ACCase resistant green foxtail (*Setaria viridis (L.) P. Beauv*) in a long-term rotation study with different in-crop herbicide use intensities.

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Background:

In 2000, the University of Manitoba established the Pesticide Free Production (PFP) experiment at Carman, Manitoba (Schoofs et al. 2005). The objective was to reduce the selection pressure for herbicide resistant weeds in a zero-tillage production system through reduced in-crop herbicide-use. Two fully-phased, crop rotations were established, one annual rotation (Flax-Oat-Canola-Wheat), and an annual/perennial rotation (Flax-Oat-Alfalfa-Alfalfa). Both rotations were repeated three times in each block with each repeat subjected to a different level of in-crop herbicide use intensity. The control treatment allowed in-crop pesticides in all crops in the rotation (Control). The first PFP treatment (PFP Oats) omitted in-crop pesticide use during the oat crop, the second PFP treatment (PFP Oats & Flax) omitted herbicide use in both the flax and oat crops. These treatments imposed different selection pressure on weeds (Table 1).

In the spring of 2009, the weed seedbank was sampled and evaluated. Based on germinated seedling densities, *Setaria* species (*S. viridis* L., *S. glauca* L., *Echniochloa crus-gali* L.) were dominant, accounting for 53.1% of total weed density. Other dominant weed species included redroot pigweed (*Amaranthus retroflexus* L.), yellow wood sorrel (*Oxalis stricta* L.) and species belonging to the *Brassica* family (Gulden et al. 2011). Weed seedbank densities were lowest in the control treatments (5,000 seeds m^{-2}) and increased to on average over 10,000 seeds m^{-2} in the PFP Oats & Flax treatments. In the annual rotation, weed seedbank densities were greatest after flax and similar in all other crops. In the rotation including alfalfa, seedbank densities were similar in all crops with the exception those following second year alfalfa which had lower seedbank densities.

For several years, visual observations after in-crop herbicide applications have indicated that some green foxtail plants were no longer sensitive to ACCase inhibitor (Group 1) herbicides which are used frequently to manage grassy weeds in this study. In this study, we characterized the nature of this biotype and it's prevalence in the seedbank.

Methods:

Characterization of the suspected resistant biotype

In the fall of 2015, green foxtail seeds were collected from plants that were not controlled by Group 1 herbicides in the PFP rotation study at the Ian Morrison Research Farm at Carman, MB. Seeds of the suspected resistant biotype and a known susceptible biotype of green foxtail were planted in pots and thinned to 6 seedlings per pot. At the 3-4 leaf stage, seedlings were treated with 0, 0.1, 1, 10, 100 and 1000x field rate of Clethodim (1x = 29.6 g ai L^{-1}) using a spray cabinet was equipped with a single flat fan nozzle, to deliver a carrier volume of 100 L ha^{-1} at 275 kPa. Green foxtail shoot biomass was determined three weeks after treatment. Dose response curve was generated as per Seefeldt (1995) and the resistance factor was determined. Seedlings of the susceptible and resistant biotypes were sampled for DNA analysis of the ACCase gene. Extracted DNA was subjected to PCR to amplify a 1087 bp ACCase gene fragment using primers ACSA and ACSAR (Delye, 2005). After sequencing the amplified gene fragment, Biolegato software (packaged bldna-TRANSLATE function) was used to compare sequences of the suspected resistant and known susceptible biotypes.

Green foxtail biotype prevalence in the seedbank

In Spring 2017, prior to seeding, 8 soil cores (10 cm diameter, 7 cm depth) were collected from each treatment of the PFP trial. Soil was mixed, placed in trays, and transferred to the greenhouse to determine the germinable portion of the green foxtail seedbank (Figure 1a). At the 4-leaf stage, the green foxtail in the trays were treated with the 1x dose of clethodim as for the dose response curve. One third of each tray was left untreated, to serve as a control and facilitate clear differentiation between green and yellow foxtail (Figure 1b). Then soils were stirred, frozen (-20 C) and the cycle was repeated. Due to low green foxtail recruitment in all subsequent cycles, herbicide treatment was not repeated. From these data, the proportion and density of herbicide resistant green foxtail and the density of all green foxtail plants were determined. These three response variables were subjected to a mixed model ANOVA. The conformation of residuals to the Gaussian distribution and heterogeneity of variance were examined and corrected if necessary. Data from both rotations were analysed together, however, each rotation was analysed as a treatment substructure to account for crop differences between the rotations. Crop, level of in-crop herbicide use and rotation were considered fixed effects while replication and the interaction of rotation with replication were considered random. Means were separated using Fisher's protected least significant difference (alpha=0.05).

Objective:

Characterize an ACCase resistant green foxtail population in a long-term rotation study and investigate the effects of crop rotation and in-crop herbicide use intensity on the prevalence of this biotype.

Table 1. In-crop herbicides applied to each crop in each rotation of the PFP long-term experiment from 2000-2016. Total group 1 herbicide use per rotation cycle is indicated. Recommended herbicide rates were used (Schoofs et al., 2004).

Rotation	Crop	Active Ingredient and Group Number of In-Crop Herbicides	Control	PFP Oats	PFP Oats & Flax
Annual Rotation	Oats	Thifensulfuron-methyl (Group 2) Tribenuron-methyl (Group 2)			
	Flax	**Sethoxydim (Group 1)** Bromoxynil (Group 6) MCPA (Group 4)	X	X	
	Wheat	Thifensulfuron-methyl (Group 2) Tribenuron-methyl (Group 2) **Clodinafop-propargyl (Group 1)**	X	X	X
	Canola	Glufosinate ammonium (Group 10)			
Annual/Perennial Rotation	Oats	Thifensulfuron-methyl (Group 2) Tribenuron-methyl (Group 2)			
	Flax	**Sethoxydim (Group 1)** Bromoxynil (Group 6) MCPA (Group 4)	X	X	
	Alfalfa year 1	**Sethoxydim (Group 1)**	X	X	X
	Alfalfa year 2	No in-crop herbicide applied			
		Total in-crop group 1 herbicide use per rotation cycle.	2	2	1

A. B.

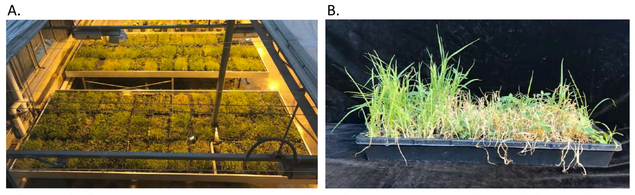

Figure 1. Greenhouse seedbank evaluation (A) and green foxtail herbicide resistance screening (B) showing the clethodim treated (right) and untreated (left) portions of the tray.

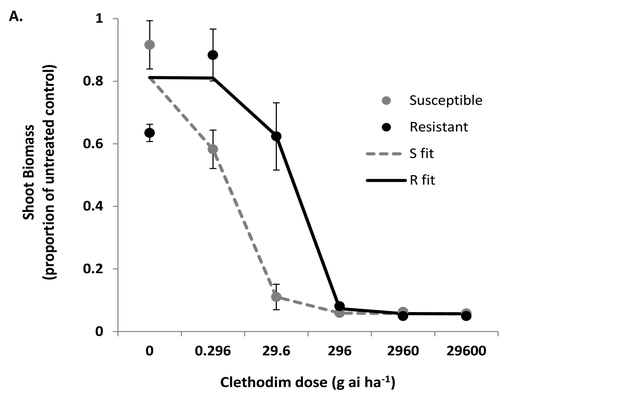

Figure 2. Shoot biomass response 3 weeks after treatment with clethodim of a known susceptible and a suspected resistant green foxtail biotype (A) and nucleotide (B. top) and translated amino acid (B. bottom) sequence for the ACCase enzyme from the known susceptible and suspected resistant green foxtail biotype. Standard errors of the mean and fitted dose response curves are indicated for each biotype in A. The top line for the nucleotide and translated amino acid sequences (B) represent the known susceptible and the suspected resistant green foxtail biotypes, respectively.

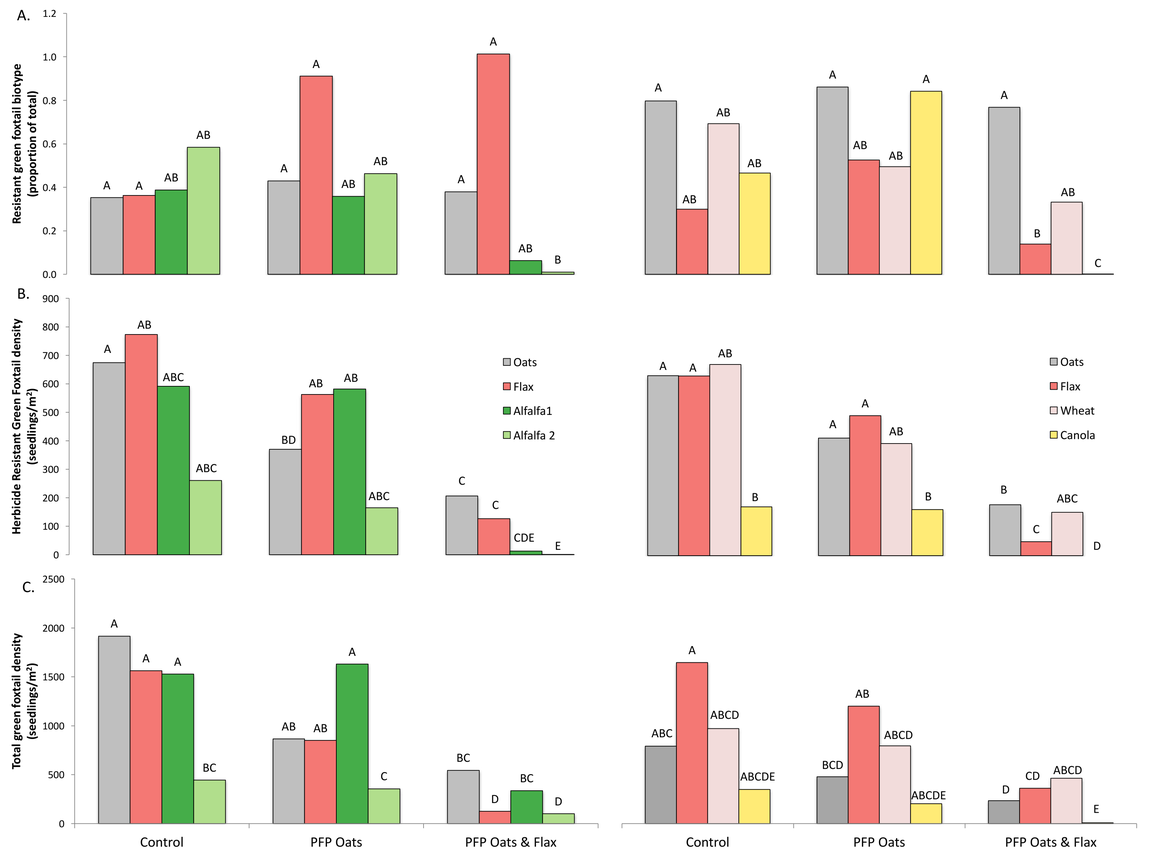

Figure 3. Proportion (A) and density (B) of the herbicide green foxtail biotype and total green foxtail density (C) in response to in-crop herbicide use intensity in an annual (left) and annual-perennial (right) rotation in a field study initiated in 2000. Different herbicide use intensities were imposed by omitting in-crop herbicides in oats only (PFP Oats), in oats and flax (PFP Oats & Flax) or no in-crop herbicide omission (Control). Within each response variable, bars with different letters are significantly different.

Results:

Characterization of the suspected resistant biotype

The dose response curves revealed that the suspected resistant green foxtail biotype was about 9-times less sensitive to clethodim than the known susceptible control biotype (Fig. 2A). Subsequent molecular analysis identified a Ile-1781-Leu substitution within the extracted gene fragment of the resistant biotype (Fig. 2B). This substitution is known to confer resistance to ACCase inhibitors in green foxtail (De Prado et al, 2004) and in *Lolium* spp. where it confers an almost identical level of resistance (Yu, Q. 2007). Similar resistance characteristics in both *Setaria* and *Lolium* genera suggest that the shared single nucleotide polymorphism is the main cause of resistance. In addition to the nucleotide substitution, a frameshift deletion was identified in the codon immediately downstream from the substitution (Fig. 2B top). The contribution of this frame shift mutation to ACCase resistance remains unknown.

Green foxtail biotype prevalence in the seedbank

Irrespective of the base rotation (annual vs. annual/perennial), the lowest densities and proportions of the ACCase resistant green foxtail biotype and total green foxtail densities were found when using the lowest number of in-crop herbicide applications (Fig. 3). Low in-crop herbicide use also resulted in the most significant differences in the three green foxtail parameters investigated here. In the annual rotation, canola consistently had the lowest total and herbicide resistant green foxtail seedbank densities in this treatment, but also showed the lowest proportion of ACCase resistant green foxtail in the seedbank. Similar green foxtail population dynamics were observed in the second year alfalfa crop in the annual/perennial rotation. Both canola and alfalfa are highly competitive against green foxtail and the effective glufosinate herbicide program in canola and cutting alfalfa for hay throughout the growing season also contributed to this. The same trends in seedbank densities were observed for both canola and second year alfalfa with increase in-crop herbicide use, although these differences were not always statistically significant. Among crops, differences in the proportion of ACCase resistant green foxtail, however, only were observed at the lowest in-crop ACCase use levels (= lowest selection pressure). With increased in-crop ACCase use, differences in the proportion of ACCase resistant green foxtail in the seedbank were no longer observed. Unfortunately, it is not possible to separate the importance of ACCase use intensity from that of ACCase use in a poorly competitive crop (flax) in this study as ACCase use frequencies were the same in the annual and annual/perennial rotations in the Control and PFP Oats treatments (Table 1).

Total weed seedbank densities (all species) reflect those observed in 2009 (Gulden et al. 2011), where, in both rotation, total weed seedbank densities were greatest in the treatments with the lowest in-crop herbicide use intensities (data not shown). The divergent trend in total weed seedbank densities and green foxtail densities in response to low in-crop herbicide use indicate that green foxtail and particularly ACCase-resistant green foxtail is less prominent in the weed community and suggests that green foxtail is even less significant under this herbicide regime.

Oats and flax were the only two crops common to both rotations. Interestingly, at the lowest in-crop herbicide use levels, a difference in the proportion of ACCase-resistant GF between oats and flax was observed only in the annual rotation with a non-significant trend in the opposite direction when alfalfa replaced wheat and canola in the annual/perennial rotation. No differences in ACCase use frequency or order of use occurred between the two rotations, however, the Group 1 active ingredients differed between the wheat (clodinafop) and first year alfalfa crops (sethoxydim) (Table 1). This ACCase resistant GF biotype has not been screened with active ingredients in the Aryloxyphenoxy proprionic acid family, however, the Ile-1781-Leu substitution is known to confer resistance to both Cyclohexanediones and Aryloxyphenoxy proprionic acids (Yu, 2007).

A broad range in the proportion of HR green foxtail in the spring seedbank was observed among the treatments in this study (>5% to 100%) (Fig 3a). Seedbank density and proportion of total density of the HR biotype were closely related (Pearson R 0.91, p-value = 0.0001) among all treatments which could have contributed to this observation. Whether this resistant green foxtail biotype was selected for in this rotation study or whether it was introduced from elsewhere is not known. Reducing the selection pressure through fewer in-crop ACCase inhibitor applications reduced the occurrence and prevalence of ACCase resistant green foxtail nevertheless.

Conclusions:

1. The presence of an ACCase resistant green foxtail biotype with a resistance factor of about 10 to clethodim was confirmed. An Ile-1781-Leu substitution was identified as the likely cause of resistance to ACCase inhibitors. The contribution of the frame shift mutation to ACCase resistance remains unknown.
2. Lower in-crop ACCase use intensities (PFP Oats & Flax) reduced the total and herbicide resistant green foxtail seedbank densities, but only affected the proportion of the herbicide resistant biotype after competitive crops with management tools that limited seed rain.
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4. Integrated weed management strategies including competitive crops, alternative herbicides or other tools (eg. (cutting for hay) that limited weed seed rain were critical for reducing the prevalence of this resistant green foxtail biotype in the seedbank.

Funding provided by:

Manitoba Pulse Soybean Growers
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NSERC CRSNG
Bayer CropScience

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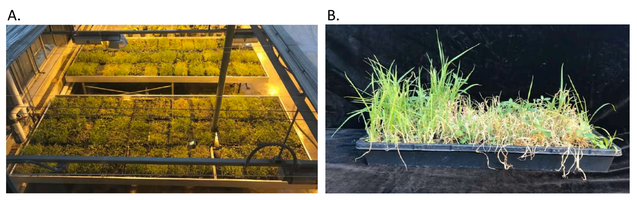
	Canola	Glufosinate ammonium (Group 10)		
Annual/Perennial Rotation	Oats	Thifensulfuron-methyl (Group 2) Tribenuron-methyl (Group 2)		
	Flax	**Sethoxydim (Group 1)** Bromoxynil (Group 6) MCPA (Group 4)	X	
	Alfalfa year 1	**Sethoxydim (Group 1)**	X	
	Alfalfa year 2	No in-crop herbicide applied		
		Total in-crop group 1 herbicide use per rotation cycle.	2	



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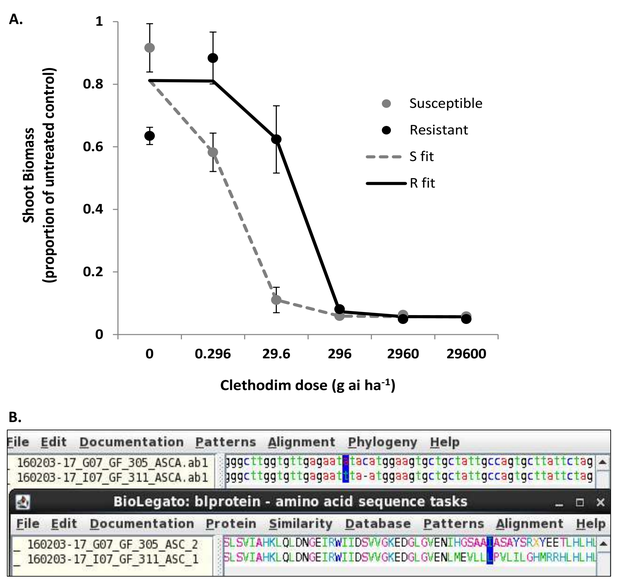

Figure 2. Shoot biomass response 3 weeks after treatment with clethodim of a known susceptible and a suspected resistant green foxtail biotype (A) and nucleotide (B. top) and translated amino acid (B. bottom) sequence for the ACCase enzyme from the known susceptible and suspected resistant green foxtail biotype. Standard errors of the mean and fitted dose response curves are indicated for each biotype in A. The top line for the nucleotide and translated amino acid sequences (B) represent the known susceptible and the suspected resistant green foxtail biotypes, respectively.

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Green foxtail biotype prevalence in the seedbank

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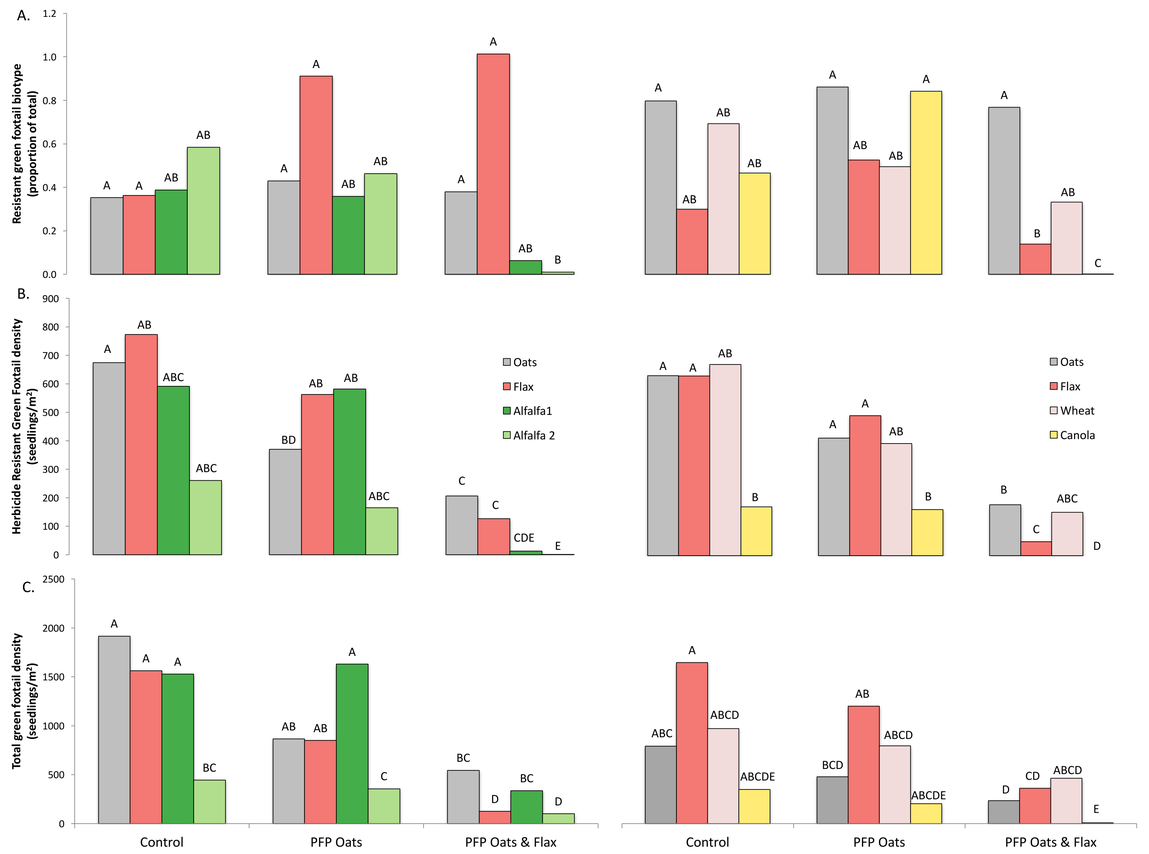

Figure 3. Proportion (A) and density (B) of the herbicide green foxtail biotype and total green foxtail density (C) in response to in-crop herbicide use intensity in an annual (left) and annual-perennial (right) rotation in a field study initiated in 2000. Different herbicide use intensities were imposed by omitting in-crop herbicides in oats only (PFP Oats), in oats and flax (PFP Oats & Flax) or no in-crop herbicide omission (Control). Within each response variable, bars with different letters are significantly different.

Conclusions:

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