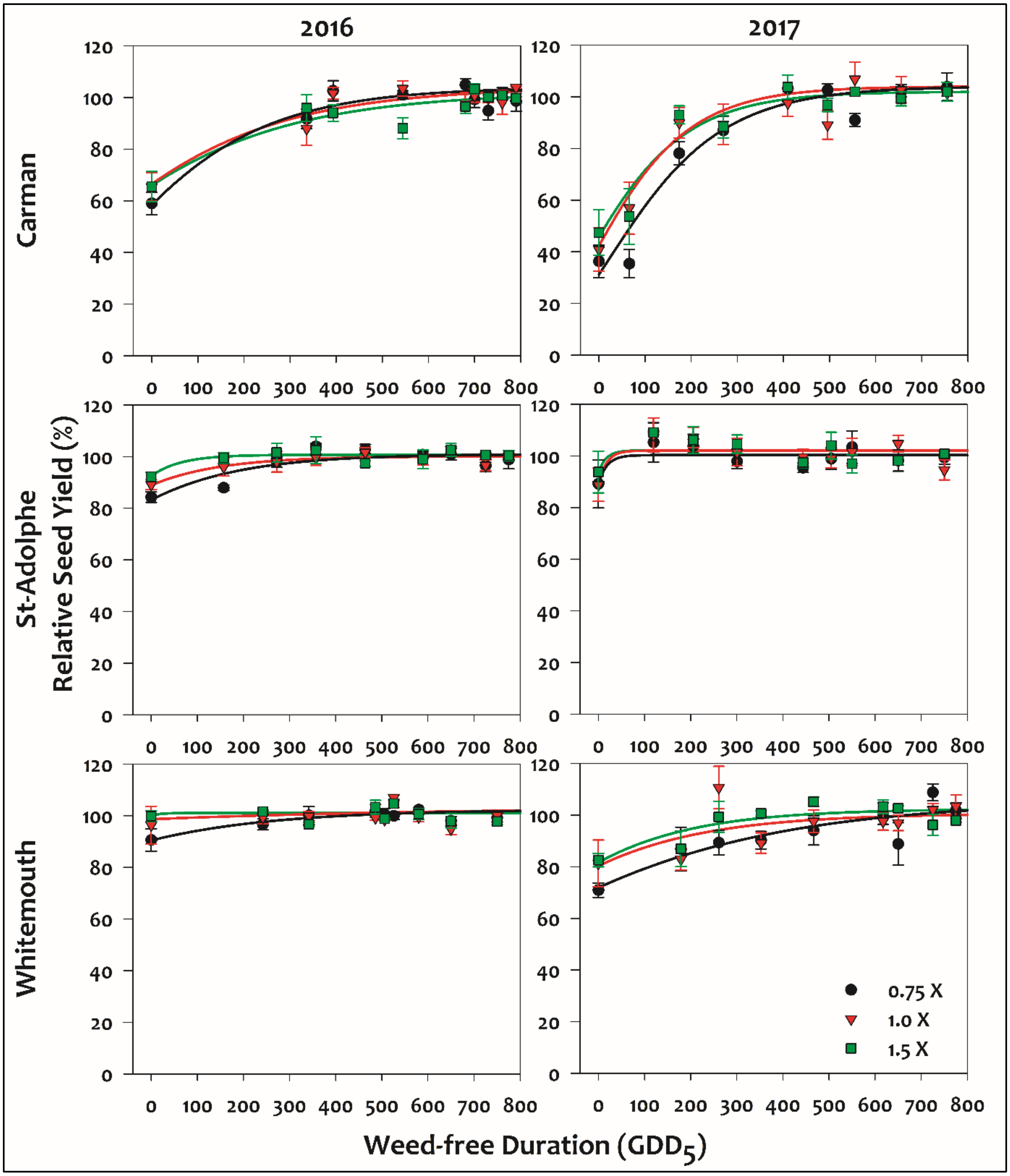


Figure B2 Graphical representation of the critical weed free period of soybean for three densities at six site-years in Manitoba. The Gompertz equation, eq. [3], was fitted to treatments within site-years. Parameter estimates and contrasts can be found in Table 3.3. Soybean density of 1.0X in the legend corresponds to 444,600 plants ha^{-1}.

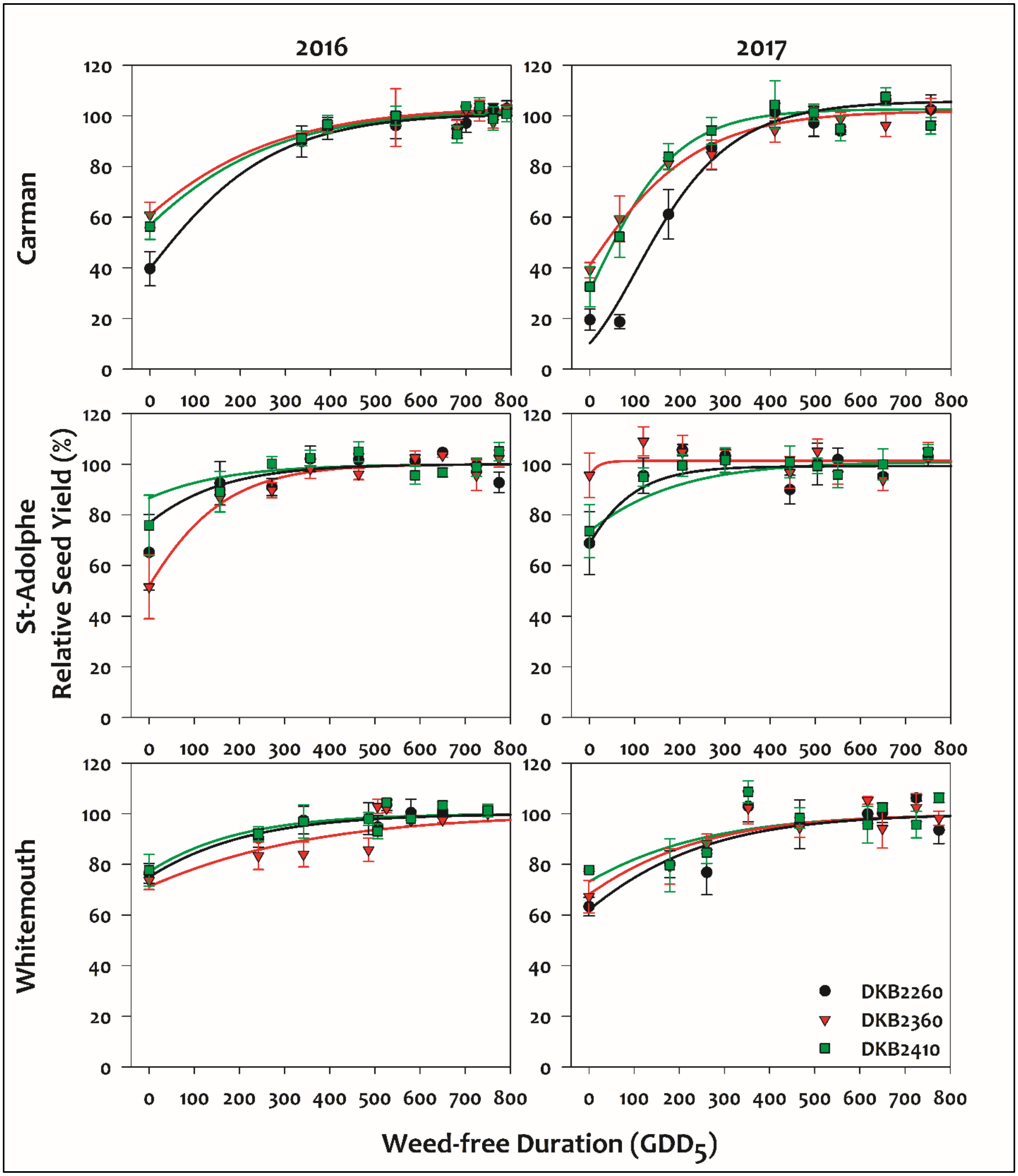


Figure B3 Graphical representation of the critical weed free period of soybean for three cultivars at six site-years in Manitoba. The Gompertz equation, eq. [3], was fitted to treatments within site-years. Parameter estimates and contrasts can be found in Table 3.3.

Table B1 Mean weedy soybean yield from three cultural weed management experiments over six site-years in Manitoba. [a]

		Soybean Yield			
Row Spacing Experiment		----------------- kg ha^{-1} -----------------			Site-Year LSD
		19 cm	76 cm		
2016	Carman	1470 c	967 d		
	St-Adolphe	3359 a	2209 b		
	Whitemouth	3099 a	2037 b		
2017	Carman	882 de	536 e		
	St-Adolphe	1061 cd	1180 cd		
	Whitemouth	1340 cd	1315 c		
Target Density Experiment [b]		0.75X	1.0X	1.5X	
2016	Carman	1951	2398	3030	b
	St-Adolphe	2821	2989	3255	a
	Whitemouth	2845	2972	3125	a
2017	Carman	776	941	1003	e
	St-Adolphe	1285	1336	1493	d
	Whitemouth	1562	1964	2073	c
	Main effect LSD	c	b	a	
Variety Experiment		DKB2260	DKB2360	DKB2410	
2016	Carman	1417	2167	2114	b
	St-Adolphe	2115	1612	2125	ab
	Whitemouth	1812	2636	2486	a
2017	Carman	413	871	729	d
	St-Adolphe	1111	1525	1298	c
	Whitemouth	1167	1397	1701	c
	Main effect LSD	b	a	a	

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation. Letters are presented beside the means if the treatment by site-year interaction was significant.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

Table B2 Mean mid-season soybean shoot biomass under weedy and weed-free conditions from three cultural weed management experiments in Manitoba. [a]

		Soybean Shoot Biomass (g m^{-2})						
		Weedy			Weed-free			SY
Row Spacing Experiment		19 cm	76 cm		19 cm	76 cm		LSD
2016	Carman	254.1	236.2		559.5	1103		c
	St-Adolphe	518.3	727.7		886.5	1516		a
	Whitemouth	475.5	870.7		778.2	1222		ab
2017	Carman	194.0	486.2		916.4	1440		ab
	St-Adolphe	370.3	621.5		771.2	1119		bc
	Whitemouth	505.1	716.4		865.5	1284		ab
Main effect LSD		d	c		b	a		
Target Density Experiment [b]		0.75X	1.0X	1.5X	0.75X	1.0 X	1.5X	
2016	Carman	318.1	623.6	630.6	1058	925.7	849.0	c
	St-Adolphe	1022	1033	836.1	1069	1116	1185	a
	Whitemouth	814.6	849.0	953.9	857.3	896.7	950.8	b
2017	Carman	437.5	463.9	500.0	1061	1038	988.5	c
	St-Adolphe	515.3	634.7	668.1	823.6	770.1	760.8	c
	Whitemouth	736.1	659.7	658.3	775.0	877.8	812.5	c
Variety Experiment		DKB2260	DKB2360	DKB2410	DKB2260	DKB2360	DKB2410	
2016	Carman	246.9	370.0	481.3	747.5	917.3	1016	d
	St-Adolphe	618.9	460.1	947.7	1186	1134	1127	a
	Whitemouth	559.2	601.4	676.0	664.3	921.7	943.1	b
2017	Carman	245.5	468.8	219.6	912.6	987.1	780.6	bc
	St-Adolphe	639.9	734.3	546.2	674.8	705.7	1079	bcd
	Whitemouth	402.8	510.8	617.3	693.0	631.9	537.6	cd
Main effect LSD					b	a	a	

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation within experiment. Letters are presented for the significant main effect within weedy, weed-free, or their interaction. Abbreviation: SY = site-year.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}

7.2.1 Leaf Area Index

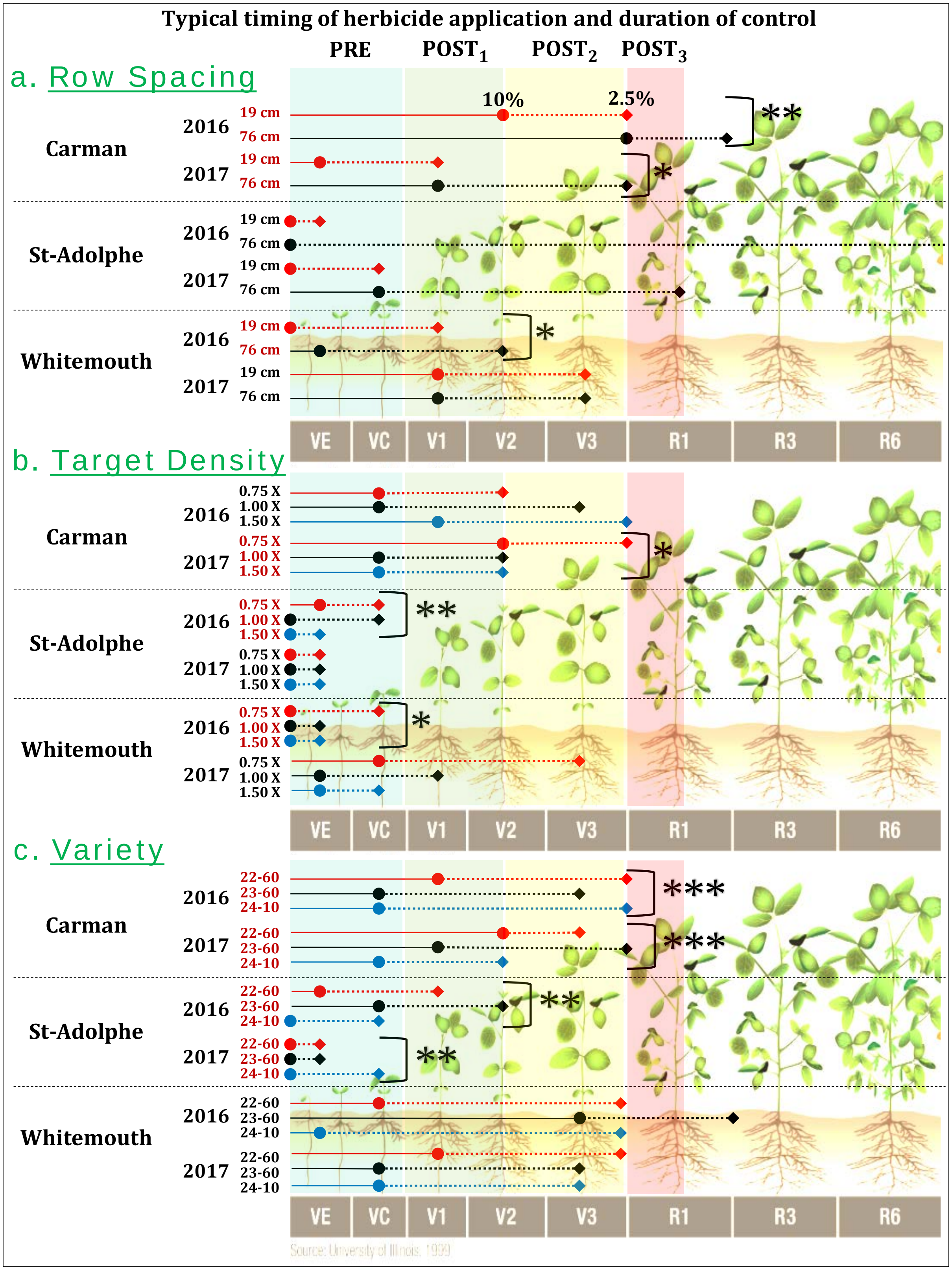
Leaf area index of soybean was calculated from five random plants within untreated (weedy) and one weed-free plot from each experimental treatment at each location in 2016. Soybean leaves were removed from the petiole and their surface area was measured with a LI-COR 3100C leaf area meter (LI-COR Biosciences, Lincoln, NE). Within individual plots, soybean leaf areas were summed and the leaf area index was calculated using the ground area estimation from five soybean plants based on their actual density. Leaf area indices were then analyzed with a mixed model approach. Fixed effects used in the mixed model were the herbicide treatment, the experimental treatment, and the location. Random effects were the block nested within the location and the experimental treatment nested within the block and location interaction. Differences among herbicide treatments were determined using the SLICE statement. Conformation of the residuals to the Gaussian 'normal' distribution was determined using the Shapiro-Wilk statistic (Littell et al. 2006). Lund's test (Lund 1975) was used to determine if extreme outliers were present and required further examination. Homoscedasticity was tested by visual inspection of residual vs. predicted values (Kozak and Piepho 2018), and corrected using the group option in the repeated statement to minimize the Aikaike information criterion (Littell et al. 2006). Using the PDMIX800 macro (Saxton 1998), Fisher's protected LSD at a significance level of 5% (α=0.05) was used to separate the means.

Table B3 Mean soybean leaf area index under weedy and weed-free conditions from three cultural weed management experiments at three locations in 2016. [a]

	Soybean Leaf Area Index						
Row Spacing Experiment	Weedy			Weed-free			SY LSD
	19 cm	76 cm		19 cm	76 cm		
Carman	2.66	1.75		4.09	3.00		b
St-Adolphe	3.60	2.07		6.43	3.50		a
Whitemouth	3.40	2.33		3.62	2.71		b
Main effect LSD	b	c		a	b		
Target Density Experiment [b]	0.75X	1.0X	1.5X	0.75X	1.0 X	1.5X	
Carman	2.36	3.79	3.22	4.09	4.44	5.68	
St-Adolphe	3.36	3.67	3.20	3.61	3.09	4.06	N.S.
Whitemouth	3.28	3.56	4.30	3.03	3.47	3.35	
Main effect LSD		N.S.		b	ab	a	
Variety Experiment	DKB2260	DKB2360	DKB2410	DKB2260	DKB2360	DKB2410	
Carman	1.36	1.77	2.78	4.10	5.46	5.55	
St-Adolphe	2.17	2.53	3.16	4.47	2.98	4.78	N.S.
Whitemouth	2.19	3.14	3.16	1.87	2.45	3.35	
Main effect LSD		N.S.		b	ab	a	

[a] Fisher's protected LSD ($p<0.05$) was used for least square mean letter separation within experiment. Letters are presented for the significant main effect within weedy, weed-free, or their interaction. Abbreviation: N.S. = not significant; SY = site-year.

[b] Soybean density of 1.0X is equivalent to 444,600 plants ha^{-1}



Source: University of Illinois, 1999

Figure B4 Image representing the end of the critical weed-free period (CWFP) in soybean development stage for row spacing, target density, and variety at each site-year. Solid lines represent the end of the CWFP at 10% acceptable yield loss (●·······) while dotted line extensions represent the end of the CWFP at a 2.5% acceptable yield loss (·······◆). Shaded areas (PRE, POST1 - 3) indicate the typical timing of herbicide applications and duration of weed management assuming limited residual activity. Significant differences among Gompertz equation (eq. 3) parameters within sites-years are indicated (p-value: * <0.05, ** < 0.01, *** <0.001).

Figure B5 Image taken at St-Adolphe 2016 in the row spacing experiment 41 days after seeding soybean in narrow (left) and wide (right) rows and kept weed free until the V3 development stage. Weed species growing in the inter-row space of the wide row soybean were *Echinochloa crus-galli* and *Setaria pumila*.

7.3 Appendix C: Structure of the Weed Community

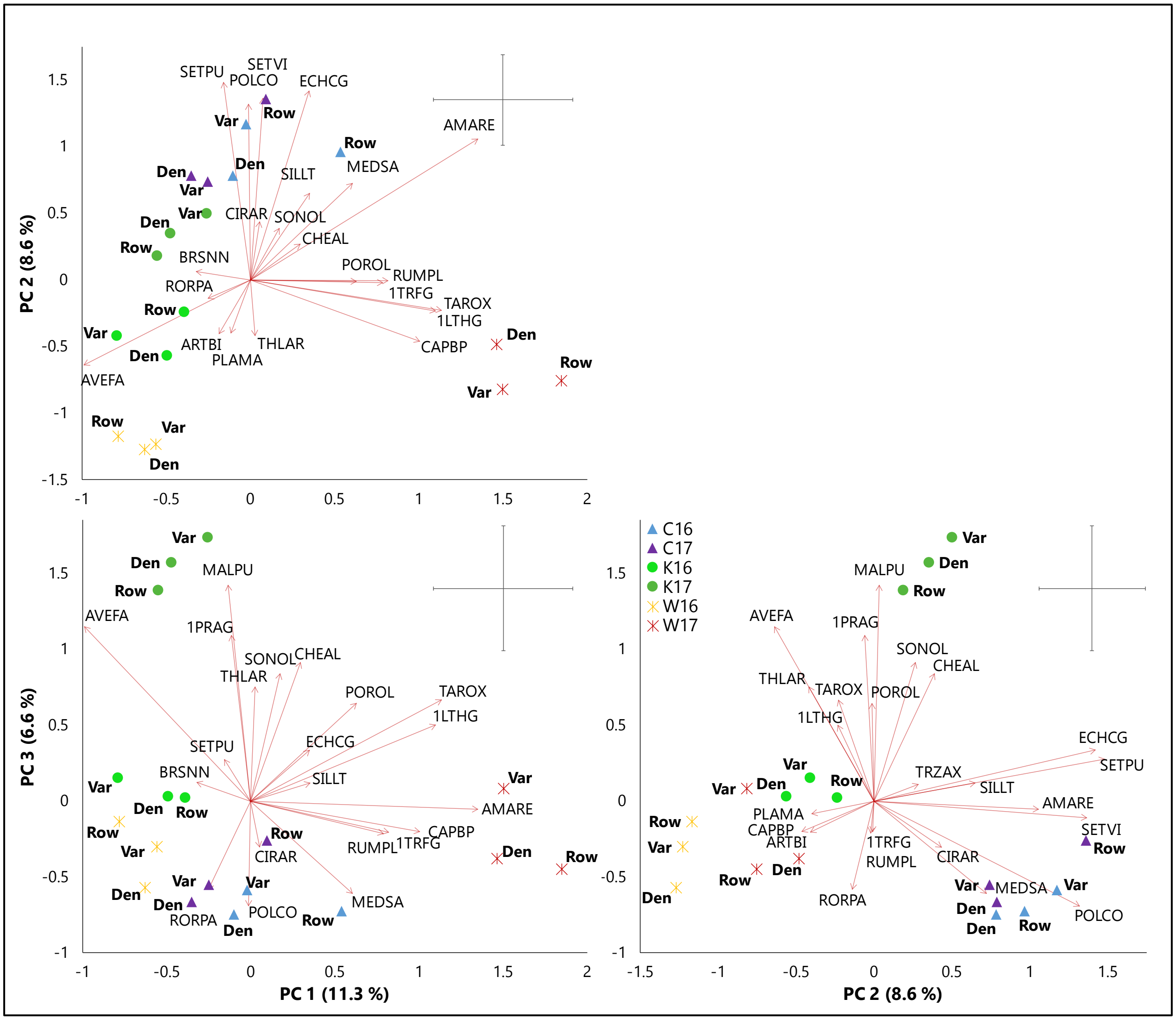


Figure C1 Biplots representing PC1 & PC2 (*top left*), PC1 & PC3 (*bottom left*), and PC2 & PC3 (*bottom right*) of field weed communities in weedy control treatments observed in three experiments at six site-years in southern Manitoba. Legend displays site-years coded by their location (**C**=Carman, **K**=St-Adolphe, **W**=Whitemouth) and year (20**16** and 20**17**). Individual soybean cultural weed management experiment's (**Row**=row spacing, **Den**=density, **Var**=cultivar) least significant means were plotted using Fisher's least significant differences (α<0.05) (lines). Code nomenclature for prominent weed species is found in Table 4.1.

Figure C2 Image of the flooding through the blocks of the target density experiment three days after an anomalous storm deposited over 150mm of precipitation.