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Seedbank change following adoption of integrated weed management (IWM) for a mixed population of ACCase-resistant and -susceptible green foxtail (*Setaria viridis*) in a rotation experiment.

by

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ABSTRACT

McLennan, Deanna J. The University of Manitoba, February 2021. Integrated weed management (IWM) in wheat, canola and soybean for a mixed population of ACCase-resistant and -susceptible green foxtail (*Setaria viridis*) in a rotation experiment.

In the spring of 2017, the germinable seedbank of a rotation experiment was characterized to determine the response of an ACCase-resistant green foxtail (*Setaria viridis* (L.) Beauv.) biotype and the total weed community to the crop phases and herbicide omission treatments applied from 2000-2016. Three herbicide omission treatments; herbicide omission during one crop phase, or two crop phases, and no herbicide omissions, were applied to two four-year crop rotations that consisted of annual and annual/perennial crop phases. In the 2017 spring seedbank, all herbicide omission treatments in the annual and annual/perennial rotations had the same average total seedbank density. In both rotations, species richness and non-foxtail weed species densities were greater when herbicides were omitted in two years of the four-year rotation. Yellow foxtail (*Setaria pumila* (L.) Beauv.) and green foxtail were the most abundant species in the seedbank. Yellow foxtail densities were reduced by 50% in the annual rotation, and 80% in the annual/perennial rotation when regular in-crop herbicide applications were made. Increased in-crop ACCase inhibitor usage resulted in greater total and ACCase-resistant green foxtail densities and a larger proportion of ACCase-resistant green foxtail in the seedbank. Crop phases that were more competitive (e.g. canola) or strategies that minimized weed seed rain (e.g. cutting for hay) resulted in lower total and ACCase-resistant green foxtail densities.

New crop phases and IWM treatments were introduced in the rotation experiment in the spring of 2017, the objective was to determine the immediate impact of the IWM treatments on the ACCase-resistant and -susceptible green foxtail biotypes, yellow foxtail, and all other weeds

in the seedbank after one year and with in-crop measurements during the field season. During the 2017 field season, the IWM treatments had no improvement in crop yield or weed suppression in soybean and canola, however, in the wheat IWM treatments there was a reduction in Setaria biomass and a trend for a small yield increase. After one year of IWM treatments, the IWM treatments in canola, wheat and soybean did not have a greater decrease for green foxtail, yellow foxtail or total seedbank densities compared with the Control treatment. The 2018 seedbank remained similar to the 2017 seedbank, green foxtail densities were greater where more ACCase inhibitor applications had been made and yellow foxtail densities were greater where more herbicide omissions had occurred. Multiple years of the IWM treatments in the annual and annual/perennial rotations may be needed before the effect become evident in the seedbank of this rotation experiment.

FOREWORD

This thesis includes an introduction, literature review, and two research chapters followed by a general discussion. The research chapters contain work completed at the University of Manitoba from April 2017 to October 2019. Chapters are written in the format of the Canadian Journal of Plant Science and follow the style defined by the Department of Plant Science, University of Manitoba, Winnipeg, MB, CA.

1.0 INTRODUCTION

Green foxtail (*Setaria viridis* (L.) Beauv.) and yellow foxtail (*Setaria pumila* (L.) Beauv.) are among the most abundant monocotyledonous weed species in Manitoba. Green foxtail and yellow foxtail ranked as the first and sixth weeds, respectively, based on the relative abundance on the 2016 Manitoba general weed survey (Leeson et al. 2016). In the past, ACCase inhibitor (Group 1) and ALS inhibitor (Group 2) herbicides were commonly relied upon for in-crop control of green and yellow foxtail in numerous crops. Consequently, green foxtail biotypes resistant to ACCase inhibitor and ALS inhibitor modes of action were identified in the 1990s (Heap and Morrison 1996; Volenberg et al. 2002). Yellow foxtail biotypes resistant to ACCase inhibitors were first reported in the 2016 herbicide-resistant weed survey (Beckie et al. 2016). As a result, these herbicide-resistant green and yellow foxtail biotypes present a primary challenge to agricultural production. In a rotation experiment at the University of Manitoba, a mixed population of ACCase-resistant and -susceptible green foxtail was identified (Murphy 2016). The rotation experiment was established in 2000, since then, three herbicide omission treatments have been applied to two four-year crop rotations. The herbicide omission treatments altered the composition of the below and aboveground weed communities (Schoofs et al. 2005; Gulden et al. 2011).

In the spring of 2017, a seedbank analysis on the rotation experiment was completed. The objective was to determine the occurrence of the ACCase-resistant green foxtail biotype in the rotation experiment in response to the in-crop herbicide omission treatments and crop phases that were applied from 2000-2016. Further objectives included characterizing yellow foxtail and the remaining weed community.

Integrated weed management (IWM) combines multiple weed management strategies to

constantly challenge weed populations and improve weed control (Liebman and Gallandt 1997). Utilizing multiple weed management methods reduces the selection pressure applied to each control method, making IWM suitable for herbicide-resistant weed biotypes (Harker and Donovan 2013). Cultural practices can be adjusted to become more effective against weeds, this includes increased seeding densities and more narrow row spacings, that help the crop canopy to close earlier in the season (Blackshaw et al. 2002). Manipulating crops for earlier canopy closure is particularly effective for management for green and yellow foxtail that are C_4 weeds and have a greater requirement for light and heat. Past research found cultural IWM strategies could be adjusted for management of green and yellow foxtail in Manitoba and North Dakota (Khan et al. 1996; Kabanyana 2004).

The rotation experiment was updated in the spring of 2017, the herbicide omission treatments were replaced with new IWM treatments and updated crop phases replaced the original crop phases. The IWM treatments consisted of cultural strategies, that had increased stand densities and narrower row spacings and were compared with standard practices. The updated crop phases in the rotation experiment were wheat (*Triticum aestivum* L.), canola (*Brassica rapa* L.), and soybean (*Glycine max* L. Merr). The objective of this study was to determine the direct impact of integrated weed management compared with standard management practices for control of a mixed population of ACCase-resistant and -susceptible green foxtail, yellow foxtail, and additional weeds. The response to the IWM treatments was measured one year later in the seedbank and supported with in-field measurements during the 2017 growing season that contributed to the 2018 seedbank.

2.0 LITERATURE REVIEW

2.1 Green and yellow foxtail

2.1.1 Distribution

Green foxtail (*Setaria viridis* (L.) Beauv.) and yellow foxtail (*Setaria pumila* (L.) Beauv.) are widespread across the Canadian prairie provinces, parts of the United States and Europe (Schröder et al. 2017). The foxtail species were likely introduced to North America from Eurasia, and were first identified in Quebec in the early 1800s (Steel et al. 1983; Douglas et al. 1985). By 1981, green foxtail was common to all prairie provinces and was present in 81% of surveyed fields, meanwhile yellow foxtail was more rare and occurred in only 0.4% of surveyed fields (Thomas and Wise 1982). In the 2016 Manitoba general weed survey, green foxtail was more abundant than yellow foxtail in most regions of the province, except yellow foxtail outranked green foxtail in the Interlake Plain and the Lake of the Woods ecoregions (Leeson et al. 2016). Across the prairie provinces, the *Setaria* species typically colonize high disturbance environments, including agricultural fields, ditches, and urban areas (Schröder et al. 2017).

2.1.2. Historical abundance

Green foxtail and yellow foxtail ranked among the top six weeds based on relative abundance for the first time in the 2016 Manitoba general weed survey (Leeson et al. 2016). Relative abundance for the Manitoba general weed surveys is calculated based on a combination of frequency, field uniformity and field density for each species identified. Green foxtail is a historically problematic weed in Manitoba that has held the number one rank since 1978 (VanAcker et al. 1999). Whereas, yellow foxtail advanced 24 ranks since the 2002 survey to the number six position (Leeson et al. 2016). The *Setaria* genus is considered among the most destructive group of annual weeds to agricultural systems worldwide (Dekker 2003). Other notable

weeds in the *Setaria* genus include knotroot foxtail (*Setaria parvifora* (Poir.) Kerguelen), bristly foxtail (*Setaria verticillate* (L.) Beauv.), and giant foxtail (*Setaria faberi* (Herm.)). All weedy *Setaria* species are related to foxtail millet (*Setaria italica* (L.) Beauv.)) a domesticated member of the *Setaria* genus (Dekker 2003).

2.1.3 Biology of green and yellow foxtail

2.1.3.1 Germination

Soil temperature and adequate moisture are critical for germination and seedling recruitment of green foxtail and yellow foxtail (Blackshaw 1979). Both foxtail species are warm-season annual grasses that use the C_4 photosynthetic pathway (Steel et al. 1983; Douglas et al. 1985), meaning photosynthesis is optimized under conditions of increased light and temperature. Germination and emergence of the foxtail species is improved at elevated soil temperatures (Vanden Born 1971), as a result, under cool Manitoba spring conditions, foxtail seedlings emerge in late May or early June once soil temperatures increase (Banting et al. 1973). The ideal temperature range for green foxtail germination is 15 ℃ to 35 ℃, whereas yellow foxtail typically germinates at a more limited temperature range of 20 ℃ to 25 ℃ (Dekker 2003). For green foxtail, soil temperature near 30 ℃ and soil moisture at field capacity resulted in uniform seedling recruitment (Peterson and Nalewaja 1992). The foxtail species can be problematic in both conventional and zero-tillage systems (Derksen et al. 2002). In conventional tillage systems, soil temperatures increase more rapidly on the darker soil surface in the spring, reaching ideal germination temperatures earlier (Douglas et al. 1985). However, if soil moisture is limited, zero-tillage systems that have improved moisture conservation may result in greater seedling recruitment (Bullied et al. 2003). Both foxtail species have an extended seedling recruitment period, with multiple flushes occurring late in the growing season, often following rainfalls

(Banting et al. 1973). Flushes that occur later in the growing season can help foxtail seedlings escape in-crop herbicide applications (VanAcker et al. 2000) and makes herbicide timing for products without some residual activity difficult (Norsworthy et al. 2012).

2.1.3.2 Shoot morphology of green and yellow foxtail

Aboveground, the foxtail species follow a standard monocot growth pattern, capable of forming tillers, and producing multiple spike-like panicles (Steel et al. 1983; Dekker 2003). Green foxtail can be distinguished from yellow foxtail by the arrangement of hairs at the leaf sheath (Douglas et al. 1985). On green foxtail, short hairs are arranged in a neat fringe along the ligule. Hairs on yellow foxtail are longer, and randomly located across the base of the leaf collar. The foxtail species respond differently to shade, green foxtail invests more biomass into leaf production, and yellow foxtail produces taller stems (Bubar and Morrison 1984). Green and yellow foxtail have a similar competitive ability when soil moisture is normal, but green foxtail competed better than yellow foxtail when soil moisture was limited (Nadeau 1984). Green and yellow foxtail plants typically grow to 45 cm in height but can reach heights of 90 cm tall. Under optimal temperature and moisture conditions, both foxtail species can produce seeds and complete their lifecycle in 30 days (Vanden Born 1971). Foxtail plants can produce upwards of 12,000 seeds, but typically each plant produces around 2,500 seeds (Vanden Born 1971). Most seeds are dispersed once green foxtail reaches fully maturity (Douglas et al. 1985), this can occur after harvest of annual crops such as canola and spring wheat (Burton et al. 2017).

2.1.3.3 Seeds and seed dormancy

Green foxtail and yellow foxtail plants produce seeds that are completely dormant at maturity (Vanden Born 1971). In fields in southern Saskatchewan, this primary dormancy was overcome about 10 weeks after seed dispersal (Banting et al. 1973). Green foxtail seeds can remain viable in the seedbank for 6-8 years, with an average of around 5 years (Dawson and Bruns 1975). The seedbank longevity of yellow foxtail is similar (Dawson and Bruns 1975). Green and yellow foxtail seeds can survive for a longer period of time at deeper soil depths, and persistence up to 39 years in green foxtail has been reported (Dekker 2003). Green foxtail and yellow foxtail seeds commonly emerge between a depth of 1-5 cm, however depths between 1.5 - 2.5 cm is preferred, and recruitment declines past depths of 7.5 cm (Dawson and Bruns 1975; Dekker 2003). Conventional and zero-tillage practices alter the vertical distribution of weed seeds in the seedbank (Ball 1992). Reduced tillage in a cropping system concentrates weed seeds near the soil surface, favouring the small-seeded foxtail species. When conventional tillage is used, seeds can be buried to the depth of tillage which is often beyond the depth for maximum seedling recruitment for the foxtail species. Green foxtail seedlings recruited from significantly deeper depths when conventional tillage was used compared with recruitment depths of green foxtail in no-tillage systems, soil disturbance caused by tillage operations were suspected to cause seed movement and alter the depth of green foxtail recruitment (Sissons 1999).

2.1.4 Crop yield losses caused by green and yellow foxtail

Green foxtail can cause significant yield losses, especially when large densities of seedlings establish prior to crop emergence (Blackshaw 1979; Peterson and Nalewaja 1992). In a greenhouse experiment, when green foxtail was seeded 4 days before wheat (*Triticum aestivum* L.), the fresh weight of the wheat was reduced by 50%. However, if green foxtail seeding was delayed until 4

days after the wheat was seeded, the fresh weight of the wheat was reduced by only 13% (Peterson and Nalewaja 1992). Similarly, in Alberta, a 5% yield loss was estimated if the density of green foxtail is 100 m^{-2} that emerged within a week of the wheat crop, but yield losses in wheat, barley (*Hordeum vulgare* L.) and canola (*Brassica rapa* L.) were minimal if green foxtail emerged after the crop (O'Donovan 1994). Estimated yield losses from green foxtail and yellow foxtail are variable in field studies across western Canada, and yield reductions can vary among locations, years, and different times of recruitment of foxtails relative to the crop (O'Donovan 1994). Recruitment and growth of green foxtail is responsive to soil nitrogen content (Blackshaw et al. 2003), greater available soil nitrogen increased yellow foxtail biomass and consequently decreased the final yield in corn (Clay et al. 2006).

2.2 Herbicide resistance

Herbicide-resistant weed biotypes develop when unresponsive individuals in a weed population are selected for after repeated applications of the same herbicide mode of action (MOA) are made (Jasieniuk et al. 1996). The first documented case of herbicide resistance was triazine resistant common groundsel (Senecio vulgaris L.) reported in 1968 (Ryan 1970), following the introduction of synthetic herbicides in the 1940's. In the last five decades, a large increase in the number of herbicide-resistant weed biotypes has been documented globally (Heap 2021). To date, at least 262 weed species have developed resistance, and resistance to every known herbicide mode of action has been identified. Altogether, 515 unique cases (species x site of action) of herbicide resistance have been reported worldwide. Among countries, Canada ranks third in the number of identified herbicide-resistant weed species (68), following the United States (166) and Australia (95). In Canada, the additional cost of weed management and the decreased crop yields due to

resistant weeds are estimated to cost producers $1.1 to 1.5 billion dollars annually (Beckie et al. 2016).

2.2.1 ACCase inhibitors (Group 1) herbicides

The first ACCase inhibitor (Group 1) herbicide was registered in 1976 (Diclofop), since then twenty ACCase inhibitor herbicides have been registered on numerous crops, including flax (*Linum usitatissimum* L.), alfalfa (*Medicago sativa* L.), and cereals (Beckie et al. 2013; Heap 2014). All ACCase inhibitor herbicide active ingredients belong to one of three chemical families, (i) Aryloxyphenoxy propionic acids (Fop), (ii) Cyclohexadione (Dim), and (iii) Phenylpyrazolin (Den). These compounds inhibit the eukaryotic version of Acetyl-CoA carboxylase, the enzyme responsible for catalyzing the first step in fatty acid synthesis (Devine 2002). Dicots have a eukaryotic and a prokaryotic version of this enzyme, while members of the Gramineae family only have the eukaryotic form (Devine 2002). This is the basis for the selectivity of the ACCase inhibitor mode of action between monocots and dicots. Moreover, certain cereals crops (e.g. spring and durum wheat) have tolerance to ACCase inhibitors applications, this selectivity is based on herbicide detoxification and makes ACCase inhibitors selective graminicides (Devine 1997). ACCase inhibitor herbicides, were widely used by producers for in-crop control of green foxtail and wild oats (*Avena fatua* L.) (Heap and Morrison 1996). Improved in-crop weed control of two difficult weed species allowed for reductions in tillage and helped producers transition to reduced tillage systems in the 1990s. ACCase inhibitor herbicides are considered as high-risk for the development of herbicide-resistant weed biotypes (Beckie 2006). Risk of herbicide-resistant weed selection is directly connected to usage, and less than ten applications of ACCase inhibitor products may select for resistant individuals.

2.2.2 ACCase-resistant green foxtail and yellow foxtail

At present, 48 weed biotypes with resistance to ACCase inhibitors (Group 1) have been reported worldwide (Heap 2021). In Manitoba, ACCase-resistant green foxtail biotypes were first identified during 1991 in south-central Manitoba (Heap and Morrison 1996). Both the 2008 and 2016 herbicide-resistant weed surveys found ACCase-resistant green foxtail in 44% of surveyed fields in Manitoba. The prevalence of ACCase-resistant green foxtail had doubled from the 2003 herbicide-resistant weed survey results for Manitoba (Beckie et al. 2016). ACCase-resistant yellow foxtail was first formally reported on the 2016 Manitoba herbicide-resistant weed survey (Beckie et al. 2016). This ACCase-resistant yellow foxtail biotype was found in 32% of the fields in Manitoba where yellow foxtail was found.

The target-site Ile-1781-Leu mutation is the only known mutation to provide resistance to ACCase inhibitors in the *Setaria* species (De Prado et al. 2004). The Ile-1781-Leu mutation is a single nucleotide polymorphism (SNP) at the 1781 codon, resulting in replacement of leucine with isoleucine (Délye et al. 2002). The amino acid replacement changes the structure of the protein, so the herbicide is no longer able to bind. The Ile-1781-Leu mutation confers resistance to all three ACCase inhibitor chemical families (fops, dims, dens), however, the level of resistance to each family may vary (Kaundun 2014). There is no fitness penalty associated with the Ile-1781-Leu mutation in *Setaria* species. Instead, this mutation has been linked to improved fitness, defined as the improved ability to survive and reproduce, when no herbicide selection pressure is applied (Wang et al. 2010). In a greenhouse experiment, the ACCase-resistant green foxtail biotype grew 1 to 2 additional tillers and consistently produced 24% more seeds than the susceptible biotype, however seeds from the resistant biotype had a lower thousand kernel weight (TKW) and potentially lower recruitment compared with the susceptible biotype (Wang et al. 2010). Likewise,

fitness penalties for the Ile-1781-Leu mutation have not been observed in other species (e.g., *Lolium rigidum*) (Vila-Aiub et al. 2005).

2.2.3 ALS-resistant green foxtail and yellow foxtail

Green foxtail and yellow foxtail have developed resistance to ALS/AHAS inhibitor (Group 2) herbicides, another extensively used herbicide mode of action (Volenberg et al. 2002; Beckie et al. 2016). ALS inhibitors bind to the acetolactate synthetase/acetohydroxyacid enzyme, preventing assembly of microtubules and the subsequent development of amino acids (Tranel et al. 2002). The ALS inhibitor herbicides are classified into 5 main chemical families, (i) sulfonylureas, (ii) triazolopyrimidines, (iii) imidazolinones, (iv) sulfonylaminocarbonyltrizolinones and (v) pyrimidinylthiobensoic acids. These compounds control both monocot and dicot weeds and can be applied to many crops including pulses and cereals (Beckie et al. 2013). More ALS inhibitor active ingredients have been registered compared with all other modes of action, and globally, more acres are treated with ALS inhibitor herbicides than ACCase inhibitors (Heap 2014). The 2016 herbicide-resistant weed survey identified ALS-resistant green foxtail on 6% of the 50 surveyed fields where green foxtail was present. ALS-resistant yellow foxtail occurred on 17% of the 60 surveyed fields (Beckie et al. 2016). At present, 165 weed species resistant to ALS inhibitors have been documented globally (Heap 2021).

2.3 Integrated weed management (IWM)

Integrated weed management (IWM) promotes the use of multiple strategies to manage weed populations (Liebman and Gallandt 1997), in a way that does not have negative economic or environmental effects. Application of diverse weed control methods varies the selection pressure and reduces the risk of herbicide resistance by "*keeping weed communities off balance*" (Harker

and Donovan 2013). Herbicide-resistant weed biotypes are often identified in systems with simple weed control methods (e.g. no-tillage, with continuous use of herbicide-resistant crops) (Owen et al. 2015). Weed management strategies combined under IWM can include tillage, crop rotation, and biological control. One common IWM strategy is to enhance the competitive ability of the crop against weeds, this can be achieved by increasing the seeding density and planting in more narrow rows, facilitating more rapid crop canopy closure (Blackshaw et al. 2002). When spring temperatures are cool, enhancement of crop competition is effective at reducing seedling recruitment and growth of C_4 weeds, including the *Setaria* species, that have increased requirements for heat and light (Vanden Born 1971). Past research found cultural strategies, including seeding rate, could be manipulated to successfully manage green and yellow foxtail. (Khan et al. 1996; Kabanyana 2004). In North Dakota, control of green foxtail using either a 2X seeding rates or early seeding dates provided equivalent control to an in-crop ACCase inhibitor application (Khan et al. 1996).

2.4 Weed seedbanks

Soil seedbanks are defined as the collection of viable seeds within the upper layer of the soil profile (Cavers 1995), where seeds are able to persist for multiple years until conditions favourable for germination occur. Seeds enter the soil seedbank when mature plants disperse seeds that either remain on the soil surface or become covered by soil with wind, freeze thaw cycles or tillage. Environmental conditions have a large influence on the survivability of seeds in the seedbank. Seeds typically survive for longer periods of time at deeper burial depths (Dawson and Bruns 1975), where they are protected from decay and predators near the soil surface (Cavers 1995) and environmental cues enforce increased seed dormancy (Baskin et al. 2017). Both green and yellow foxtail seeds had greater longevity when buried at 20 cm depth than at 10 cm depth,

although seedling recruitment in germinated seeds was limited at both of these depths (Dawson and Bruns 1975). The majority of seeds exit the seedbank by germinating, making the seedbank the major source of weeds that infest crops. A direct relationship has been observed between the size of the soil seedbank and aboveground weed densities in field. Seedbank densities of 100-1,000 seeds m^2 within the 0-10 cm depth of the soil profile led to recruitment of 400 seedlings/m^2 (Forcella et al. 2014). Seedbank densities greater than 1,000 seeds m^2 resulted in large weed populations that required multiple management methods including tillage and herbicide applications (Forcella et al. 2014).

2.4.1 Agronomic influences on the seedbank

Crop choice and the associated herbicide applications and other agronomic practices that occur, largely influence which weed species survive and the number of viable seeds that enter the weed seedbank (Ball 1992). Equally important, environmental conditions such as temperature and precipitation experienced during the growing season serve as ecological filters that further determine the inputs to the seedbank (Clements et al. 1994). Because of this, the size and composition of the soil seedbank is known to reflect the history of past management practices that have occurred (Benoit et al. 1989; Cavers 1995). As previously mentioned, tillage directly affects the vertical distribution of seeds in the seedbank (Ball 1992; Sissons 1999). Besides, more weed seeds near the soil surface, zero-tillage systems may support greater weed diversity and contain more perennial weeds (Murphy et al. 2006). In Quebec, reduced herbicide rates and fewer herbicide treatments led to greater seedbank diversity and species richness, compared with more frequent herbicide applications at increased rates (Légère et al. 2005). IWM approaches, such as growing crops at increased stand densities decreased the number of weed species in the seedbank (Clements et al. 1994).

2.4.2 Herbicide-resistant weeds and seedbanks

Dormant seeds can survive in the seedbank over multiple years, allowing seeds with specific traits (e.g. resistance to ACCase inhibitors) to germinate and reproduce during future growing seasons (Warwick 1991). Herbicide-resistant weed seeds can quickly build up in the seedbank and survive in the system long after the selection pressure was applied. For example, one resistant palmer amaranth plant (*Amaranthus palmeri* S.Wats) can produce over 500,000 seeds that enter the seedbank (Norsworthy et al. 2014). The resistant palmer amaranth population could take over an agricultural field in as little as three growing seasons. Because of that, minimizing weed seed rain from herbicide-resistant weed species is commonly recommended as part of the management for resistant weed species. On the contrary, seeds from herbicide susceptible plants that remain in a seedbank, after the susceptible aboveground plants have been eliminated may buffer the onset of a resistant population (Warwick 1991). This occurs when susceptible and resistant seeds germinate at the same time and the presence of the susceptible individuals slows the selection for resistance. However, susceptible biotypes may not be able to fully recover, as most ACCase and ALS inhibitor mutations do not confer a fitness penalty, meaning, when the herbicide is no longer applied, the resistant biotypes will remain the field and continue reproducing (Sibony and Rubin 2003).

2.4.3 Seedbanks and diversity indices

Diversity of species in the weed seedbank can be characterized by species richness, species evenness and species diversity indices (e.g. Shannon-Wiener, Simpson's) (McGill and Magurran 2011). Species richness refers to the number of different species identified in the sampling area. Species evenness measures how balanced the species in a community are relative to each other. Many diversity indices are calculated using species richness and species evenness, including the

Shannon-Wiener diversity index. For instance, if two communities had the same species richness, the one with greater evenness would be considered more diverse (less even = more dominance) (McGill and Magurran 2011). Diversity index values in agricultural systems, whether aboveground or in the seedbank, are typically low (<2), as often just a few weed species are present (Légère et al. 2005). Many weed species occur at lower densities in agricultural systems, including newly introduced species or volunteers from crops (Buhler 1997). As a result, in agricultural fields, typically only a few weed species account for 70-90% of the seedbank (Buhler 1997) and are often similar to the main crops in rotation (Bullock 1992).

3.0 CHARACTERIZATION OF AN ACCASE-RESISTANT GREEN FOXTAIL BIOTYPE AND THE TOTAL SEEDBANK OF A ROTATION EXPERIMENT.

Abstract

In the spring of 2017, the germinable seedbank of a rotation experiment was characterized to determine the response of an ACCase-resistant green foxtail (*Setaria viridis* (L.) Beauv.) biotype and the total weed community to the herbicide omission treatments and crop phases applied from 2000-2016. The three herbicide omission treatments were: (i) omission during one crop phase, (ii) omission during two crop phases, (iii) and no herbicide omissions. All three herbicide omission treatments were applied to two four-year crop rotations that consisted of annual and annual/perennial crop phases. In the 2017 seedbank, the average total seedbank densities were the same among the three in-crop herbicide omission treatments in the annual and annual/perennial rotations. Green foxtail densities were greater in the control treatment that received frequent in-crop ACCase inhibitor herbicide applications. Further, the increased in-crop ACCase inhibitor usage resulted in greater densities and a larger proportion of an ACCase-resistant green foxtail biotype in the seedbank of the control treatment. Species richness and densities of yellow foxtail and non-foxtail weed species were greater when in-crop herbicides were omitted in the annual and annual/perennial rotations. Crop phases that were more competitive (e.g. canola) and management practices that reduced seedbank inputs resulted in lower seedbank densities for all weed species, including the ACCase-resistant green foxtail biotype.

3.1 Introduction

Worldwide, Canada ranks third in the number of unique herbicide-resistant weed biotypes (Heap, 2020), and across the western Canadian prairies, herbicide-resistant weed biotypes occur on over 50% of the arable cropland (Beckie et al. 2016). In western Canada, green foxtail (*Setaria viridis* (L.) Beav.) ranks amongst the most ubiquitous herbicide-resistant weed species, found in 44% of surveyed fields in Manitoba in 2016 (Beckie et al. 2016). Green foxtail has developed resistance to multiple widely used herbicide modes of action. At this time, there are green foxtail biotypes known to be resistant to ACCase inhibitors (Group 1), ALS inhibitors (Group 2) and microtubule assembly inhibitors (Group 3) (Heap and Morrison 1996)**,** and biotypes with resistance to ACCase inhibitors and microtubule assembly inhibitors have been reported across western Canada (Jasieniuk et al. 1994). Green foxtail is a widespread weed and has been the number one mid-season weed based on relative abundance in the Manitoba general weed survey since 1978 (Leeson et al. 2016).

In response to concerns over herbicide resistance, the University of Manitoba established a rotation experiment near Carman, Manitoba in 2000 (Schoofs et al. 2005). The objective was to reduce the selection pressure for herbicide-resistant weed biotypes through reduced in-crop herbicide applications. Two fully phased, crop rotations were established in a zero-tillage production system, one annual rotation [flax (*Linum usitatissimum* L.)-oat (*Avena sativa* L.)-canola (*Brassica rapa* L.)-wheat (*Triticum aestivum* L.)], and an annual/perennial rotation [(flax-oat-alfalfa (*Medicago sativa* L.)-alfalfa)]. Three herbicide omission treatments were applied to both crop rotations. Weed community analyses five and nine years after establishment found that in-crop herbicides could be omitted during the production of competitive crops (e.g. oat), without compromising yield when compared with in-crop herbicide use each year (Schoofs et al. 2005;

Gulden et al. 2011). Two herbicide omissions every four years (in oat and flax), however, led to yield reductions and increased weed populations.

The 2009 analysis of the rotation experiment found the *Setaria* species group, mainly green foxtail and yellow foxtail (*Setaria glauca* (L.) Beauv.), but also barnyard grass (*Echinochloa crusgalli* (L.) Beauv.) to a lesser extent, accounted for 53% of the seedbank density (Gulden et al. 2011). Further, in 2015, an ACCase-resistant green foxtail biotype was confirmed within this rotation experiment (Murphy 2016). The Ile-1783-Leu mutation was identified, and the ACCase-resistant green foxtail biotype was found to be insensitive to the 266 g ai L^{-1} (9X field rate) of clethodim. It is not known when or how the ACCase-resistant green foxtail biotype became part of the study, however, but we presume it has been there long enough for the population to be distributed throughout the rotation experiment.

In the spring of 2017, a second seedbank analysis was completed. The main objective was to determine the occurrence of the ACCase-resistant green foxtail biotype in the rotation experiment in response to the in-crop herbicide omission treatments and crop phases. Further objectives included characterizing the remaining weed community in relation to green foxtail. The hypotheses tested in this study were: (i) More frequent in-crop applications of ACCase inhibitor herbicides resulted in a greater density of the ACCase-resistant green foxtail biotype, and a larger proportion of the green foxtail population in these treatments would be resistant to ACCase inhibitors, (ii) Weed species sensitive to ACCase inhibitors will display the opposite response, with greater densities occurring when ACCase inhibitor usage was reduced, (iii) Reduced in-crop herbicide usage will result in greater weed diversity and greater densities of all weeds and individual weed species.

3.2 Materials and Methods

3.2.1 Experimental Location

The rotation experiment was established at the University of Manitoba Ian N. Morrison Research Station near Carman, Manitoba (49.497614, -98.040209). The soil at the rotation experimental site is an Orthic Black Hochfield Chernozem. Further information on the climate, soil characteristics, and past experiment management are described in (Schoofs et al. 2005; Gulden et al. 2011).

3.2.2 Experimental Design

Two fully-phased, four-year crop rotations were established in 2000. The first rotation consisted of annual crop phases only (flax-oat-canola-wheat). The second rotation was a mixture of annual and perennial crop phases (flax-oat-alfalfa-alfalfa). Both crop rotations were repeated three times in each block and three different in-crop herbicide omission treatments were applied, one to each repeat of each rotation. The first treatment (NO) omitted in-crop herbicide use during the oat crop phase only, the second treatment (NOF) omitted in-crop herbicide use in both the flax and oat crop phases. The Full Herbicide treatment (FH) dictated in-crop herbicide use in all crop phases in the rotation. These treatments imposed different levels of selection pressure on the weed species in the rotation experiment (Table 1). In addition to the two crop rotations, a chemical fallow and prairie grassland treatment were established for comparison. Altogether each block consisted of 26 treatments (4 crops x 2 rotations x 3 herbicide use intensity treatments plus one prairie grassland and one chemical fallow treatment).

Table 3.1 Active ingredient, group number and rate of the in-crop herbicides applied to the annual and annual/perennial crop rotations of the rotation experiment from 2000-2016. Application of ACCase inhibitor (Group 1) herbicides are shown in bold font. Total ACCase inhibitor herbicide use per rotation cycle is indicated for each herbicide omission treatment.

Rotation	Crop phase	Active ingredient	Group number	Rate (g a.i. ha^{-1})	Herbicide omission treatment		
					FH	NO	NOF
Annual	Oats	Thifensulfuron-methyl	2	10	x		
		Tribenuron-methyl	2	5			
	Flax	**Sethoxydim**	**1**	**500**			
		Bromoxynil	**6**	280	**x**	**x**	
		MCPA	**4**	280			
	Wheat	Thifensulfuron-methyl	2	10			
		Tribenuron-methyl	2	5	**x**	**x**	**x**
		Clodinafop-propargyl	1	**56**			
	Canola	Glufosinate ammonium	10	500	x	x	x
Annual/ Perennial	Oats	Thifensulfuron-methyl	2	10	x		
		Tribenuron-methyl	2	5			
	Flax	**Sethoxydim**	**1**	**500**			
		Bromoxynil	**6**	280	**x**	**x**	
		MCPA	**4**	280			
	Alfalfa year 1	**Sethoxydim**	**1**	**500**	**x**	**x**	**x**
	Alfalfa year 2	No in-crop herbicide applied	N/A	N/A			
Total in-crop ACCase inhibitor herbicide applications per rotation cycle.					2	2	1

3.2.3 Soil core collection

During late April of 2017, shortly after snowmelt and before germination or recruitment of weed seedlings, soil cores were collected for seedbank evaluation from each plot of the rotation experiment. Eight soil cores were taken randomly within each plot (= one experimental unit), but not within 1 meter of the plot boundaries. Each soil core was 10 cm in diameter and taken to a depth of 7 cm. The 7cm depth was selected as the majority of the weed seeds in a zero-tillage system were expected to be located within the top 5 cm of the soil profile (Ball 1992) and green foxtail seedlings were unlikely to recruit from a depth greater than 7 cm. The day after soil cores were recovered from the field, soil cores from each experimental unit were combined and homogenized, placed in trays (50cm L, 25cm W, 2.37 6cm H, The HC Companies), and transferred to the greenhouse (Plant Science Conservatory). Trays were watered daily and exposed to ambient greenhouse temperatures, with a daily average temperature of 21°C, and a daylength of 14 hours.

3.2.4 Clethodim treatment

At the four-leaf stage, the green foxtail seedlings were treated with 29.6 g a.i. L^{-1} clethodim (1x field rate) mixed with Amigo Adjuvant (phosphate ester surfactant) (0.005 V/V), using a spray cabinet (Agassiz Scientific Ltd., Saskatoon Canada) equipped with a single flat fan nozzle (80015, TeeJet XR, Wheaton, IL) to deliver a carrier volume of 100L ha^{-1} at 275 kPa. One-third of each tray was left untreated, to serve as a control and facilitate clear differentiation between green and yellow foxtail. Fourteen days after the clethodim application, weeds in each tray were quantified. In the clethodim treated section of the tray, the number of green foxtail plants that survived the herbicide application were counted using three 10cm x 10cm quadrats. In the unsprayed portion of each tray, green and yellow foxtail seedlings were enumerated in two 10 cm x10 cm quadrats. Foxtail densities were large enough and homogenous so this could be done with confidence. Densities of all other weed seedlings were determined by species within the quadrats in the unsprayed portion of the tray.

Once the counts were completed, the soil in each tray was stirred and this entire procedure was repeated for a total of four germination cycles, until the seedbank was exhausted. Between the second and third germination cycle, the soil was frozen for 3 weeks at -21 °C (Cardina and Sparrow, 1996). Due to low green foxtail recruitment in all subsequent germination cycles, the herbicide treatment was not repeated, and only total green foxtail recruitment was evaluated.

3.2.5 Statistical analysis

The statistical analysis was completed using SAS University software (version 9.4). From the green foxtail resistance data, the density and proportion of ACCase-resistant green foxtail and the density of all green foxtail plants were determined. Species richness was determined by summing the number of different weed species identified within each plot. The relative abundance

of each species was determined from the total density for each plot and was used to calculate the Shannon-Wiener Diversity Index (H):

(1) $$H = -\sum_{i=1}^{k} p_i \, lnp_i$$

Where p_i is the proportion of the total seedbank density represented by species i. p_i is multiplied by the natural logarithm of this proportion (lnp_i). The values for each species in the seedbank are summed and multiplied by negative 1.

Weed densities, the diversity indices, and species richness were subjected to a mixed model ANOVA. Data from both rotations were analyzed together, however, each rotation was analyzed as a treatment substructure to account for crop differences between the rotations. Crop phase, level of in-crop herbicide use (FH, NO, NOF) and rotation were considered fixed effects while replication and the interaction of rotation with replication were considered random. The kenwardrogers (KR) ddfm option was used. Means were separated using Fisher's protected least significant difference (alpha=0.05). The conformation of residuals to the Gaussian distribution and heterogeneity of variance were examined and corrected if necessary. Differences between the two crop rotations were analyzed by completing a separate mixed model analysis, using only the data from the oat and flax crops. All response variables and model parameters were the same in this analysis. The proportion of ACCase-resistant green foxtail was analyzed using the beta distribution model in Glimmix, the fixed and random effects for the model were the same as the mixed model analysis described above. The residual option and the logit link function were selected in the model statement, and treatment means were separated with Fisher's protected lsd.

3.3 Results

3.3.1 Total seedbank density

After sixteen years, the average total germinable seedbank densities did not differ among the three in-crop herbicide omission treatments (FH, NO, NOF) in either rotation. In the annual rotation, the average total seedbank density ranged from 3,300 seeds m^{-2} to 5,400 seeds m^{-2} in the FH treatment. The NO and NOF treatments had a larger range in total seedbank densities that was from 2,900 seeds m^{-2} to about 6,300 seeds m^{-2}. In the annual/perennial rotation, the average total seedbank density ranged from 3,600 seeds m^{-2} to 7,600 seeds m^{-2} in the FH treatment. There was a similar range of 4,200 seeds m^{-2} to 8,000 seeds m^{-2} in the NO and NOF treatments.

In the annual rotation, total seedbank densities were lowest in the canola phase (Fig. 3.1) and in the annual/perennial rotation total seedbank densities were lowest in the first-year alfalfa phase of the rotation experiment (Fig. 3.2). In the NOF treatment, total seedbank densities following the first-year alfalfa phase were lower compared with all other crop phases in the annual/perennial rotation. While total seedbank densities after the first-year alfalfa phase were lower compared with the oat phase in the NO treatment and both the oat and flax phases in the FH treatment, and all other crop phases resulted in intermediate densities. In the NO treatment of the annual rotation, the total seedbank density following the canola phase was about 2.2X lower compared with the wheat phase, and the total seedbank densities in the flax and oat phases were intermediate. In the NOF and FH treatments, the range in total seedbank densities between the lowest (canola) and greatest (oat) crop phases was about 2.1X and about 1.6X, respectively. Despite the large range in the total seedbank densities there were no statistical differences among crop phases within the NOF and FH treatments. Lack of statistical separation among the crop

phases may have been caused by the limitation of three replicates in the rotation experiment and the inherent spatial variability and relatively low sampling intensity in seedbank experiments.

3.3.2 Total green foxtail seedbank densities

Green foxtail was a main weed species in the seedbank of the rotation experiment and in both rotations, total green foxtail densities were directly related to ACCase herbicide use intensity (= # of applications per rotation cycle) (Fig. 3.1, Table 3.1). Averaged among crop phases, the total green foxtail densities were about 600 seeds m^{-2} in the NOF treatment (Fig. 3.1&3.2), which received only four in-crop ACCase inhibitor herbicide applications in both rotations over the duration of the experiment. One additional in-crop ACCase inhibitor herbicide application per rotation cycle in the NO and FH treatments increased the total green foxtail seedbank densities about 4-fold in the annual rotation. The influence of ACCase inhibitor usage on total green foxtail densities was less pronounced in the annual/perennial rotation, one additional ACCase inhibitor herbicide application in the NO and FH treatments increased total green foxtail densities about 3-fold (Fig. 3.1A&B). No differences in the total green foxtail seedbank densities were observed between the FH and NO treatments in both rotations.

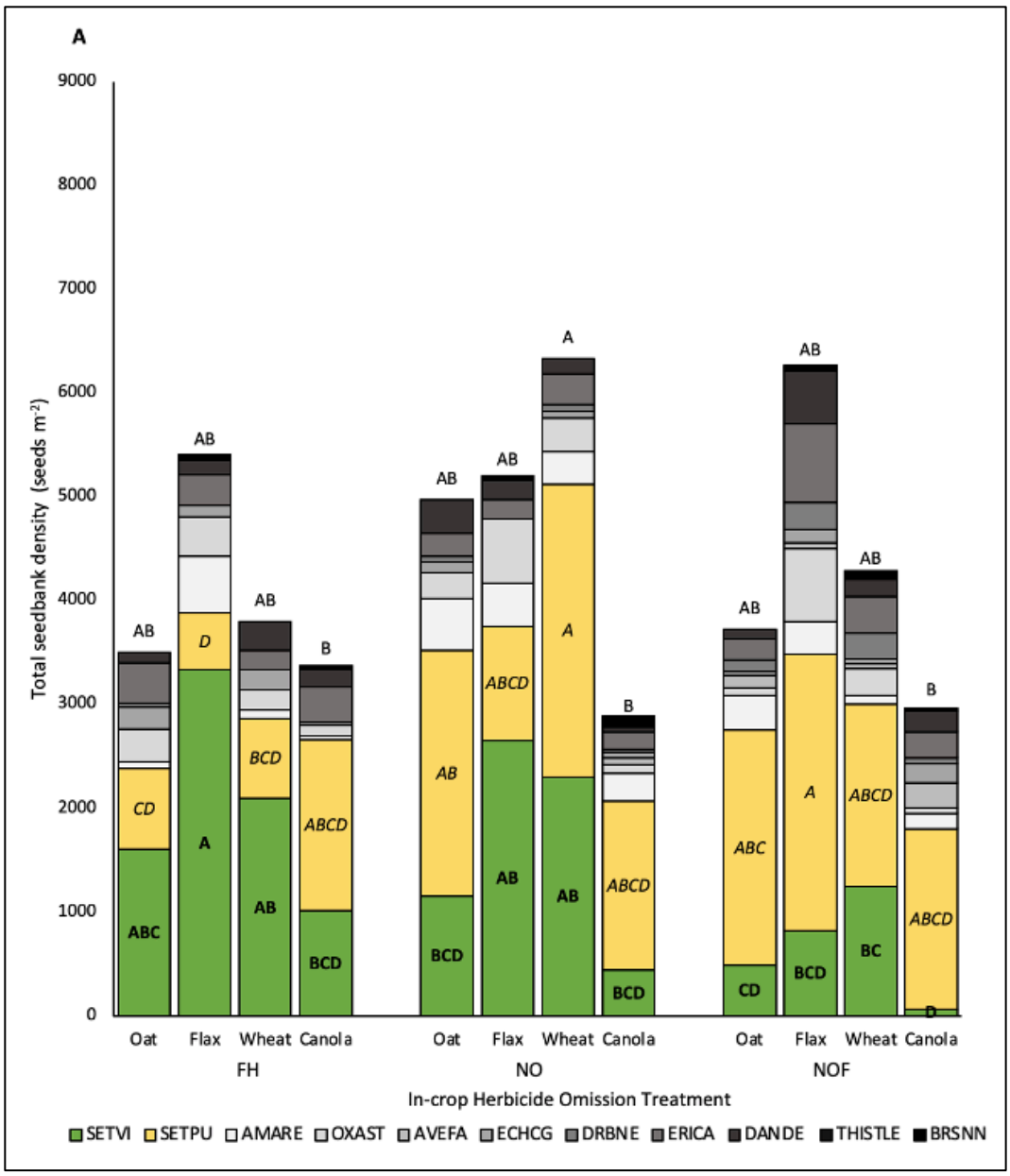

Figure 3.1 Total seedbank density in response to in-crop herbicide use for the annual rotation of the crop rotation experiment. Different herbicide use intensities were imposed by omitting in-crop herbicides in oat only (NO), in oat and flax (NOF), compared with in-crop application each year (FH). Densities for the individual weed species are indicated by different colours. Letter separations are shown for green foxtail (bold), yellow foxtail (italic), and total seedbank (normal font). Within each response variable, bars with different letter values are significantly different. Add NO

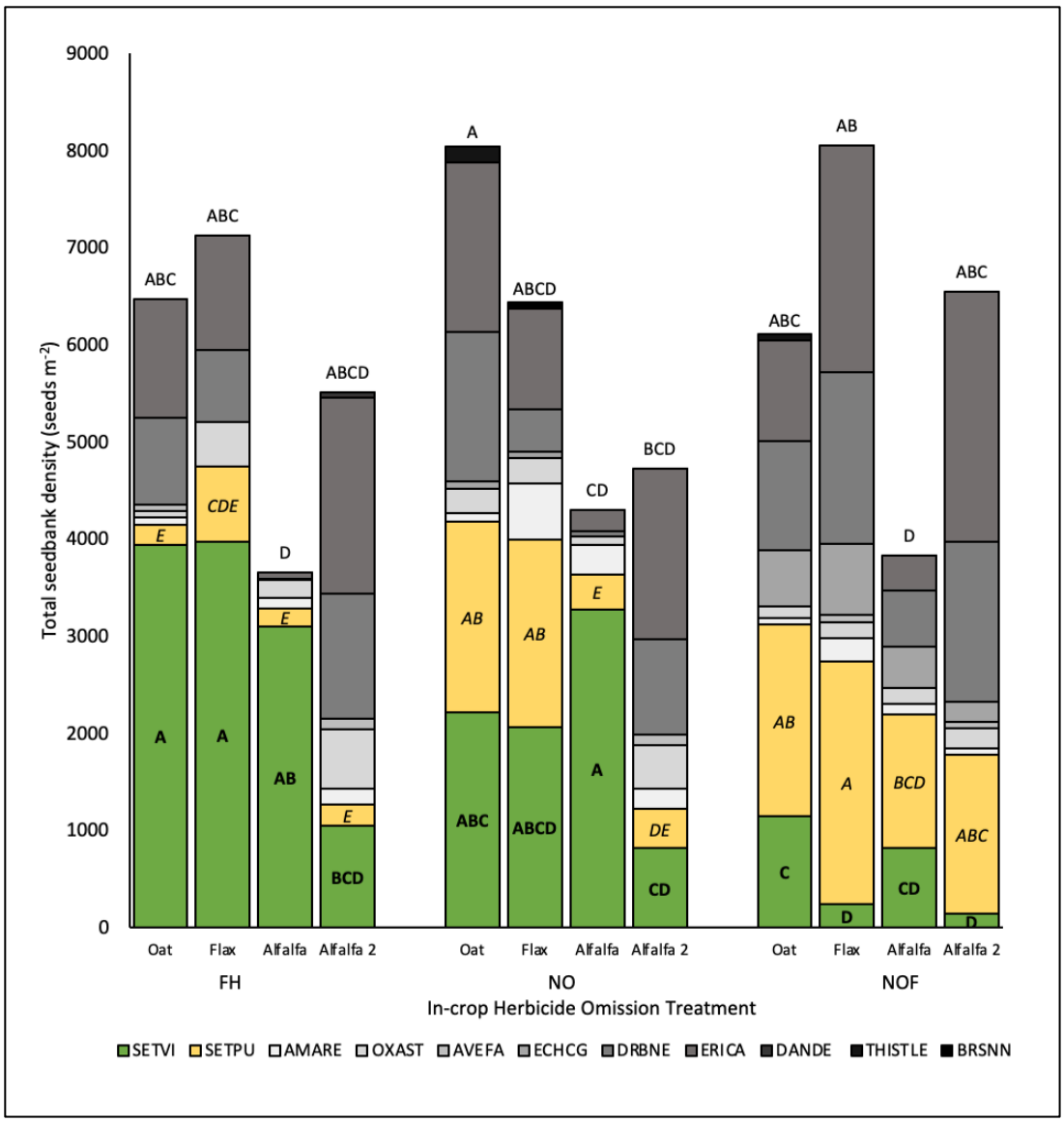

Figure 3.2 Total seedbank density in response to in-crop herbicide use intensity for the annual/perennial rotation of the rotation experiment. Different herbicide use intensities were imposed by omitting in-crop herbicides in oat only (NO), in oat and flax (NOF), compared with in-crop application each year (FH). Densities for the individual weed species are indicated by different colours. Letter separations are shown for green foxtail (bold), yellow foxtail (italic), and total seedbank (normal font). Within each response variable, bars with different letter values are significantly different.

Second-year alfalfa lowered the total green foxtail densities in the 2017 seedbank in the annual/perennial rotation. This differed among the in-crop herbicide omission treatments (Fig 3.2). In the FH treatment, total green foxtail densities in the second-year alfalfa phase were 4-fold lower compared with both the oat and flax phases, and 3-fold lower compared with the first-year

alfalfa phase, although the difference between the first- and second-year alfalfa phases was not significant. In the NO treatment, the crop phase with the greatest green foxtail densities was the first-year alfalfa phase, as a result, green foxtail densities in the second-year alfalfa phase were 4-fold lower compared with the first-year alfalfa phase, and 2-fold lower compared with the oat and flax phases. The increased total green foxtail densities in first-year alfalfa phase of the NO treatment may have been caused by an application of an ACCase inhibitor herbicide, that likely failed to control part of the green foxtail population. Green foxtail densities were generally lower in the NOF treatment, as a result, total green foxtail densities were equal in flax (248 seeds m^{-2}) and second-year alfalfa (133 seeds m^{-2}) and green foxtail densities in both phases were 8-fold lower than in the oat phase (1,100 seeds m^{-2}). Overall, second-year alfalfa was the only crop that reduced green foxtail densities in all of the herbicide omission treatments of the annual/perennial rotation.

Total green foxtail densities in the 2017 seedbank were often lowest in the canola phase in the annual rotation (Fig 3.1). In the FH treatment, total green foxtail seedbank densities in the canola phase (800 seeds m^{-2}) were lower compared with the flax phase (3,300 seeds m^{-2}). In the NOF treatment, total green foxtail densities were lower in the canola phase (70 seeds m^{-2}) compared with the wheat phase (1,000 seeds m^{-2}). In all other crop phases (oat and wheat in the FH treatment, oat and flax in the NOF treatment) green foxtail densities were not different from the flax or wheat phases in the FH and NOF treatments, respectively. In the NO treatment, there was a similar trend and a 6-fold range in green foxtail seedbank densities between the greatest (flax) and lowest (canola) crop phases but did not result in significant differences in green foxtail seedbank densities among the four crop phases. By comparison, the range in green foxtail densities among crop phases in the FH treatment was about 3-fold and in the NOF treatment was about 12-

fold. Large variation within treatments (NO, SEM=412.7 seeds m^{-2}, FH, SEM = 293.9 seeds m^{-2}) likely contributed to the lack of statistical separation in green foxtail densities among the crop phases. Among the crop phases in the annual rotation, only canola was able to reduce green foxtail seedbank densities, but not consistently among all in-crop herbicide omission treatments.

3.3.2 ACCase-resistant green foxtail seedbank densities

ACCase-resistant green foxtail seedbank densities were related directly to ACCase inhibitor herbicide use intensity (= # of applications per rotation cycle) (Table 3.1) similar to that observed in the total green foxtail seedbank densities. In both rotations, the lowest ACCase-resistant green foxtail densities were observed when in-crop herbicide and ACCase inhibitor applications were limited (NOF treatment) which received only one in-crop ACCase inhibitor application during each four-year rotation cycle compared with the other in-crop herbicide omission treatments (NO & FH) which received 2 ACCase applications in the four-year cycle (Fig. 3.2A). On average the NOF treatment, contained 50 seeds m^{-2} and 75 seeds m^{-2} of ACCase-resistant green foxtail in the annual and annual/perennial rotations, respectively. Two in-crop ACCase inhibitor applications per rotation cycle increased the ACCase-resistant green foxtail seedbank densities in the NO treatment by about 12-fold and increased densities by about 21-fold in the FH treatment of both rotations.

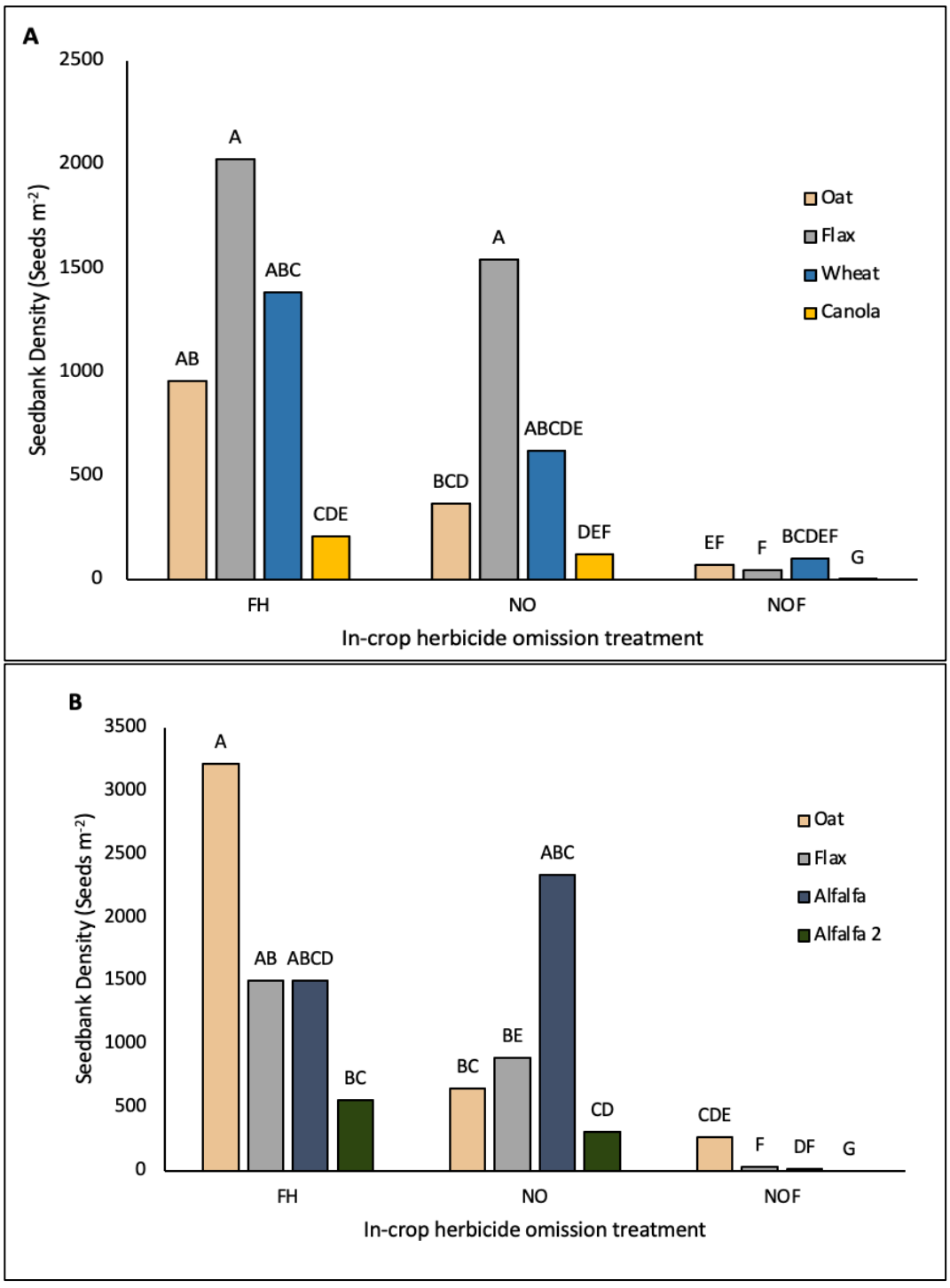

Figure 3.3 Density of the ACCase-resistant green foxtail biotype in response to in-crop herbicide use intensity in an annual (A) and annual/perennial (B) crop rotation in a field study initiated in 2000. Different herbicide use intensities were imposed by omitting in-crop herbicides in oat only (NO), in oat and flax (NOF), compared with in-crop application each year (FH). Bars with different letters are significantly different.

The canola and second-year alfalfa phases slowed the increase of the ACCase-resistant green foxtail seedbank densities when herbicide selection pressure was low (NOF) (Fig 3.3). This also was similar to the total green foxtail seedbank densities (Fig 3.1&3.2). In the NOF treatment, the canola phase of the annual rotation and the second-year alfalfa phase of the annual/perennial rotation contained 0 seeds m^{-2} of ACCase-resistant green foxtail (Fig 3.3). The other crop phases in both rotations had greater ACCase-resistant green foxtail densities in the NOF treatment that ranged from 11 to 250 seeds m^{-2}. In the NO and FH treatments, the second-year alfalfa and canola phases slowed the accumulation of ACCase-resistant green foxtail densities to a lesser extent. The second-year alfalfa phase had lower ACCase-resistant green foxtail densities compared with the oat and first-year alfalfa phases in the FH and NO treatments respectively. Similarly, the canola phase had lower ACCase-resistant green foxtail densities compared with the oat and flax phases in the FH treatment and lower ACCase-resistant green foxtail densities compared with the flax phase in the NO treatment. In both rotations, the canola and second-year alfalfa phases were successful at reducing theincrease in ACCase-resistant green foxtail densities particularly when the ACCase inhibitor selection pressure was low.

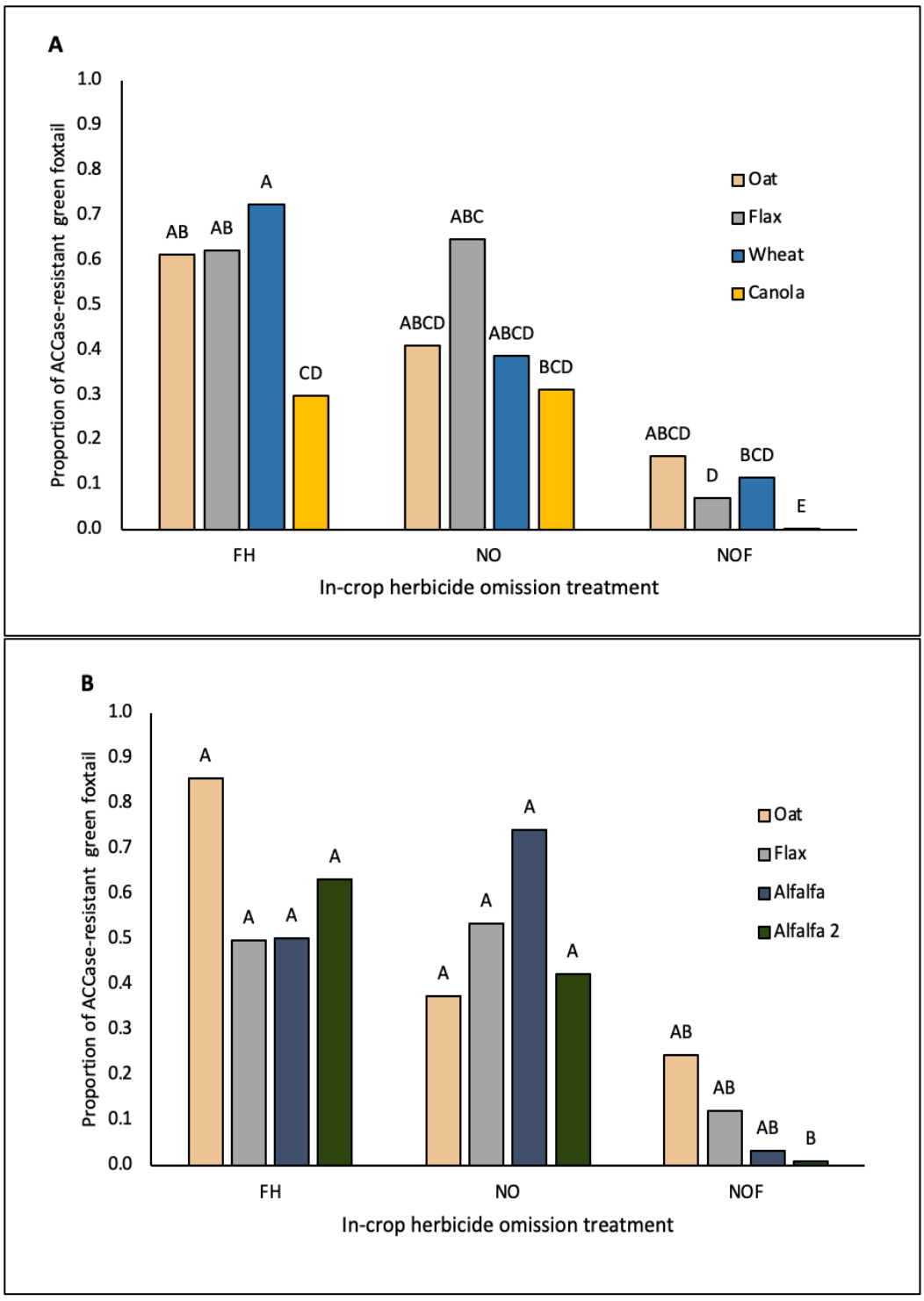

Figure 3.4 Proportion of the ACCase-resistant green foxtail biotype in response to in-crop herbicide use intensity in an annual (A) and annual/perennial (B) crop rotation in a rotation experiment initiated in 2000. Different herbicide use intensities were imposed by omitting in-crop herbicides in oat only (NO), in oat and flax (NOF), compared with an in-crop herbicide application every year (FH). Bars with different letters are significantly different.

Decreased in-crop ACCase inhibitor herbicide usage (NOF) resulted in a lower proportion of ACCase-resistant green foxtail only in the seedbank after the canola phase in the FH and NOF treatments, but not in any other crop phase (Fig. 3.4A&B). Lower ACCase selection pressure led to an ACCase-resistant green foxtail proportion of about 0 to 24% of the total green foxtail population in both rotations. Increased ACCase inhibitor herbicide application intensity in the NO and FH treatments resulted in a proportion of ACCase-resistant green foxtail that ranged from 30% to 86%. Closely related to the ACCase-resistant green foxtail density trends, the seedbank after the canola phase had a lower proportion of ACCase-resistant green foxtail in the FH and NOF treatments, but this difference was not significant in the NO treatment. Canola is competitive against green foxtail and was the only crop phase that received an in-crop herbicide that was effective against ACCase-resistant green foxtail, these factors likely contributed to the lower proportion of ACCase-resistant green foxtail in the seedbank following the canola phase. In the annual/perennial rotation, there was a 40% range in the ACCase-resistant green foxtail proportion in the seedbank in the FH and NO treatments. Large variation within treatments (NO, oat phase, annual rotation, SEM=0.21 units, FH, flax phase, annual/perennial rotation, SEM = 0.29) led to the lack of statistical separation in the ACCase-resistant green foxtail proportion in the seedbank among crop phases. Although, the second-year alfalfa and canola phases reduced ACCase-resistant green foxtail densities, canola was the only phase among both rotations that lowered the proportion of ACCase-resistant green foxtail in the seedbank of the rotation experiment.

3.3.3 Yellow foxtail seedbank densities

Yellow foxtail was the second most abundant weed species in the 2017 seedbank, and yellow foxtail densities were lowest in the FH treatment in all crop phases except the canola phase (Fig. 3.1A&B). In the oat and flax phases of the annual/perennial rotation, regular in-crop

herbicide applications (FH) lowered yellow foxtail densities by about 5-fold compared with the herbicide omission treatments (NO & NOF). Likewise, in the second- and first-year alfalfa phases, regular in-crop herbicide applications (FH) reduced yellow foxtail densities by about 7-fold compared with the NOF treatment. However, yellow foxtail densities in the first- and second-year alfalfa phases, remained low when herbicides were omitted in the oat phase only (NO). In the annual rotation, the average yellow foxtail densities in the FH treatment of the oat, flax and wheat phases were about 3X lower compared with the NO and NOF treatments. Suppression of yellow foxtail densities following the canola phase did not occur in any of the herbicide omission treatments with the same densities of yellow foxtail among herbicide omission treatment. This was opposite to the trend observed in total seedbank and green foxtail densities in the canola phase where reduced in crop herbicide use in other crops resulted in lower green foxtail densities.

3.3.4 Non-foxtail weed species seedbank densities

Analysis of oat and flax, the crop phases common to both crop rotations, confirmed the annual/perennial rotation had a larger total seedbank densities in the common crop phases compared with the annual rotation (Table 3.2). Seedbank densities of green foxtail and yellow foxtail were similar in the annual rotation and annual/perennial rotations, however, densities of non-foxtail weed species were greater in the annual/perennial rotation compared with the annual rotation. The non-foxtail weed species with greater densities in the annual/perennial rotation included Canada fleabane (*Conyza canadensis L.*), yellow woodsorrel (*Oxalis stricta L.*) and yellow whitlow grass (*Draba aizoides L.*). Despite the increased density of non-foxtail weed species in the annual/perennial rotation, average species richness and the average Shannon-Wiener diversity indices did not differ between the rotations.

Table 3.2 Seedbank densities of total and ACCase-resistant green foxtail, yellow foxtail, non-foxtail weeds and the total seedbank in the oat and flax phases that were common to the annual and annual/perennial crop rotations of the rotation experiment. Seedbank densities with a p-value less than 0.05 (bold) had significantly different densities in the annual and annual/perennial rotations.

Seedbank density	Annual rotation	Annual/perennial rotation	P-value
Total seedbank (seeds m^{-2})	4883	7265	**0.0075**
Green foxtail (seeds m^{-2})	1664	2195	0.2459
ACCase-resistant green foxtail (seeds m^{-2})	389	523	0.2483
Yellow foxtail (seeds m^{-2})	1604	1481	0.6327
Non-foxtail weeds (seeds m^{-2})	1614	3588	**0.0132**
Species richness	7.8	8.1	0.8755
Shannon-Wiener diversity index	1.34	1.23	0.3811

In total, 15 weed species were identified in the 2017 seedbank of the rotation experiment. The annual/perennial rotation contained all 15 weed species, while only 13 of these weed species were identified in the annual rotation (Fig. 3.1A&B). Green and yellow foxtail were identified as dominant weed species in both crop rotations. Together, green and yellow foxtail accounted for 70% of the seedbank density in the annual rotation and 50% of the seedbank density in the annual/perennial rotation. Other significant weed species in the rotation experiment were redroot pigweed (*Amaranthus retroflexus L.*), yellow woodsorrel, yellow whitlowgrass, and Canada fleabane (Table 3.3). Three species, wild buckwheat (*Polygonum convolvulus* L.), green smartweed (*Polygonum lapathifolium* L.) and volunteer alfalfa were considered rare because they were present in less than 20% of the plots, these species were included in the species richness, Shannon-Wiener diversity index and the total seedbank density analysis but were not analyzed individually. Hemp nettle (*Galeopsis tetrahit* L.) and purslane (*Portulaca oleracea* L.) were excluded as they were associated principally with the prairie grassland and chemical fallow treatments, respectively.

Of the nine non-foxtail species analyzed, lambsquarters (*Chenopodium album L.*) and barnyardgrass (*Echinochloa crus-galli (L.) P. Beauv.*), were found more frequently when

herbicides were omitted (NOF) (Fig. 3.1A&B). Lambsquarters increased by 14X which was almost significant (p=0.0643) in the annual rotation and increased by 19X (p=0.0079) in the annual/perennial rotation in the NOF treatment compared with the FH and NO treatments. Likewise, in the NOF treatment, barnyardgrass was 14X greater in the annual rotation (p=0.0001) and was 36X greater in the annual/perennial rotation compared with the FH and NO treatments (p=0.0295). Redroot pigweed, yellow whitlow grass and Canada fleabane showed a consistent trend of increased weed densities in the NOF treatment.

Crop phase affected the abundance of redroot pigweed, yellow woodsorrel, lambsquarters, and barnyardgrass in the 2017 seedbank (Fig. 3.1A&B). In the annual rotation, redroot pigweed (p=0.0212) and yellow woodsorrel (p=0.0027) had greater densities following the flax phase. Three additional weed species tended to be more abundant in the flax phase; these were yellow whitlow grass, dandelion and Canada fleabane. The canola phase often contained greater densities of lambsquarters and volunteer canola, and the wheat and oat phases often had greater densities of grassy weeds. In the annual/perennial rotation, the second-year alfalfa and flax phases resulted in greater weed densities, with greater lambsquarters densities in the second-year alfalfa phase (p=0.0011), and increased barnyardgrass densities in the flax phase (p=0.0028).

Table 3.3 Common name, binomial name and EPPO code for weed species identified in the 2017 seedbank of the rotation experiment.

Common name	Binomial name	Bayer code
Green foxtail	*Setaria viridis*	SETVI
Yellow foxtail	*Setaria pumila*	SETPU
Barnyardgrass	*Echinochloa crusgalli*	ECHCG
Redroot pigweed	*Amaranthus retroflexus*	AMARE
Yellow woodsorrel	*Oxalis stricta*	OXAST
Lambs-quarter's	*Chenopodium album*	CHEAL
Wild oat	*Avena fatua*	AVEFA
Volunteer canola	*Brassica napus*	BRSNN
Canada fleabane	*Erigeron canadensis*	ERICA
Yellow whitlowgrass	*Draba aizoides*	DRBNE
Dandelion	*Taraxacum officinale*	TAROF

3.3.2 Seedbank diversity

Species richness and the Shannon-Wiener index increased in response to reduced herbicide inputs in specific crop phases in the 2017 seedbank of the rotation experiment (Fig 3.5A&B). In the wheat phase of the annual rotation, when in-crop herbicides were omitted from the oat and flax phases (NOF), the number of species was 9.0 compared with the FH treatment which contained 6.6 species. Species richness was greater in all crop phases in the annual/perennial rotation. On average, reduced in-crop herbicide applications in the NOF treatments of the annual/perennial rotation resulted in an average of about 3 more weed species in each phase compared with the FH treatment. In both rotations, the NO treatment contained an intermediate number of species. Species richness was not affected by crop phase in either rotation. In the oat phase of the annual/perennial rotation, the Shannon-Wiener diversity index increased in response to the in-crop herbicide omissions. The Shannon-Wiener diversity index value in the FH treatment (0.98) of the oat phase indicated greater dominance or lower weed diversity compared with the NO (1.30) and NOF treatments (1.45). The Shannon-Wiener diversity index was not influenced by herbicide inputs in the annual rotation or crop phase in either rotation.

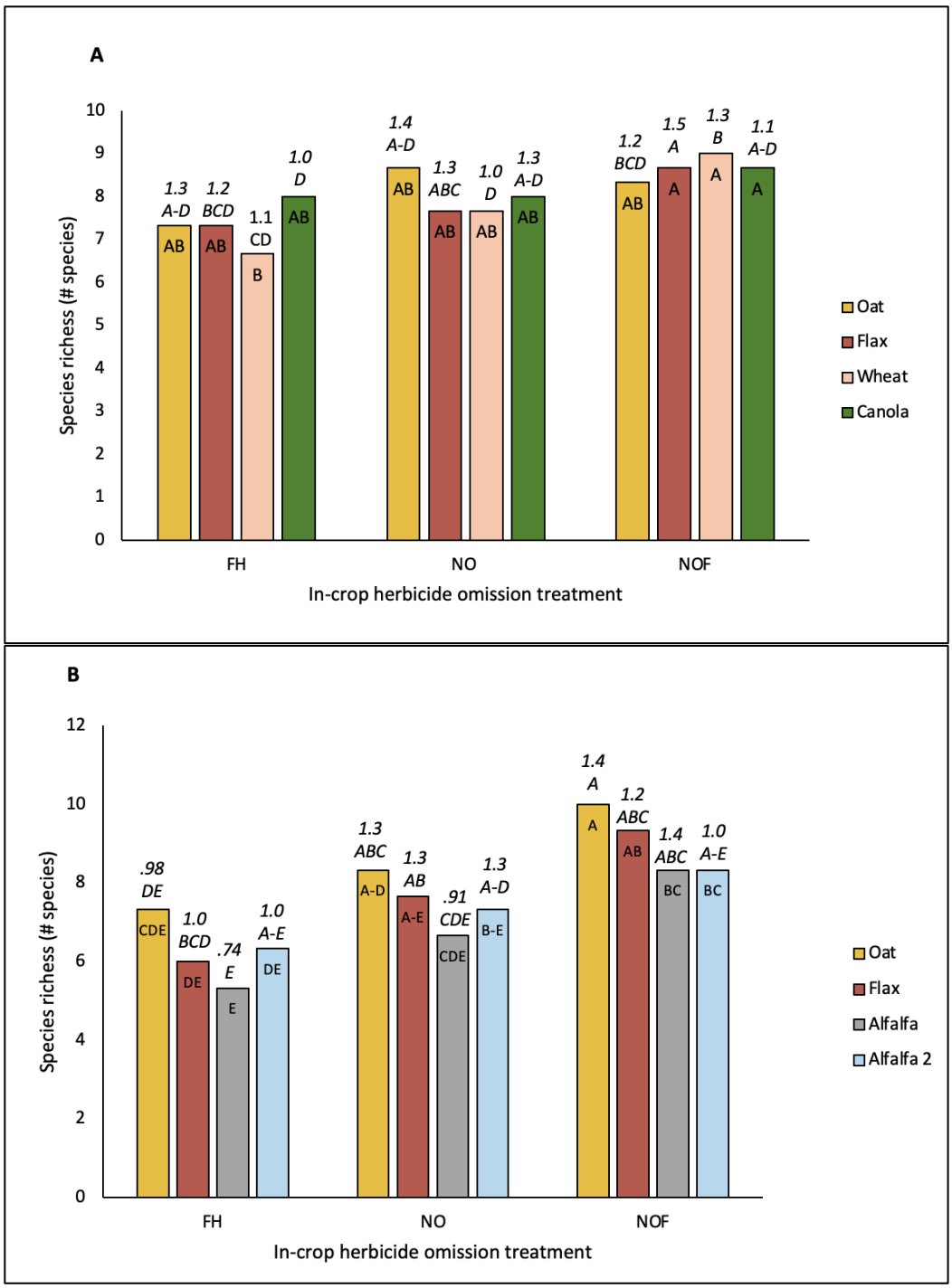

Figure 3.5 Species richness and the Shannon-Wiener diversity index (italic font) in response to in-crop herbicide use intensity in an annual (A) and annual/perennial (B) crop rotation in a rotation experiment initiated in 2000. Different herbicide use intensities were imposed by omitting in-crop herbicides in oat only (NO), in oat and flax (NOF), compared with an in-crop herbicide application every year (FH). Bars with different letters are significantly different.

3.4 Discussion

3.4.2 Rotation effect differences

The two-years of alfalfa in the rotation experiment was not representative of an alfalfa crop that would be terminated after 3-5 years of production (Malhi et al. 2007), this may explain why the annual/perennial rotation had a greater 2017 total seedbank density in 2017 compared with the annual rotation. Alfalfa is sensitive to weed competition at early developmental stages, therefore proper weed management is recommended prior to alfalfa establishment (Dillehay et al. 2011). In the rotation experiment, the crop planted before alfalfa was flax, that had a greater seedbank compared with other crops in the 2009 analysis (Gulden et al. 2011). Moreover, alfalfa is a legume species that hosts symbiotic bacteria capable of fixing soil nitrogen. Because of this, soil nitrogen level may have been greater following the alfalfa phases in the annual/perennial rotation, although this was not measured. Increased nitrate nitrogen at the soil surface, following the alfalfa termination may have increased weed seedling recruitment densities (Malhi et al. 2007). Yellow foxtail seeds soaked in a potassium nitrate solution had greater germination compared with seeds soaked in water (Peters et al. 1963). Increased soil nitrogen levels promotes above or belowground growth of many weeds including green foxtail (Blackshaw et al. 2003). In a greenhouse experiment, when soil nitrogen was doubled, green foxtail growth increased by 42%, whereas wheat growth increased by 12% (Peterson and Nalewaja 1992). Increased soil nitrogen following two years of alfalfa may have promoted weed growth in the oat phase (7,154 seeds m^{-2}) of the annual/perennial rotation, compared with lower weed densities in the oat phase in the annual rotation (4,098 seeds m^{-2}).

3.4.3 ACCase-resistant green foxtail biotype

It is not known from where the ACCase-resistant green foxtail biotype in the rotation experiment originated nor is the duration of time this biotype has been in the rotation experiment known. One possibility is the ACCase-resistant green foxtail biotype could have been selected for in the rotation experiment. The ACCase inhibitor mode of action is considered to be high-risk for selection of resistant biotypes, with as few as 10 applications resulting in selection (Beckie 2006). One case documented, seven ACCase inhibitor applications (diclofop-methyl and sethoxydim) within a decade selected for ACCase-resistant green foxtail biotypes (Heap and Morrison 1996). Due to the high risk of selection, consecutive applications of ACCase inhibitors is not recommended (Beckie and Harker 2017), however back-to-back ACCase inhibitor herbicide applications occurred in both rotations of the rotation experiment. During the first 16 years of the rotation experiment, ACCase inhibitors were applied up to 4 times to each of the NOF treatments, and up to 9 times to the FH and NO treatments, potentially enough to select for resistant individuals. Further, there is a greater risk of herbicide-resistance evolving in zero-tillage systems (Beckie et al. 2008), as reliance on chemical control is increased. Alternatively, this ACCase-resistant biotype may have been introduced from elsewhere on the Ian N. Morrison Research Station farm. Failure of ACCase inhibitors on green foxtail had been observed in other areas of the research farm for a number of years. Regardless, it is known that by 2017, at the time of this seedbank analysis, the ACCase-resistant biotype appeared to be distributed throughout the rotation experiment and was reflected in the treatment-dependent distribution found in this study.

ACCase-resistant green foxtail densities were greater in the annual/perennial rotation (740 seeds m^2), compared with the annual rotation (443 seeds m^2). There was no difference in the order of application between the two rotations, however, the ACCase inhibitor active ingredients

differed between the wheat (clodinafop) and first-year alfalfa (sethoxydim) crop phases. Resulting in two applications of sethoxydim in the annual/perennial rotation, instead of one clodinafop and one sethoxydim treatment per 4-year cycle in the annual rotation. Another difference with the annual/perennial rotation was the second year alfalfa phase did not receive an in-crop herbicide application (Schoofs et al. 2005). Due to this difference, ACCase inhibitors were the only in-crop herbicides applied that controlled grassy weeds in the annual/perennial rotation. Whereas in the annual rotation, ACCase-resistant green foxtail populations would have been reduced by the application of glufosinate to canola.

3.4.4 Seedbank differences between green and yellow foxtail

In the 2017 seedbank, yellow foxtail densities were similar to green foxtail, however the species responded differently to the crop phases in the rotation experiment. In the annual/perennial rotation, both species acted similarly among all the crop phases, with lower densities of green and yellow foxtail following the alfalfa phases. In the annual rotation, yellow foxtail densities were not reduced following the canola phase, unlike green foxtail where the reduction was significant. The herbicide applied to canola, glufosinate, has the same efficacy on both foxtail species when applied at 291 and 409 g ai ha^{-1} (Corbett et al. 2004). Slight differences in the time of seedling recruitment between the two species, however, could have influenced the efficacy of the glufosinate application on these species. Green foxtail seeds germinate more rapidly than yellow foxtail seeds at temperatures from 10 ºC to 35 ºC, and germination of yellow foxtail seeds is delayed more at cooler temperatures compared with green foxtail seeds (Manthey and Nalewaja 1987). Yellow foxtail seeds have improved germination at increased temperatures (Dekker 2003). Thus, delayed seedling recruitment in yellow foxtail may have allowed more yellow foxtail seedlings to escape the glufosinate application compared with green foxtail. Another consideration

is competition of yellow foxtail is improved under high moisture conditions (Nadeau 1984). The 2014-2016 growing seasons all received above normal precipitation potentially favouring yellow foxtail (Environment Canada, 2020).

3.4.5 Differences among crop phases

Canola and first-year alfalfa were effective at reducing the total seedbank density and the ACCase-resistant green foxtail densities in their respective rotations, and canola kept the proportion of ACCase-resistant green foxtail in the seedbank low. Canola is a C_3 crop and once established is competitive against green foxtail and other summer annual weeds (Blackshaw et al. 2002). Besides increased crop competition, the glufosinate herbicide applied to canola has a different mode of action that is effective against the ACCase-resistant green foxtail biotype (Group 10, glutamine synthetase inhibitors). Rotating the MOA of herbicides is commonly recommended to mitigate the development of herbicide-resistant weeds (Beckie and Harker 2017). In alfalfa, the use of mechanical weed control (cutting for hay) in conjunction with an in-crop herbicide limited weed seed rain. Harvesting alfalfa for hay is a good management tactic for reducing weed populations (Beckie et al. 2014) The management of canola and alfalfa in the rotation experiment align with recommended management practices for managing herbicide-resistant weeds (Beckie and Harker 2017).

3.4.1 2017 total seedbank density

Methodology likely did not cause different total average seedbank densities in the rotation experiment between the 2009 analysis (9,000 seeds m^{-2}) and the 2017 analysis (5,000 seeds m^{-2}). In 2009, around 7,700 cm^3 of soil was sampled from each plot, by taking 16 soil cores that were 6.4 cm wide to a 15 cm depth (Gulden et al. 2011). In contrast, the 2017 analysis collected 8 soil cores, that were 10 cm in diameter to a 7 cm depth from each plot, this captured a smaller soil

volume of 4,400 cm^3 or 56% of the soil volume collected in 2009. The soil core sizes were changed for the 2017 analysis because the main focus of this seedbank experiment was green foxtail, that has smaller sized seeds that germinate from 0.5 – 8 cm depths (Vanden Born 1971); further, in a zero-tillage production system the vast majority of these seeds would be located in the top 7 cm of soil (Ball 1992). In small research plots the weed seedbank can be accurately determined by collection of as few as 5 soil cores that are each 10 cm in diameter followed by grow-outs to determine the germinable portion of the seedbank (Benoit et al. 1989). Regardless of the soil volume collected, when completing a soil grow-out a minimum surface area of 250 cm^2 should accurately estimate the seedbank (Forcella 1992). Therefore, differences in methodology should have no effect on the seedbank density estimates, potential reasons for the differences between the analyses include weather conditions during the previous year and spring temperatures prior to soil core collection (Forcella et al. 1992).

3.4.6 Seedbank diversity

Both crop rotations supported a similar number of weed species, with 13 species in the annual rotation and 15 species in the annual/perennial rotation of the rotation experiment. In a similar rotation experiment, greater diversity of crop lifecycles (i.e. annual and perennial crops) within conventional cropping systems also did not increase species richness (Ulber et al. 2009). Species richness was only affected when changes were made to weed management practices. In the rotation experiment, intensity of herbicide input was more influential than previous crop or rotation on the number of species. This is supported by the significantly greater number of weed species in the NOF treatment of the annual/perennial rotation. Generally, cropping systems with limited herbicide inputs contain a greater number of weed species (Hyvonen and Salonen 2002).

In the rotation experiment, values for the Shannon-Wiener diversity index were low, typical for conventional agricultural systems (Légère et al. 2005), but were slightly greater than the Shannon-Wiener index values from the 2009 analysis (Gulden et al. 2011). The small increase, signifying greater diversity, is likely due to an increase in the number of species identified from an average of 7 in the 2009 seedbank to an average of 8 in the 2017 seedbank. Herbicide usage had a larger effect on the diversity indices compared with crop phase. Other research has found that herbicide or tillage were more important than previous crop at influencing weed species diversity (Bàrberi et al. 1997).

Long-term crop rotation experiments are important for providing a "realistic assessment", and can provide deeper insights on the effects of agronomic management practices on crop yield (Johnston and Poulton 2018). Long-term field experiments have studied soil properties and fertility (Roth), crop rotation and tillage (Bàrberi and Lo Cascio 2001; Cardina et al. 2002), and entire management systems (Davis et al. 2005). Similar studies have focussed on seedbank composition changes in response to herbicide inputs over time (Ball 1992; Sosnoskie et al. 2009). This rotation experiment is the first continuously running crop rotation experiment where a herbicide-resistant biotype has been characterized in response to herbicide input treatments. Continuous rotation of these herbicide input treatments and crop phases, allowed confirmation that repeated herbicide applications of the same MOA led to greater seedbank densities of the herbicide-resistant weed species.

3.5 Conclusion

After 16 years of crop rotation and herbicide omission treatments, the seedbank of the rotation experiment was analyzed after, including characterizing an ACCase-resistant green foxtail biotype. The seedbank analysis confirmed that repeated applications of ACCase inhibitors let to

increased densities and an increased proportion in the seedbank of ACCase-resistant green foxtail. On average, two ACCase inhibitors herbicide applications in every 4 years increased herbicide-resistant green foxtail densities by 17X compared with one ACCase inhibitor application every 4 years. The herbicide omission treatments applied in the rotation experiment demonstrates the direct relationship between herbicide usage and the of herbicide-resistant biotypes. In the rotation experiment, crop management strategies such as another effective herbicide MOA or a mechanical form of weed management reduced the ACCase-resistant green foxtail densities and proportions. Integrated weed management strategies for ACCase-resistant green foxtail biotypes that rely on a combination of cultural and herbicidal control should be evaluated.

4.0 RESPONSE OF AN ACCASE-RESISTANT AND -SUSCEPTIBLE GREEN FOXTAIL BIOTYPE AND ADDITIONAL WEEDS ONE YEAR AFTER IWM TREATMENT IMPLEMENTATION.

Abstract

Previously, ACCase-resistant and -susceptible green foxtail biotypes, yellow foxtail and additional weeds were characterized in a rotation experiment that had imposed three herbicide omission treatments on two crop rotations for sixteen years. In the spring of 2017, new crop phases and IWM treatments were implemented in the rotation experiment. The objective was to determine the immediate impact of the IWM treatments on the entire seedbank, with a focus on green foxtail, after one year and with in-crop measurements during the field season. During the 2017 field season, the IWM and Control treatments had the same final yield in soybean and canola and the same level of weed suppression. However, in the wheat IWM treatments there was a reduction in *Setaria* species biomass and a trend for a small yield increase compared with the Control treatment. After one year, the IWM treatments did not have a greater seedbank reduction for any green foxtail, yellow foxtail or non-foxtail weed densities compared with the Control treatments. Multiple years of the IWM treatments in the annual and annual/perennial rotations may be needed before the effect of the IWM treatments becomes evident in the seedbank of this rotation experiment. Overall, after one year of the IWM treatments, the 2018 seedbank was similar to the 2017 seedbank. ACCase-resistant and total green foxtail densities were greater where more ACCase inhibitor applications had occurred in the original experiment and weed species sensitive to ACCase inhibitors had greater densities where more herbicide omissions had occurred.

4.1 Introduction

Green foxtail (Setaria *viridis* (L.) Beauv.) is a summer annual monocotyledonous weed, that has been the top-ranked mid-season weed species based on the relative abundance in Manitoba for the past 40 years (Leeson et al. 2016). A closely related *Setaria* species, yellow foxtail (*Setaria pumila* (Poir) Roem & Schult.), advanced from the 32nd rank in 2012 to the 6th rank in 2016 (Leeson et al. 2016). From the 1980s to the 1990s green and yellow foxtail populations were managed readily using ACCase inhibitor (Group 1) and ALS inhibitor (Group 2) herbicide modes of action in Manitoba (Heap and Morrison 1996). Both foxtail species have developed resistance to ACCase and ALS inhibitor modes of action (Heap and Morrison 1996; Beckie et al. 2016), and green foxtail biotypes resistant to microtubule assembly inhibitors (Group 3) also exist in western Canada (Jasieniuk et al. 1994). In Manitoba, the 2016 herbicide-resistant weed survey identified the ACCase-resistant green foxtail biotype in 44% of surveyed fields, and the ACCase-resistant yellow foxtail biotype in 32% of surveyed fields (Beckie et al. 2016). Considering the abundance and widespread resistance of green and yellow foxtail, effective management practices that reduce the selection pressure on in-crop herbicides need to be evaluated in current Manitoba cropping systems.

Integrated weed management (IWM) combines multiple approaches to manage weeds (Liebman and Gallandt 1997). This reduces the selection pressure applied against the individual control method, making IWM appropriate for the management of herbicide-resistant biotypes (Harker and Donovan 2013). One common IWM strategy is to enhance the competitive ability of the crop against weeds, this can be achieved by increasing the seeding density and planting the crop at more narrow row spacing, allowing the crop canopy to close earlier in the season (Blackshaw et al. 2002). At low spring temperatures, enhancement of crop competition is effective on C_4 weeds, including the *Setaria* species, that have increased requirements for heat and a greater

saturation level for light (Vanden Born 1971). Past research found cultural strategies, including crop seeding rate, could be manipulated to successfully manage green and yellow foxtail in Manitoba and North Dakota (Khan et al. 1996; Kabanyana 2004).

Previous studies have described the abundance and diversity of individual weed species in a rotation experiment, where two crop rotations were subjected to three herbicide omission treatments for 16 years (Schoofs et al. 2005; Gulden et al. 2011). The rotation experiment contains a population of ACCase-resistant green foxtail (Murphy 2016), the density and proportion of the ACCase-resistant biotype was characterized in the seedbank in the spring of 2017 (Chapter 2). Further, at this time, yellow foxtail was the second most abundant weed species, and both foxtail species accounted for a large portion of the total seedbank population. In the spring of 2017, the rotation experiment was updated where new, integrated weed management (IWM) treatments replaced the herbicide omission treatments, and updated crop-phases were overlaid on the original crop rotations. The IWM treatments compared increased crop stand densities and narrower row spacings to standard practices for the management of all weeds with a focus on green and yellow foxtail. The crop phases in the rotation experiment were changed to wheat (*Triticum aestivum* L.), canola (*Brassica rapa* L.), and soybean (*Glycine max* L. Merr) to match the top three commercial crops currently produced in Manitoba (Statistics Canada 2019).

The objective of this study was to investigate the immediate impact of integrated weed management compared with standard management practices on control of a mixed population of ACCase-resistant and -susceptible green foxtail, yellow foxtail, and additional weeds. The response to the treatment changes was measured the following year in the seedbank in the spring of 2018 and supported with in-field measurements during the 2017 growing season that contributed to the 2018 seedbank. We hypothesized that IWM strategies would effectively reduce the density

and proportion of the ACCase-resistant biotype in the 2018 seedbank compared with the standard management practices. Further, the IWM treatments were expected to be effective on yellow foxtail and additional weeds. Differences were expected among crop phases; the canola and wheat phases were expected to have a greater total seedbank reduction compared with soybean. However, the application of ACCase inhibitor herbicides in wheat was expected to further increase ACCase-resistant green foxtail.

4.2 Materials and methods

4.2.1 Experimental site (2000-2016)

The rotation experiment was established at the University of Manitoba Ian N. Morrison Research Station near Carman, Manitoba in 2000. Two fully phased, four-year crop rotations were initiated, one composed of annual crop phases (flax-oat-canola-wheat), the second with annual and perennial crop phases (flax-oat-alfalfa-alfalfa). Three herbicide omission treatments were applied to both crop rotations. The first herbicide omission treatment (NO) omitted the application of in-crop herbicides during the oat phase of the rotation, the second herbicide omission treatment (NOF) omitted in-crop herbicide use in the flax and oat phases of the rotation. The Full Herbicide treatment (FH) included an in-crop herbicide application in all crop phases in rotation. These treatments imposed different levels of herbicidal selection pressure, resulting in different weed densities and communities after nine years of rotation (Gulden et al. 2011). Further information on the rotation experimental site, the methods used from 2000-2016, and initial results are described in (Schoofs et al. 2005). The 2017 seedbank composition and occurrence of the ACCase-resistant green foxtail biotype were detailed in Chapter 3.

4.2.2 Experimental design

The experimental layout of the rotation experiment remained unchanged; a Randomized Complete Block Design (RCBD) with the original 4 x 12 m experimental units. In 2017, the former annual rotation that consisted of flax-oat-canola-wheat was replaced with soybean-wheat-canola-wheat. The annual/perennial rotation of flax-oat-alfalfa-alfalfa was changed to a three-year rotation of wheat-canola-soybean. Due to the switch to a three-year rotation, the second-year alfalfa plots were removed from the annual/perennial rotation. For clarity within this thesis, the original crop rotation names (annual, and annual/perennial) were not changed.

Table 4.1 Arrangement of the original three herbicide omission treatments (FH, NO, NOF*) into the two integrated weed management treatments (Control, IWM1, IWM2) implemented in spring 2017 at the rotation experiment. Seeding rates and row spacing distances for the new Control and IWM treatments are listed below for wheat, canola, and soybean.

Treatment Name (2000 – 2016)	Full Herbicide (FH)		No herbicide in oat (NO)	No herbicide in oat and flax (NOF)
Treatment Name (2017 – Present)	Control		IWM1 and IWM2	
Crop	Seeding rate	Row spacing (cm)	Seeding rate	Row spacing (cm)
Wheat	300 seeds m^{-2}	28	450 seeds m^{-2}	19
Canola	100 seeds m^{-2}	19	150 seeds m^{-2}	19
Soybean	444,600 seeds ha^{-1}	76	666,900 seeds ha^{-1}	38

* FH = In-crop herbicide applied to every crop. NO = In-crop herbicide omitted during oat. NOF = In-crop herbicide omitted during oat and flax.

The two original herbicide omission treatments (NO & NOF) were replaced with an integrated weed management treatment relying on a combination of cultural weed management techniques (Table 1) that included narrower row spacings and increased seeding densities. Although the same IWM treatment was applied was to both former herbicide omission treatments (NO & NOF), these treatments will continue to be separated due to potential legacy effects. The former NO treatment became IWM1, and the former NOF treatment became IWM2. The former

Full Herbicide treatment remained a Control treatment that used standard production practices for these crops (wider row spacings and lower seeding densities).

4.2.3 Field work

4.2.3.1 Seeding

Before seeding, plots were fertilized using a disc drill (R-Tech Industries Ltd., Homewood, MB) to apply urea, monoammonium phosphate, and ammonium sulfate fertilizers based on soil-test recommendations (Agvise Laboratories, Northwood, North Dakota). All crops were seeded using the same disc drill. Wheat (Cardale, CWRS) and soybean (23-60 RY, Dekalb) were seeded on May 20, 2019. Granular inoculant (Cell-Tech, Monsanto BioAg**,** *Bradyrhizobium japonicum*) was applied with the soybean seed at a rate of 3.6 kg ha^{-1}. Canola (233P, InVigor® hybrid, BASF) was seeded on May 24, 2019. In Manitoba, canola is typically grown at a narrow row spacing, so only the seeding densities were adjusted in the canola IWM treatments. Row spacing and seeding densities for wheat and soybean and the densities for the canola that were used for the Control and IWM treatments can be found in Table 1.

4.2.3.2 Weed management

Before crop emergence, glyphosate (Roundup Transorb ®, Monsanto Canada Inc.) was applied at a rate of 900 g a.i ha^{-1} (540 g a.e./L) over the entire experiment using a tractor mounted sprayer equipped with flat fan nozzles to deliver an output of 100 L ha^{-1} at 276 kPa. In-crop herbicide applications were made on June 25, 2017. Wheat was treated with 10 g a.i. ha^{-1} thifensulfuron methyl and 5 g a.i. ha^{-1} tribenuron methyl (Refine® SG, FMC Canada) with clondiafop at a rate of 56 g a.i. ha^{-1} (Horizon® NG, Syngenta® Canada). Soybean was treated with 900 g a.i. ha^{-1} glyphosate (Roundup Transorb® HC, Bayer CropScience Canada). Canola was treated with glufosinate formulated as Liberty® 150SN (BASF Canada) at the rate of 500 g ai ha^{-}

[1]. All in-crop herbicides were applied using a bike sprayer with a 4 m boom equipped with flat fan nozzles, delivering a volume of 110 L ha^{-1} at a pressure of 276 kPa.

4.2.4 In-field measurements

4.2.4.1 Weed emergence

To determine initial weed abundance, weed densities were determined on June 20**,** 2017, shortly before the in-crop herbicide applications. Due to early developmental stages, green foxtail, yellow foxtail, and barnyardgrass were not differentiated, instead, they were grouped as "*Setaria* and related species". The *Setaria* and related species were counted in four randomly placed 10 cm x 10 cm quadrats within each plot. Broadleaf weeds and other grass weeds were counted using 20 cm x 20 cm quadrats, placed randomly in four locations of each plot all more than 1m from the edges of each experimental unit.

4.2.4.2 Plant height and stem thickness

Plant height measurements were taken at the R5 stage in soybean (August 17, 2017), full pod development in canola, and full flower in wheat (August 1, 2017). The distance from the soil surface to the highest point was measured on eight randomly selected plants within each plot. Immediately after harvest, the stem thickness of canola and soybean plants was measured. Width at the stem base, just above the soil surface, was determined using calipers on 8 randomly selected plants. In wheat, the number of reproductive tillers was determined after harvest; 8 plants were pulled in each experimental unit, and the tillers on each plant were enumerated.

4.2.4.3 Aboveground biomass

Crop and weed biomass samples were collected on the same date as the plant height measurements, at the time each crop had reached maximum aboveground biomass. Two 30 cm x 30 cm quadrats of aboveground biomass were removed at the soil surface, at the front and back of

each plot. Biomass samples were separated into the following four categories: crop, *Setaria* and related species, other grassy-weeds, and broadleaf weeds. Great densities made it not possible to speciate *Setaria* and *Echinochloa* species. The biomass samples were placed in paper bags and oven-dried at 60 ºC for 48 hours, after which the dried biomass samples were weighed.

4.2.4.4 Yield

Crops were harvested separately as they reached maturity; wheat was harvested on August 28, 2017, canola on September 18, 2017, and soybean on October 3, 2017. Each plot was harvested with a plot combine (Kincaid Equipment Manufacturing, St Haven, KS, USA) and the harvested grain was air-dried. Grain samples were cleaned using a Clipper M2BC seed cleaner (Blount/Ferrell- Ross, Bluffton, IN, USA), and if necessary, a Carter Dockage tester (XT7, Carter Day International, Minneapolis, Minnesota) was used to remove wild oats (*Avena fatua* L.). Grain moisture content was determined gravimetrically. Air-dry weights of grain subsamples were recorded, subsamples were oven-dried for 48 hours, dry weights were determined and grain yield was corrected to seed moisture contents of 13.5, 12, 13 % for wheat, canola, and soybean, respectively, (Purdue, 2016) and converted to kg ha^{-1}.

4.2.5 Seedbank collection

The soil core collection and greenhouse grow-out methods were identical to those described in the previous chapter (Chapter 2). In brief, soil cores were collected in late April of 2018, from each plot 8 soil cores (10 cm diameter) were taken to a depth of 7 cm. The soil cores from each plot were homogenized, distributed evenly in a 50 cm x 25 cm tray with drainage holes and moved to the greenhouse. Once the majority of green foxtail had reached the 3-4 leaf stage, two–thirds of each tray was treated with 29.6 g a.i. L^{-1} of clethodim using a spray cabinet (Agassiz Scientific Ltd., Saskatoon Canada) to quantify the ACCase-resistant green foxtail. To quantify the

abundance of yellow foxtail and other weed species, one-third of each tray was left untreated. Within the treated section, the number of ACCase-resistant and -susceptible green foxtail was counted in two 10 cm by 10 cm quadrats, fourteen days after application. Within the untreated section, other weeds and yellow foxtail were counted in three 10 cm by 10 cm quadrats. Once emergence ceased, the process was repeated for three additional germination cycles. Before the third germination cycle, the greenhouse trays were frozen at -21 ºC for three weeks.

4.2.6 Statistical analysis

4.2.6.1 Field data

Weed densities in the field were converted to the number of weeds m^{-2}, and values for all species were summed to determine total weed density for each plot. Species with a relative abundance of less than 10% were excluded in the statistical analysis as they were considered uncommon (Poos and Jackson 2012). Plant height and stem thickness measurements were averaged for each plot. Aboveground biomass for each category (crop, *Setaria* and related species, grassy weeds, broadleaved weeds) were converted to kg ha^{-1} prior to analysis. Data analysis was conducted using SAS University Edition (9.4Version). The response variables were total and individual weed densities, plant height, stem thickness, biomass, and yield. A mixed model analysis was used to compare these response variables across the three integrated management treatments (Control, IWM1, and IWM2) and the current crop phase (soybean, wheat, or canola). These and their interaction were considered as fixed effects. In the annual rotation, the two wheat crop phases were separated based on the preceding crop phase (flax or canola) to detect legacy effects should they occur. Replicate and the interaction of rotation and replicate were modeled as random effects. The crop rotations were analyzed together, but the data was divided by including a treatment substructure that separated the data based on previous crops and former herbicide

omission treatments. Data were inspected by reviewing the AIC values and the pattern of residuals to ensure the ANOVA assumptions were met. The repeated statement was used to correct for heteroscedasticity among treatments if necessary. Estimated means were separated using Fisher's protected least significant difference (alpha=0.05).

4.2.6.2 Seedbank data

Within each tray, the density of ACCase-resistant green foxtail seedlings per m^{-2} was determined from the counts on the treated section, and the density of all other weed species was determined in the untreated section. Density values for all species were summed to determine total germinable seedbank density. The proportion of ACCase-resistant green foxtail was calculated from the ACCase-resistant and total green foxtail densities. Relative abundance was calculated as the ratio between the individual weed density to the total weed density. The densities of ACCase-resistant green foxtail, all other weed species and total weed densities were subjected to a mixed model ANOVA. In this model, the fixed effects were the previous crop phase, herbicide omission treatment (FH, NO, NOF), crop rotation and their interactions. Random effects were replication and the interaction of rotation with replication. The annual and annual/perennial rotations were analyzed together, but rotational differences were accounted for by including a treatment substructure. Each response variable, the pattern of residuals and heterogeneity of variance were inspected and corrected when necessary by minimizing the AIC values. Means were separated using Fisher's protected least significant difference (alpha=0.05).

The proportion of ACCase-resistant green foxtail and the relative abundance of green foxtail, yellow foxtail and all other weed species in the 2018 seedbank and the change from the 2017 to 2018 seedbank were modeled using the Beta Distribution model using the Glimmix procedure. Fixed and random effects were the same as in the mixed model analysis previously

described. The “logit” link function and the Wald covtest statement were invoked. The residual option was selected in the random statement, and treatment means were separated with Fisher’s protected LSD. Differences between the between the 2017 and 2018 seedbank in the ACCase-resistant green foxtail proportion and relative abundance of green foxtail, yellow foxtail and all other weed species were determined by calculating the difference between 2017 and 2018 seedbank data in each experimental unit. To determine whether the difference between years was significant, in specific, greater than zero, the ls means t-test that compares each treatment mean to zero was used.

4.3 Results

4.3.1 Total green foxtail seedbank densities

Green foxtail densities were lower in all treatments in the spring 2018 seedbank compared with the spring 2017 seedbank. From 2017 to 2018, the green foxtail seedbank had an average reduction of 1,133 seeds m^{-2} or 53% in the annual/perennial rotation and an average reduction of 833 seeds m^{-2} or 29% in the annual rotation (Fig. 4.1A). During the 2017 field season, from April through August, the experimental site received 42.5% less precipitation compared with the 30-year average (Table 4.2). This limited moisture likely reduced weed growth and seedbank inputs. In addition to the overall decrease in the green foxtail seedbank densities, the relative abundance of green foxtail decreased on average by 11% in the annual rotation and by 13% in the annual/perennial rotation from 2017 to 2018 (Fig. 4.2A&B), meaning, green foxtail occupied a smaller proportion of the total seedbank in 2018 compared with 2017.

Table 4.2 Long-term (1981-2010) and average temperature and precipitation in 2016 and 2017 from the Carman UM Weather Station from April to September (Environment Canada 2020)

		April	May	June	July	August	September	
Air Temperature (ºC)	Long-term Average	4.5	11.6	17.2	19.4	18.5	13.4	Average 14.1
	2017	5.2	12.1	17.1	19.4	17.6	13.5	14.1
	2016	2.6	13.4	17.1	19.1	18.6	14.1	14.0
Precipitation (mm)	Long-term Average	29.5	69.6	96.4	78.6	74.8	49.0	Total 397.9
	2017	18.6	25.2	64.4	23.3	22.8	74.6	228.9
	2016	55.3	90.2	113.3	78.8	57.7	64.7	460.0

In the 2018 seedbank, total green foxtail densities were often greater in the Control treatment compared with the IWM treatments, but this was only significant in the wheat(flax) phase of the annual rotation (Fig. 4.1B). Increased total green foxtail densities in the Control treatment (1800 seeds m^{-2}) of the wheat(flax) phase, were related to ACCase inhibitor herbicide applications in 2016 and 2017 that were likely ineffective on part of the green foxtail population. Compared with lower total green foxtail densities in the IWM1 (1000 seeds m^{-2}) and IWM2 (600 seeds m^{-2}) treatments, where fewer in-crop herbicide applications were made. The overall trend in the annual/perennial rotation was similar, however, an interaction was observed in the soybean(oat) phase, where total green foxtail densities in the IWM2 (800 seeds m^{-2}) and Control (800 seeds m^{-2}) treatments were the same, while green foxtail densities were lower in the IWM1 (250 seeds m^{-2}) treatment. Increased green foxtail densities in the IWM2 treatment of the soybean(oat) phase may be related to the tendency of greater green foxtail densities in the IWM2 treatment following the oat phase in the 2017 seedbank. The total green foxtail densities were often greatest in the Control treatment. This was caused by the selection of ACCase-resistant green foxtail by more frequent ACCase inhibitor herbicide applications in the Control treatment during the previous 16 years of the experiment.

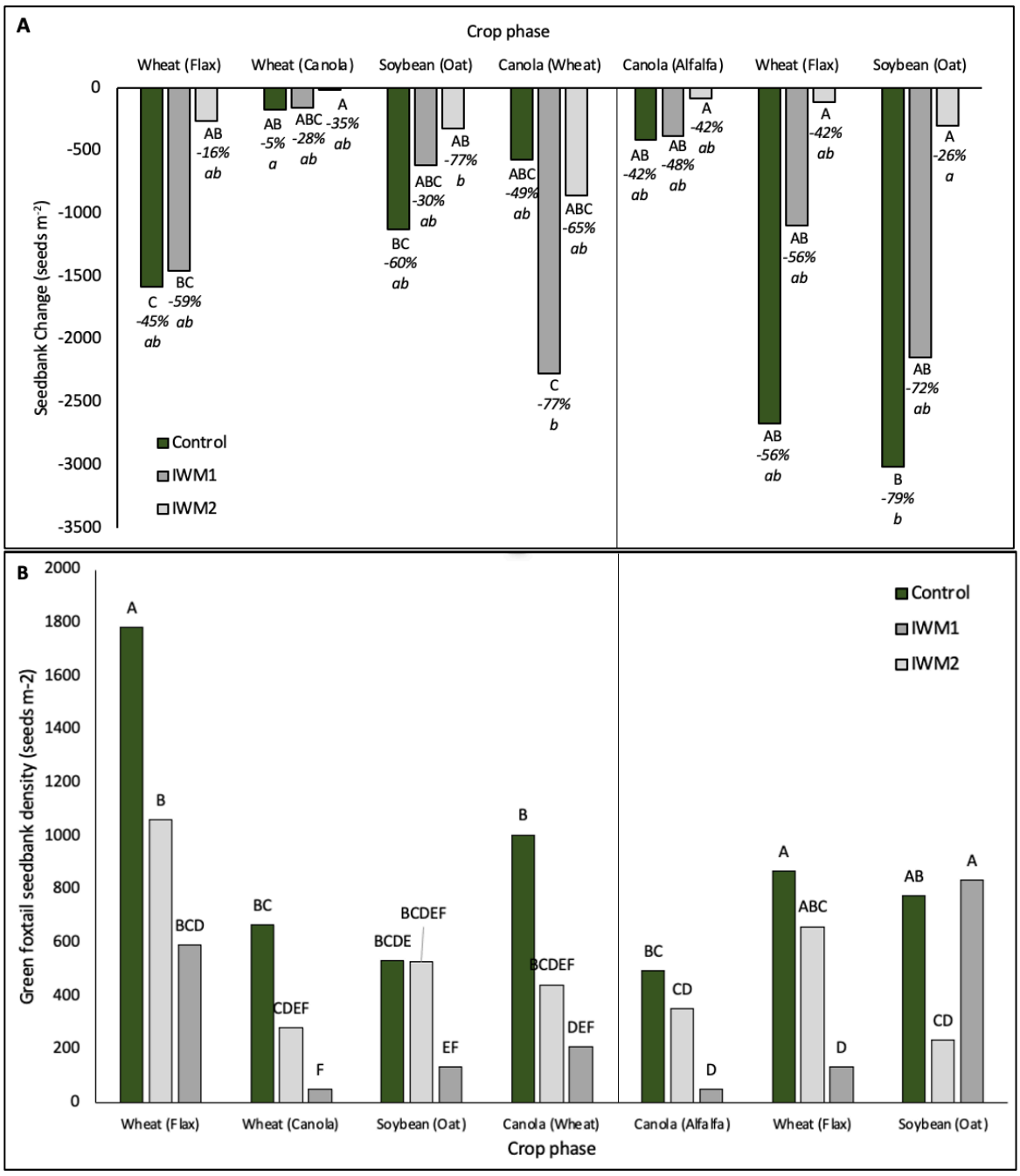

Figure 4.1 Change in the total green foxtail seedbank density from 2017 to 2018 (A) and the 2018 seedbank densities (B) in three crops in response to integrated cultural weed management techniques implemented in 2017 in the annual (left) and annual/perennial rotation (right). Numbers below bars, on Figure A, are the proportional change between 2017 and 2018. Crop phase and IWM treatment followed by different letters are significantly different based on Fisher's Protected LSD, within each crop rotation (uppercase letters represent the reduction in absolute densities mean separations and the lowercase letters represent the reduction in proportional density mean separations).

After one year, the canola IWM treatments did not have a greater reduction (absolute and proportional) in total green foxtail than in the Control, but the relative abundance of green foxtail declined in the IWM1 and IWM2 treatments in the annual rotation (Fig. 4.1A). In the canola(wheat) phase of the annual rotation, the relative abundance of green foxtail in the 2018 seedbank decreased in the IWM1 ($p=0.0250$, ls means t-test of the difference to zero) and the IWM2 treatments ($p=0.0424$, ls means t-test) compared with 2017, however no change in the relative abundance of green foxtail in the seedbank of the Control treatment was observed between 2017 and 2018 seedbank ($p=0.6733$, ls means t-test) (Fig.4.2). Additionally, in the IWM1 treatment of the canola(wheat) phase the green foxtail densities showed a trend of larger reductions compared with the Control and IWM2 treatments. The absolute density reduction of total green foxtail was about 2X larger and the proportional reduction of total green foxtail was at least 10% greater in the IWM1 treatment compared with the Control and IWM2 treatments (Fig 4.1A). Indicating a small effect of the IWM1 treatment on green foxtail densities in the canola(wheat) phase. Although, in the canola(alfalfa) phase of the annual/perennial rotation, the IWM treatments did not result in a larger total green foxtail seedbank reduction compared with the Control treatment. Potentially as total green foxtail reductions in the canola(alfalfa) phase were smaller. It may take multiple years of the IWM treatments before significant effects in the seedbank are found in this experiment.

IWM in wheat did not reduce total green foxtail seedbank densities (absolute and proportional) after one year of implementation (Fig 4.1A), however, the relative abundance of green foxtail decreased in the seedbank from 2017 to 2018 in the IWM1 treatment of the annual rotation (Fig 4.2). In the wheat(flax) phase of the annual rotation, the relative abundance of green foxtail in the IWM1 treatment declined in the seedbank from 2017 to 2018 ($p=0.0232$, ls means t-

test), however, no changes in the Control (p=0.5410, ls means t-test) and IWM2 (p=0.3628, ls means t-test) treatments from 2017 to 2018 were detected. In the IWM1 treatment, the decrease in the relative abundance of green foxtail did not translate to absolute or proportional reductions of green foxtail densities that were larger than in the Control treatment (Fig 4.1A) Moreover, in the wheat(canola) phase of the annual rotation, the absolute reduction of total green foxtail densities was low compared with the other crop phases. Because of this, the proportional reduction of total green foxtail densities that was about 6X greater in the IWM treatments compared with the Control treatment was relatively small, therefore it was not statistically significant. Generally, the effectiveness of IWM treatments on green foxtail in wheat were negligible in the seedbank after one year of implementation.

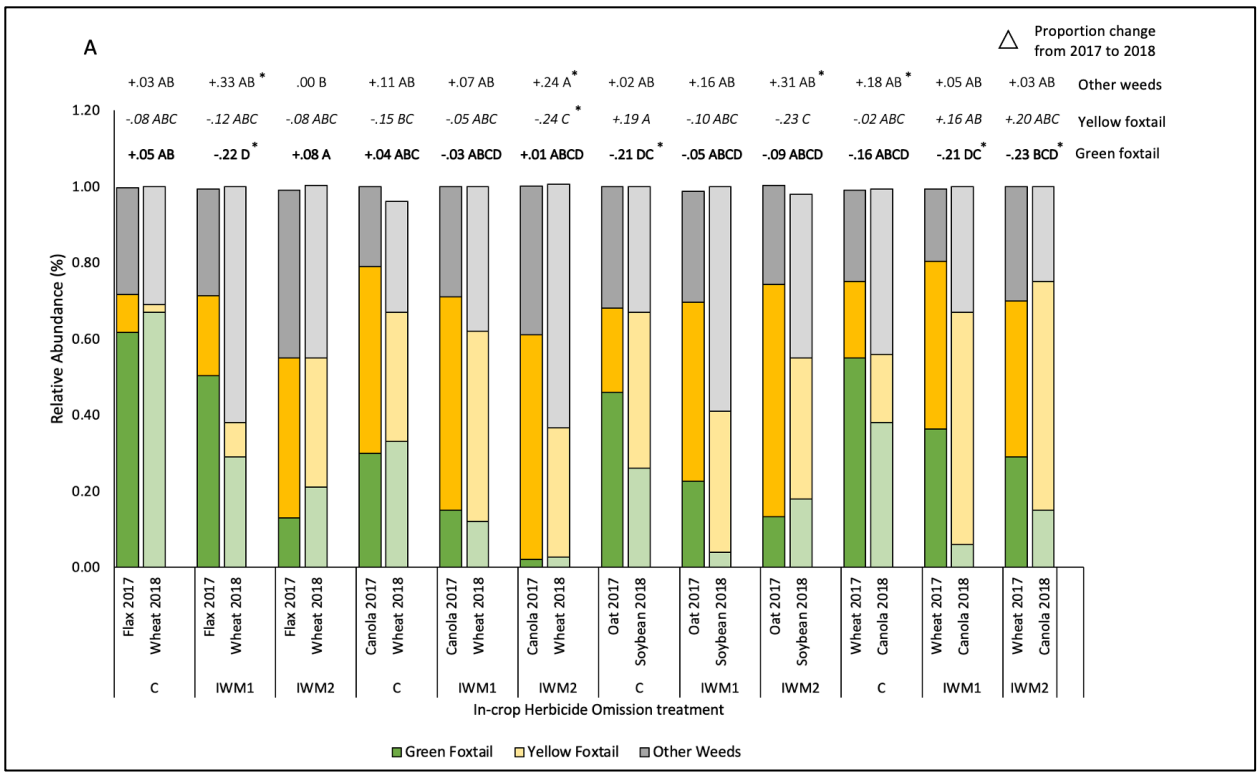

Figure 4.2. Relative abundance of green foxtail, yellow foxtail and all other weed species combined as influenced by preceding crop and weed management treatment in the annual rotation the 2017 and 2018 spring seedbank. Weed management treatments initiated in 2017 are indicated (Control, IWM1 and IWM2) and crop phases for the annual rotation. Relative abundance values for green foxtail, yellow foxtail and other weeds, within each crop phase in the 2017 (left bar) and crop and weed management treatment implemented in 2018 (right bar) are indicated. The change in proportion and statistical analysis between 207 and 2018 are indicted above the bars with other weeds (top), yellow foxtail (middle) and green foxtail (bottom). Within rows, crop phase and IWM treatment combinations with different letter values are significantly different in relative abundance from the 2017 to 2018 seedbank analyses, in specific green foxtail (bold), yellow foxtail (italics), or other weeds (normal font) based on Fisher's Protected LSD, within each crop rotation. Proportional changes that are different from zero are marked with an asterisk.

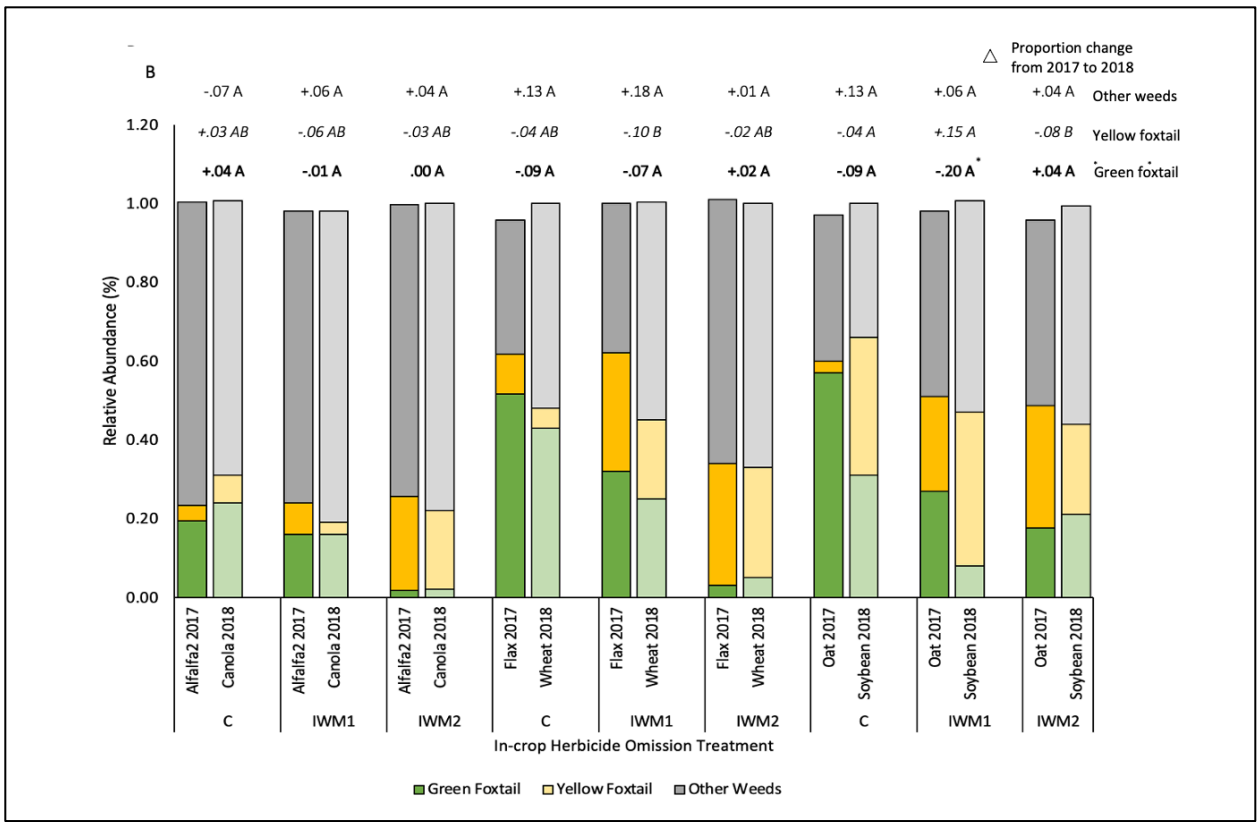

Figure 4.3 Relative abundance of green foxtail, yellow foxtail and all other weed species combined as influenced by preceding crop and weed management treatment in the annual/perennial rotation in the 2017 and 2018 spring seedbank. Weed management treatments initiated in 2017 are indicated (Control, IWM1 and IWM2) and crop phases for the annual/perennial rotation. Relative abundance values for green foxtail, yellow foxtail and other weeds, within each crop phase in the 2017 (left bar) and crop and weed management treatment implemented in 2018 (right bar) are indicated. The change in proportion and statistical analysis between 207 and 2018 are indicted above the bars with other weeds (top), yellow foxtail (middle) and green foxtail (bottom). Within rows, crop phase and IWM treatment combinations with different letter values are significantly different in relative abundance from the 2017 to 2018 seedbank analyses, in specific green foxtail (bold), yellow foxtail (italics), or other weeds (normal font) based on Fisher's Protected LSD, within each crop rotation. Proportional changes that are different from zero are marked with an asterisk.

After one year of the IWM treatment implementation, IWM in soybean did not reduce the total green foxtail densities (absolute and proportional) in the seedbank (Fig. 4.1A), but the relative abundance of total green foxtail decreased in the IWM1 treatment in the annual/perennial rotation (Fig 4.2). In the soybean(oat) phase of the annual/perennial rotation, the absolute and proportional reductions in total green foxtail densities were about 10X and 3X greater, respectively, in the Control treatment compared with the IWM2 treatment (Fig 4.1A). The total green foxtail reduction

(absolute and proportional) in the IWM1 treatment was intermediate, resulting in a decrease in the relative abundance of green foxtail from 2017 (p=0.0439, ls means t-test). No change in the relative abundance of green foxtail in the Control (p=0.3681, ls means t-test) or the IWM2 (p=0.3505, ls means t-test) treatments from 2017 was observed (Fig 4.2). This suggests the IWM1 treatment was somewhat effective at reducing green foxtail seedbank densities. In the annual rotation, the reduction (absolute and proportional) in total green foxtail density did not differ in the IWM and Control treatments (Fig 4.1A). In the 2018 seedbank, the relative abundance of green foxtail decreased in the Control treatment compared with the 2017 seedbank (p=0.0291, ls means t-test) and remained unchanged in the IWM treatments (IWM1 p=0.5663, IWM2 p=0.2809, ls means t-test) (Fig 4.2). Based on the changes in the proportional and absolute green foxtail seedbank densities, the IWM treatments did not result in improved reductions in the spring seedbank densities of green foxtail one year after treatment initiation in soybean.

4.3.2 ACCase-resistant green foxtail seedbank densities

In both rotations, the proportion of the total green foxtail seedbank that was resistant to ACCase inhibitor herbicides was lower in the IWM2 treatments compared with the Control and the IWM1 treatments in all crop phases (Fig 4.3). In the IWM2 treatment between 15% and 45% of the total green foxtail seedbank was occupied by the ACCase-resistant biotype while in the Control and IWM1 treatments, the ACCase-resistant biotype accounted for 60-80% of the seedbank in most cases. Despite an overall reduction in the total green foxtail seedbank densities between 2017 and 2018, the ACCase-resistant biotype proportion of the total green foxtail seedbank increased between 2017 and 2018 in the wheat(flax) phase (ls means t-test, p=0.0392) of the annual/perennial rotation and in the wheat(canola) phase (ls means t-test, p=0.0237) of the

annual rotation. The proportion of ACCase-resistant green foxtail in the seedbank remained constant from 2017 to 2018 in all other treatments of the rotation experiment.

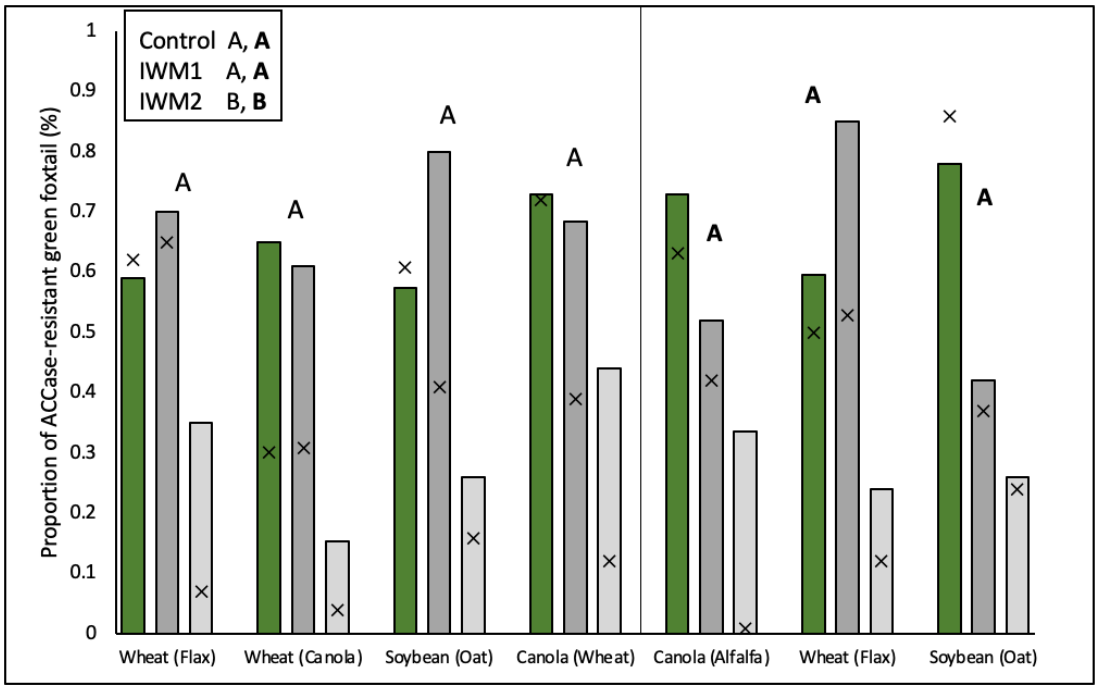

Figure 4.4 Proportion of the ACCase-resistant green foxtail biotype of total green foxtail in response to in-crop herbicide use intensity in an annual (left) and annual/perennial (right) crop rotation in the 2018 seedbank analysis of the rotation experiment. The bars indicate the proportion of ACCase-resistant green foxtail in the 2018 seedbank for the Control (green), IWM1 (dark grey) and IWM2 (light grey) treatments. The proportion of ACCase-resistant green foxtail in the 2017 seedbank are indicated with the X mark within each bar. Crop phase and IWM treatment followed by different letters had a significantly different proportion of ACCase-resistant green foxtail in the 2018 seedbank based on Fisher's Protected LSD, within each crop rotation.

ACCase-resistant green foxtail densities in the 2018 seedbank were greatest in the Control treatment and lowest in the IWM2 treatment (Fig. 4.4A). ACCase-resistant green foxtail densities in the IWM1 treatment were intermediate in most crop phases. There was a 2.5-fold range in ACCase-resistant green foxtail densities among the Control treatments. The greatest ACCase green foxtail density, over 1000 viable seeds m^{-2}, were observed in the Control treatment in the wheat(flax) phase of the annual rotation and the lowest ACCase-resistant green foxtail density (380 viable seeds m^{-2}) in the Control treatment was observed in the canola(alfalfa) phase of the

annual/perennial rotation. The annual rotation wheat(flax) phase had received ACCase inhibitor herbicide applications in 2016 and 2017 and that selection pressure of repeated ACCase inhibitor applications contributed to the elevated ACCase-resistant green foxtail densities in this phase, whereas reduced ACCase inhibitor use in the canola(alfalfa) treatment limited the accumulation of ACCase-resistant green foxtail. In comparison, ACCase-resistant green foxtail densities in the IWM2 treatment ranged from about 15 viable seeds m^{-2} in the wheat(canola) treatment of the annual rotation to more than 300 viable seeds m^{-2} in the wheat(flax) of the annual rotation. Despite the change in treatment structure in 2018, increased densities of ACCase-resistant green foxtail continue to be found in treatments that had received increased ACCase inhibitor applications since the initiation of the study in 2000.

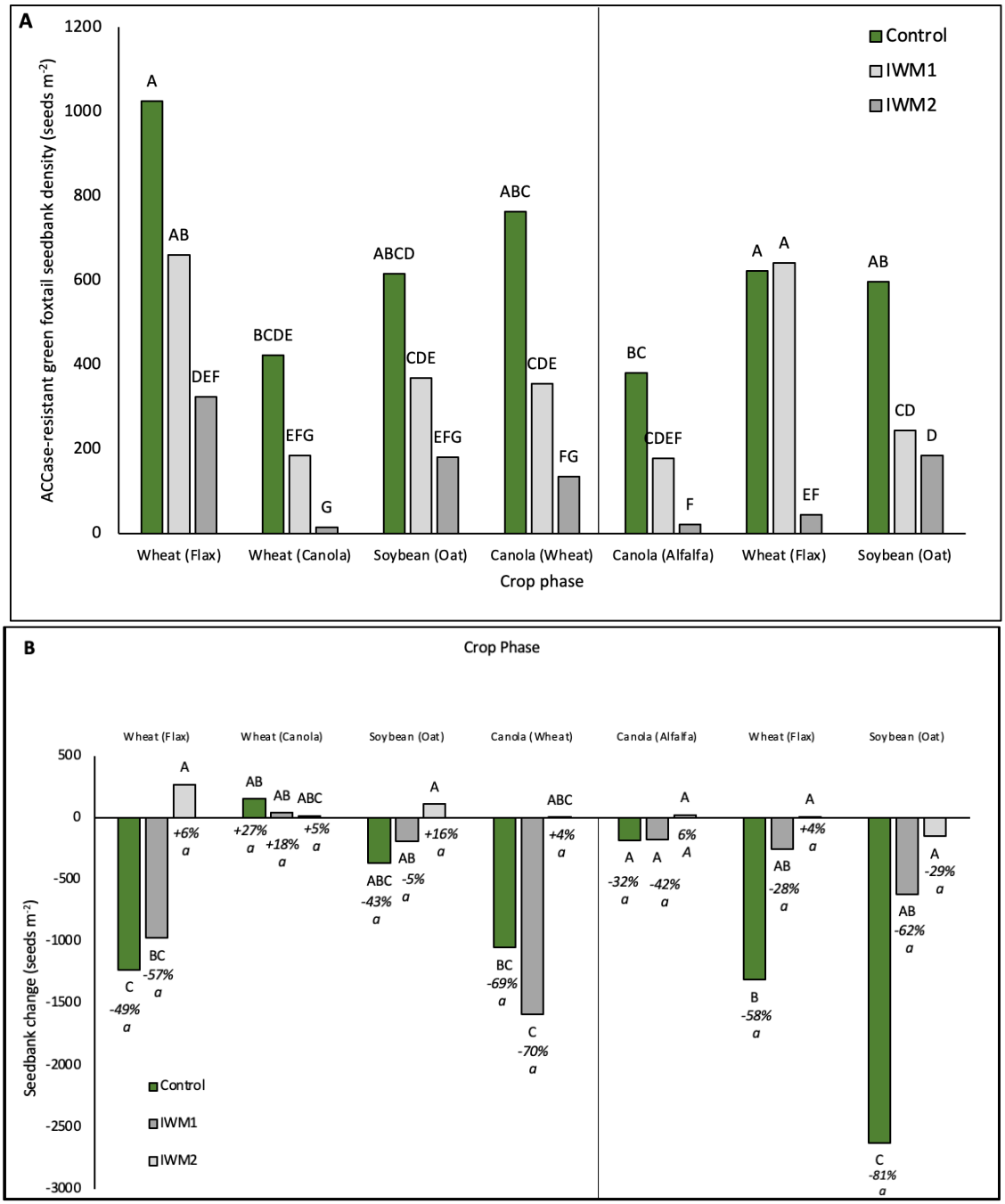

Figure 4.5 The 2018 seedbank densities (A) and the change in the ACCase-resistant green foxtail seedbank density from 2017 to 2018 (B) in three crops in response to integrated cultural weed management techniques implemented in 2017 in the annual (left) and annual/perennial rotation (right). Numbers below bars, on Figure A, are the proportional change between 2017 and 2018. Crop phase and IWM treatment followed by different letters are significantly different based on Fisher's Protected LSD, within each crop rotation (uppercase letters represent the reduction in absolute densities mean separations and the lowercase letters represent the reduction in proportional density mean separations).

Similar to total green foxtail, the IWM treatments in soybean, wheat and canola, did not reduce ACCase-resistant green foxtail densities (absolute and proportional) more than in the Control treatments after the first year of treatment implementation (Fig. 4.3B). In fact, in the soybean(oat) phase of the annual/perennial rotation, the absolute reduction of ACCase-resistant green foxtail densities was larger in the Control treatment compared with the IWM1 and IWM2 treatments. Similarly, in the wheat(flax) phase of the annual/perennial rotation, the reduction in absolute density of ACCase-resistant green foxtail was greater in the Control treatment than in the IWM2 treatment, but densities in the IWM1 treatment were intermediate. Besides these crop phases, there was no difference in the changes in absolute ACCase-resistant green foxtail densities and no differences in the proportional change of ACCase-resistant green foxtail densities were observed among any of the treatments. This was surprising as the treatment means ranged from a 27% increase in proportion of ACCase-resistant green foxtail in the Control treatment of the wheat(canola) phase in the annual rotation to an 81% decrease in the Control treatment of the soybean(oat) phase in the annual/perennial rotation. The large range in values without statistical separation indicate a high degree of variation within treatments. The IWM2 treatment (SEM=0.43) had the greatest variation, this may have been due to variation among plots for ACCase-resistant green foxtail in these treatments and the low number of replications. Overall, the IWM treatments in soybean, canola, and wheat did not reduce ACCase-resistant green foxtail densities more than the Control in the seedbank after one year of treatment implementation.

4.3.3 Yellow foxtail seedbank densities

In the 2018 seedbank, yellow foxtail densities were often greater in the IWM1 and IWM2 treatments, where in-crop herbicide applications were omitted in 25 or 50% of the rotation cycle from 2000-2016, compared with the Control treatment, but this was only significant in two crop

phases (Fig. 4.5A). The canola(wheat) phase of the annual rotation and the soybean(oat) phase of the annual/perennial rotation contained yellow foxtail densities in the IWM treatments that were about 3X greater than in the Control treatment. The other crop phases had a similar trend in both rotations, with lower yellow foxtail densities in the Control treatment compared with the IWM treatments, but in these treatments, the apparent differences were not significant. Greater yellow foxtail densities in the IWM treatments may be a legacy effect from the herbicide omission treatments in the rotation experiment.

There was a tendency for increased yellow foxtail density reductions in the IWM1 and IWM2 treatments, where yellow foxtail densities in the 2017 seedbank were greater, but no significant differences were observed (Fig. 4.5B). In the soybean(oat) phase of the annual/perennial rotation, the absolute reduction of yellow foxtail densities was 8X larger in the IWM1 treatment and 16X larger in the IWM2 treatment compared with the Control treatment. The canola(alfalfa) and wheat(flax) phases in the annual/perennial rotation and the wheat(flax) phase of the annual rotation followed the same trend, although the fold differences in the IWM treatments were smaller. Despite the apparent change in yellow foxtail densities (proportion and absolute) from the 2017 to the 2018 seedbank, there was no significant differences in main effects. There was large variation in the IWM2 treatments of the annual (SEM= 262 seeds m^{-2}) and annual/perennial (SEM= 404 seeds m^{-2}). The variation may have been caused by inconsistent stand establishment due to dry conditions in 2017 based on visual observations and the low number of replicates in the rotation experiment.

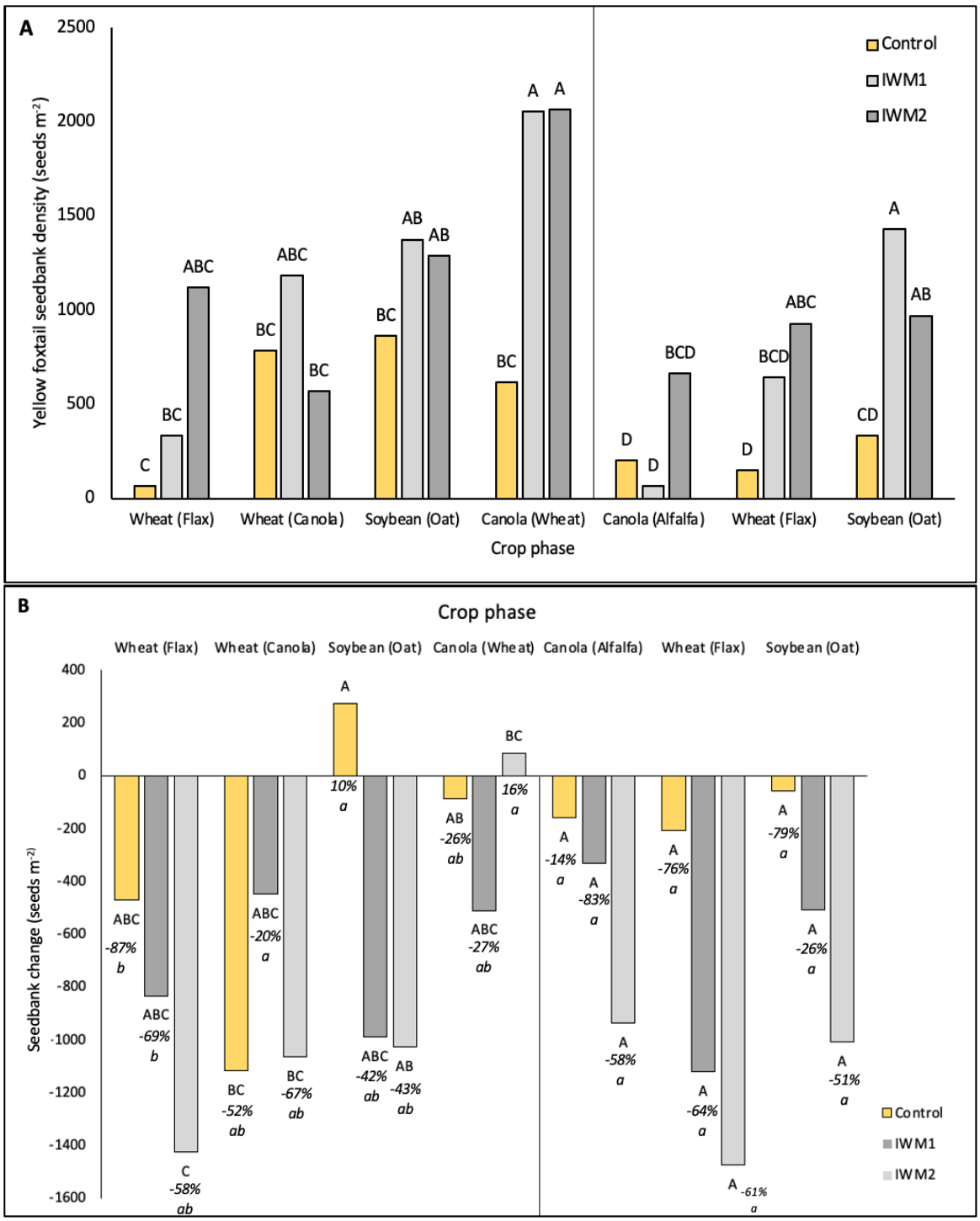

Figure 4.6 The 2018 seedbank densities (A) and the change in the yellow foxtail seedbank density from 2017 to 2018 (B) in three crops in response to integrated cultural weed management techniques implemented in 2017 in the annual (left) and annual/perennial rotation (right). Numbers below bars, on Figure A, are the proportional change between 2017 and 2018. Crop phase and IWM treatment followed by different letters are significantly different based on Fisher's Protected LSD, within each crop rotation (uppercase letters represent the reduction in absolute densities mean separations and the lowercase letters represent the reduction in proportional density mean separations).

4.3.4 Total weed seedbank densities

In the spring of 2018, the average total seedbank density (2,912 seeds m^{-2}) in the rotation experiment was lower compared with the 2017 average total seedbank (5,259 seeds m^{-2}). Similar to the *Setaria* species, total weed seed production likely was reduced due to lower-than-normal precipitation during the 2017 growing season, in contrast with above normal precipitation during 2016 that contributed to the 2017 seedbank (Table 4.2). The relative abundance of all non-foxtail weed species increased by 13% in the annual rotation and there was an increase of 12% in the annual/perennial rotation relative to green foxtail and yellow foxtail (Fig. 4.2). Despite the overall reduction in the total seedbank densities, the non-foxtail species occupied a larger proportion of the 2018 seedbank compared with 2017.

The 2018 total seedbank densities tended to be lower in the Control treatment, but this was only significant in the soybean(oat) phase in both rotations (Fig. 4.6B). In the soybean(oat) phase of the annual and annual/perennial rotations, the total seedbank density in the Control treatments was around 2000 seeds m^{-2} compared with the total seedbank densities in the IWM1 and IWM2 treatments that ranged from around 3300 to 4500 seeds m^{-2}. In 2018, lower total seedbank densities in the Control treatment in the soybean(oat) phases of both crop rotations may have caused by smaller total seedbank densities in the Control treatment in the spring of 2017.

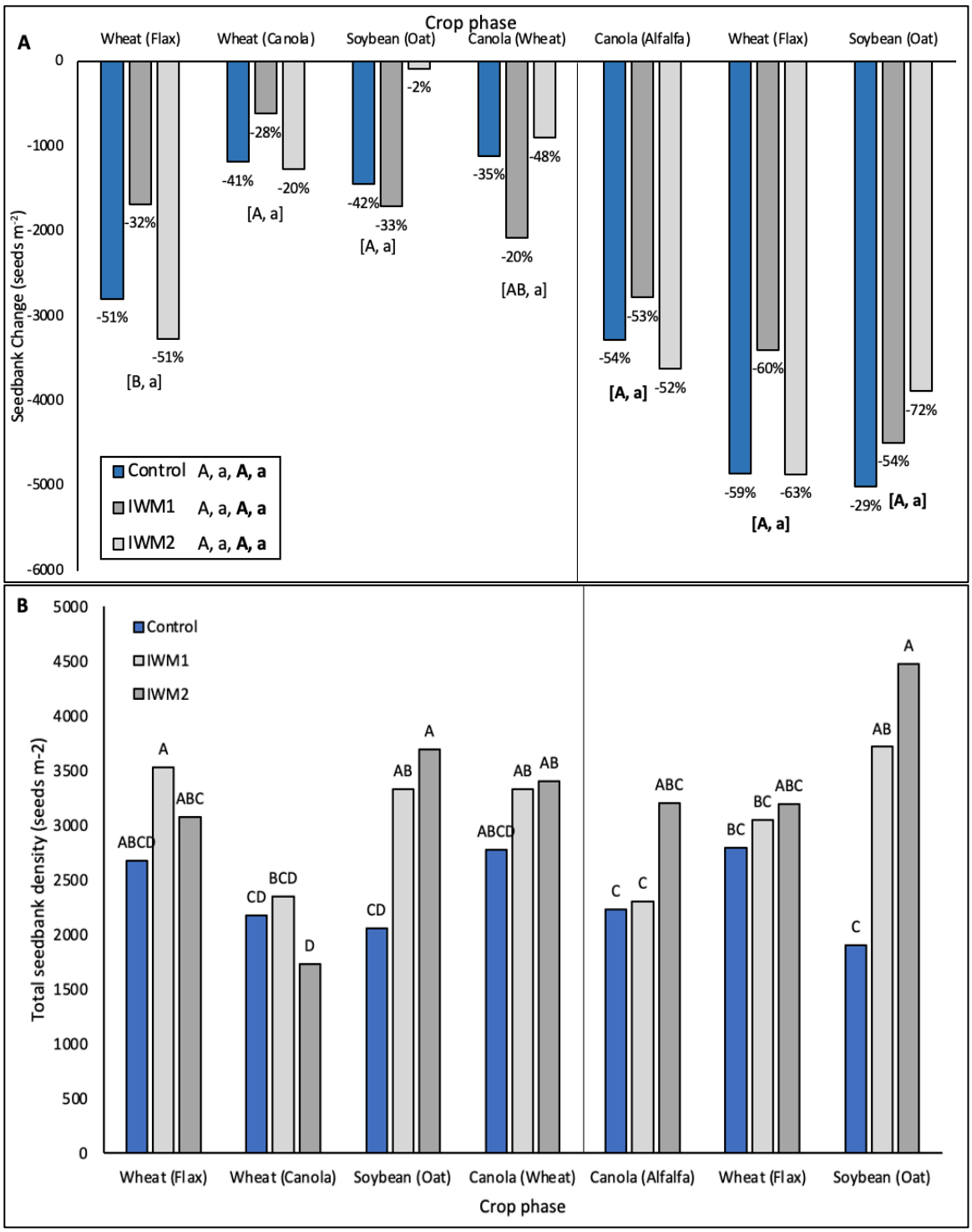

Figure 4.7 Change in the total seedbank density from 2017 to 2018 (A) and the 2018 seedbank densities (B) in three crops in response to integrated cultural weed management techniques implemented in 2017 in the annual (left) and annual/perennial rotation (right). Numbers below bars, on Figure A, are the proportional change between 2017 and 2018. Crop phase and IWM treatment followed by different letters are significantly different based on Fisher's Protected LSD, within each crop rotation (uppercase letters represent the reduction in absolute densities mean separations and the lowercase letters represent the reduction in proportional density mean separations).

4.3.5 In-crop measurements

For the most part, crop and weed biomass, stem thickness, plant height, and seed yield in canola and soybean during the 2017 field season were similar in the Control and the IWM treatments. Canola plant height, biomass, and seed yield were the same in the Control and the IWM treatments. However, the canola plants in the Control treatment had stems that were at least 0.5 cm thicker compared with the canola in the IWM treatments (Table 4.4), due to the plastic growth habit of canola leading to greater branching and larger plants when planted at lower densities. Further, in canola the biomass of grassy-weed, *Setaria* species, and all other species were equal in the Control and the IWM treatments (Table 4.3). Plant height, stem thickness, and seed yield in soybean were the same in the Control and the IWM treatments. In the annual/perennial rotation, soybean biomass was greater in the Control treatment and lower in the IWM2 treatment. The soybean IWM treatments contained more total weed biomass compared with the Control treatment in the annual rotation. This was due to uncontrolled late emerging wild oat and volunteer canola that escaped in-crop herbicide applications (Table 4.3). During the 2017 field season, IWM in canola and soybean did not provide greater crop seed yield or weed suppression compared with the Control treatment, although the crops may have been limited by dry conditions (Table 4.2).

Table 4.3 Aboveground biomass dry weight of the crop and Setaria, broadleaved and other grassy weeds in the IWM treatments of the rotation experiment during the 2017 field season. IWM treatments followed by different letters are significantly different based on Fisher's Protected LSD, within each crop rotation.

Rotation	Biomass type (kg ha^{-2})	IWM Treatment	Soybean (oat)		Wheat (flax)		Canola (wheat)		Wheat (canola)
Annual	Crop	Control	624.3		1080.0		726.6	B	946.7
		IWM1	579.3		1243.3		836.6	AB	750.0
		IWM2	667.3		1723.3		1173.3	A	1106.
	Setaria and related species	Control	101.7		233.3		170 .0		96.7
		IWM1	163.3		90.0		173.3		23.3
		IWM2	121.7		63.3		223.3		0.0
	Broadleaved weeds	Control	13.6		13.3		0.0		6.6
		IWM1	21.3		9.0		0.0		10.0
		IWM2	6.0		3.3		26.6		43.3
	Other grassy-weeds	Control	130.3		26.6		203.3		23.3
		IWM1	181.6		43.3		16.6		183.3
		IWM2	193.3		63.3		30.0		433.3

Rotation	Biomass type (kg ha^{-2})	IWM Treatment	Soybean (oat)		Wheat (flax)		Canola (alfalfa)
Annual/ Perennial	Crop	Control	2403.7	A	1066.7		1756.7
		IWM1	1669.0	B	1266.7		1660.0
		IWM2	991.3	C	1613.3		1690.0
	Setaria and related species	Control	72.0		513.3	A	3.3
		IWM1	67.2		90.0	B	3.3
		IWM2	107.6		46.6	B	26.6
	Broadleaved weeds	Control	0.0	B	3.3		6.0
		IWM1	38.9	AB	6.6		0.0
		IWM2	57.0	A	16.6		20.0
	Other grassy-weeds	Control	9.0		20.0		0.0
		IWM1	22.0		43.3		43.3
		IWM2	168 .0		63.3		6.6

In wheat, based on in-crop measurements, the IWM treatments were not effective against *Setaria* and non-foxtail species relative to the Control treatment. On average, the wheat IWM treatments contained almost 4-fold and 10-fold less less *Setaria* biomass compared with the Control treatment in the annual and the annual/perennial rotation, respectively (Table 4.3). However, the IWM treatments did not contain less broadleaved weed or other grassy-weed biomass compared with the Control treatment indicating that *Setaria* species were replaced by other weeds in the IWM treatments. Wheat plant height and biomass were the same in the IWM

and Control treatments (Table 4.3), and the wheat plants in the Control treatment had at least one more tiller (Table 4.4). Wheat in the IWM1 and IWM2 treatments did not have increased seed yield compared with the Control treatment, however, there was a trend for greater seed yield in the IWM1 and IWM2 treatments compared with the Control treatment (Table 4.3) and seed yield potential may have been limited due to low precipitation (Table 4.2). In the 2017 field season, the wheat IWM treatments did not reduce total weed seedbank densities, increase seed yield or decrease in-crop weed biomass.

Table 4.4 Yield, crop height and stem thickness of the soybean, wheat and canola phases in the annual and annual/perennial rotations of the rotation experiment during the 2017 field season.

Rotation	In-crop measurement	Treatment	Soybean (oat)	Wheat (flax)	Canola (wheat)	Wheat (canola)
Annual	Yield (kg ha^{-1})	Control	175.6	1028.4	441.6	1097.5
		IWM1	536.5	2058.8	409.1	1792.2
		IWM2	370.7	3220.4	328.6	1385.8
	Plant Height (cm)	Control	49.3	76.0	98.1	78.9
		IWM1	56.7	79.5	98.9	86.6
		IWM2	52.8	80.2	99.6	83.0
	Stem thickness (cm)*	Control	4.09	3.38 A	6.28	3.33 A
		IWM1	4.32	2.42 B	5.81	1.83 B
		IWM2	3.88	2.88 B	5.72	2.50 B
Rotation	In-crop measurement	Treatment	Soybean (oat)	Wheat (flax)	Canola (alfalfa)	
Annual/ perennial	Yield (kg ha^{-1})	Control	819.4	1544.8	1429.9	
		IWM1	907.5	1728.7	1067.7	
		IWM2	717.7	2091.5	1335.2	
	Plant Height (cm)	Control	87.8	81.6	114.8	
		IWM1	77.4	77.0	108.1	
		IWM2	78.3	80.4	112.6	
	Stem thickness (cm)*	Control	6.34	4.13 A	8.26	
		IWM1	5.19	2.54 B	7.34	
		IWM2	5.76	2.96 B	7.42	

* The number of tillers on individual wheat plants were counted instead of stem thickness.

4.4 Discussion

4.4.1 Environmental conditions

The environmental conditions experienced during the 2017 field season may have reduced seedbank inputs leading to a lower total germinable seedbank in 2018. (Chapter 2). Precipitation received from April to August in 2017 was 57.5% of the long-term average precipitation at

Carman, Manitoba (Environment Canada 2020). Reduced moisture may have limited weed seedling recruitment and growth (Robinson and Gross 2010), directly affecting weed seed rain into the seedbank in the fall of 2017 (Liebman and Mohler 2009). In *Setaria* species, seed production is linked to plant biomass accumulation (Bussan et al. 2009). *Setaria* species have shallow fibrous root systems and would be unable to reach deep soil moisture compared with tap-rooted species (Dalley et al. 2016). Further, environmental conditions (ex. light quality, temperature, and soil moisture) experienced by seeds during maturation can alter the dormancy characteristics (Baskin and Baskin 1973). For instance, increased soil moisture during seed maturation in cotton (*Gossypium arboreum* L.) reduced germination of cotton seed (Carver, 1936) and wild oat seed produced under such conditions (Sexsmith, 1969) compared with plants that matured in drier conditions. Although soil moisture levels were not monitored, rainfall received during September, when seeds of green foxtail and yellow foxtail ripen and disperse (Beckie et al. 2018) was 1.5X greater compared with the long-term average. Increased soil moisture and other environmental conditions or a combination may have influenced seed dormancy characteristics and may have contributed to reduced germinability of seeds in the 2018 seedbank evaluation.

4.4.2 Seedbank composition

The seedbank in this rotation experiment contained increased densities of C_4 weeds (e.g., redroot pigweed and oxalis) compared with the plant densities observed in the field during the 2017 growing season where greater densities of C_3 weeds were observed (e.g. wild oat, Canada fleabane (*Erigeron canadensis* L.)). Differences in temperature and available soil moisture between early-spring Manitoba field conditions and the greenhouse environment likely contributed to differential recruitment among weed species between the field and the greenhouse. Furthermore, weed densities in the field were determined at one time point, in early-June only when springtime

temperatures were relatively cool and C4 weed seedling recruitment may not have completed. Green foxtail emergence was found to begin around 350 GDD in conservation tillage systems in Manitoba (Bullied et al. 2003). This GDD range coincides with May 19th, 2017 for the location of the rotation experiment, therefore green foxtail emergence may not have been completed when weed densities were determined in early June. This may have resulted in an underestimation of the densities of green foxtail and other C_4 species in the field, whereas the seedbank densities in the greenhouse were determined over four germination cycles, facilitating enumeration of a greater portion of the seedbank. The additional counts completed during the greenhouse seedbank evaluation may have more accurately defined the species present in the rotation experiment (Ball and Miller 1989; Cardina and Sparrow, 1996).

Seedbank densities of the rotation experiment were comparable to seedbank densities in other North American studies (Sosnoskie et al. 2009; Forcella et al. 2014). The average seedbank density in 2017 was above 6000 seeds m^{-2} in the annual/perennial rotation, and above 4000 seeds m^{-2} in the annual rotation. Seedbank densities in a long-term rotation experiment near Ohio ranged from 2750 seeds m^{-2} to 7230 seeds m^{-2} (Sosnoskie et al. 2009) and agricultural fields in Minnesota contained seedbanks with densities of 50 to 16,000 seeds m^{-2} (Forcella et al. 2014). In Minnesota, when seedbank densities exceeded 1000 seeds m^{-2} multiple weed control efforts (e.g. herbicide and cultivation) were recommended to avoid negative effects on crop yield (Forcella et al. 2014). As the rotation experiment seedbank densities were within expectations, the IWM treatments with a single in-crop herbicide application should provide sufficient weed control if the applications are timed correctly (Buhler 1992). It is possible that the in-crop applications in the rotation experiment were applied too early during the growing season to control late recruiting *Setaria* and other species.

4.4.3 IWM in wheat

The IWM practices implemented in wheat led to a non-significant yield increase, but the IWM practices did not reduce seedbank densities compared with the Control after one year. This is in agreement with other studies in western Canada that found increased seeding rate in wheat does not always result in improved seed yield (Blackshaw et al. 2005). Narrower row spacings improved barley yield in Scott Saskatchewan, and reduced biomass of volunteer canola (*Brassica rapa* L.), wild mustard (*Sinapis arvensis* L.) and wild oat (Kirkland 1993). Apart from yield, improved weed suppression was observed when narrower rows and increased seeding densities were used alone (Blackshaw et al. 2005; Drews et al. 2009) or together (Liebman and Gallandt 1997; Champion et al. 1998). One study on a long-term crop rotation experiment in Europe found that crop rotation and tillage practices altered weed emergence and aboveground biomass, but were not visible in the seedbank after 12 years of rotation, due to the buffering effect caused by seeds with great longevity (Bàrberi and Lo Cascio 2001). In the rotation experiment, changes in aboveground weed suppression from the wheat IWM practices were detected in the first year, but depending on the soil characteristics and weed seedbanks, definite changes in the seedbank may take longer.

The IWM treatments in wheat may have been under greater biotic stress, as the in-crop ACCase inhibitor application would have been ineffective on part of the green foxtail population in the rotation experiment. Application of a non-ACCase inhibitor graminicide could have further reduced green foxtail biomass and may have improved yield of the wheat IWM treatments. In this rotation experiment, green foxtail seedling densities in the field were frequently above 500 plants m^{-2} which equates to at least 23% yield loss in spring wheat (O'Donovan 1994). Pairing the current IWM strategies with an in-crop herbicide effective against ACCase-resistant green foxtail may

have further improved yield and lessened the effect of ACCase-resistant green foxtail on the wheat crop.

4.4.4 IWM in soybean

Soybean is a relatively new crop in Manitoba, with production exceeding 1 million acres for the first time in 2013 (Soy Canada, 2019). Consequently, few studies have evaluated IWM practices for soybean in Manitoba (Geddes and Gulden 2018). In the 2017 field study, the IWM soybean treatments did not have greater soybean biomass, soybean yield or weed suppression compared with the Control treatment soybeans. Given the size of seedbank in the rotation experiment, the row spacing for soybean in the Control and IWM treatments perhaps should have been even more narrow. For this experiment, selection of 20 cm rows instead of 38 cm for the IWM1 & IWM2 treatments may have improved weed suppression in the IWM treatments. In Manitoba, the Critical Weed Free Period (CWFP) was shortened by up to three developmental stages when soybean was planted in 20 cm and 38 cm rows compared with 76 cm rows (Rosset and Gulden 2020), however, other Manitoba studies found that narrow row spacing in soybean was not effective against volunteer canola (Gregoire 2017; Geddes and Gulden 2018). Row spacing was found to be more effective at reducing the CWFP in soybean than increased seeding densities (Rosset and Gulden 2020). Increased soybean densities (666,900 seeds m^{-2}) did not shorten the CWFP in comparison to the standard seeding rate (444,600 seeds m^{-2}). Seeding densities for the IWM soybean treatments were similar to the general recommended densities for soybean in Manitoba (MPSG). Based on this, the IWM soybean treatments in this rotation experiment could be made more competitive by switching to a more narrow row spacing.

4.4.5 IWM in canola

Among the different crop phases, canola had the lowest total seedbank density in 2018. Compared with other crops, current hybrid canola varieties rank as equally competitive to barley, especially against dicot weeds under cool growing conditions (Harker et al. 2011). However, during the 2017 season, no difference in total weed biomass, crop yield or seedbank reduction in the 2018 seedbank between the Control and IWM treatments was observed in canola. Although, other studies in western Canada with similar weed biomass levels have found that increased seeding rates of canola reduced weed interference and increased seed yield in canola (Harker et al. 2003). One possibility was the dry conditions in 2017 led to delayed and uneven emergence that affected the competitive ability of canola, this was supported by visual observations and reduced crop biomass during the 2017 field season. Another possibility, is the efficacy of glufosinate is reduced on weeds at more advanced growth stages (Harker et al. 2000), so early emerging weeds may not have been controlled successfully. In general, the IWM1 and IWM2 treatments met recommendations for canola production, including use of a hybrid variety, and a planting density of 150 seeds m^{-2} (Harker et al. 2000). However, in 2017 dry conditions led to poor stand establishment in canola and reduced the competitive ability.

4.4.6 Practical application of IWM

Implementation of indirect weed management tactics (e.g. row spacing) to cropping systems that have primarily relied on herbicidal weed control can be effective at reducing densities of problematic weed species (Blackshaw et al. 2005). IWM methods increase stress against competing weeds (Liebman and Gallandt 1997) and utilization of multiple management strategies reduces the chance of the development of tolerance to a single management method (Harker and Donovan 2013). IWM practices work multiplicatively together, and become more effective when

used in combination (Harker et al. 2003). Decreased reliance on herbicides as the sole weed management method may remove the need for additional herbicide applications and could help conserve herbicide efficacy and manage herbicide-resistant weeds on farms. IWM practices are advantageous to producers, especially as the cost of herbicide inputs increases if additional modes of action are required and fewer effective herbicide options are available. Adjustment of seeding rate and row spacing are effective strategies that farmers can implement to facilitate weed management. However, it may take longer than one year of IWM changes to become noticeable in the seedbank.

4.5 Conclusion

After one year, the IWM treatments implemented in soybean, wheat and canola in this rotation experiment did not consistently compete better against weeds, including the ACCase-resistant green foxtail biotype, compared with the Control treatment. Precipitation received during the 2017 field season was below the long-term average, this may have caused reduced and uneven crop emergence in sensitive crops (e.g., canola), that impeded the efficacy of the IWM treatments. However, the wheat IWM treatments reduced *Setaria* species biomass, but did not reduce the biomass of non-foxtail weed species. In the seedbank, ACCase-resistant and total green foxtail had greater reductions in the treatments and crop phases where initial green foxtail densities were higher. More time may be needed before the impact of the IWM treatments are visible in the seedbank. Continued evaluation of the IWM and Control treatments in future field seasons will help show the long-term impact of IWM practices in canola, soybean and wheat.

5.0 GENERAL DISCUSSION

5.1 Rotation experiment

The rotation experiment provided a realistic representation of Manitoba cropping systems during the early 2000s. The original rotation experiment included cereal, oilseed and perennial forage crop phases that received ACCase inhibitor (Group 1) herbicide applications at a frequency similar to a typical conventional producer. Moreover, green foxtail was a dominant weed species in the rotation experiment and an ACCase-resistant green foxtail biotype was identified in 2015 (Murphy 2016). Likewise, in Manitoba, green foxtail has been repeatedly identified as an abundant weed on Manitoba weed surveys, and the ACCase-resistant green foxtail biotype occurred on 42% of fields surveyed for green foxtail (Beckie et al. 2016; Leeson et al. 2016). These characteristics made this rotation experiment an ideal platform to characterize an ACCase-resistant green foxtail biotype and to evaluate IWM practices that producers in Manitoba could adopt in crop rotations that are currently dominated by soybean, wheat and canola.

5.2 ACCase-resistant green foxtail

To my knowledge, the rotation experiment is the longest continuously running study where an herbicide-resistant weed biotype has been characterized in response to herbicide inputs. Seedbank results from the rotation experiment provide direct support that herbicide-resistant weed populations are greater following repeated inputs of the same herbicide mode of action. Specifically, in this rotation experiment, greater frequency of ACCase inhibitor herbicide applications were connected to increased densities of ACCase-resistant and -susceptible green foxtail and a greater proportion of the ACCase-resistant green foxtail biotype in the seedbank. Conversely, this rotation experiment demonstrated that the herbicide omission treatments that

received half of the ACCase inhibitor herbicide applications had lower densities and proportions of the ACCase-resistant green foxtail biotype. Besides fewer ACCase inhibitor herbicide applications, management strategies such as cutting alfalfa for hay and alternate herbicide modes of action were effective on this ACCase-resistant green foxtail biotype.

5.3 IWM practices

There was minimal change in the rotation experiment aboveground and in the seedbank one year after the IWM treatments were implemented. Wheat was the only crop phase that had a nonsignificant yield increase, but wheat did not suppress biomass for all weed species. The IWM treatments in soybean and canola did not have greater weed suppression or yield compared with the Control treatment. Minimal impact in the aboveground weed community may have been caused by lower than normal precipitation from April until August that contributed to poor and uneven emergence and may have limited final yield. Further, evaluation of the IWM treatments at one experimental site with three replications may have made it more difficult to discern differences. Previous studies have demonstrated the effectiveness of IWM on Setaria spp, but more data, and in this case, more years of rotation may be needed before we see definite changes aboveground and in the seedbank.

5.4 Future Research

At present, the IWM treatments and crop phases that were implemented in the spring of 2017 are still being applied in the rotation experiment. Multiple years of rotation may be required to determine the impact of the IWM treatments on the ACCase-resistant and -susceptible green foxtail biotypes, yellow foxtail and additional weeds in the seedbank, this is because seeds present from multiple years of seed rains delays the seedbanks response to management practices (Schwartz et al. 2015). In other long-term experiments, seedbank changes in response to

management practices were detected 4 years after implementation (Sosnoskie et al. 2009), but many seedbank analyses are completed 12 or 18 years after changes are made (Bàrberi and Lo Cascio 2001; Légère et al. 2011). In the spring of 2020, three years after the IWM treatment changes were introduced, soil cores were collected for another seedbank analysis using the same methodology. This seedbank analysis may give a more accurate picture of whether the IWM treatments will reduce densities of ACCase-resistant and total green foxtail, yellow foxtail and all non-foxtail weeds in the seedbank of this rotation experiment.

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