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Source: Canadian Journal of Plant Science, 102(5): 1001-1006

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjps-2021-0269

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## Triticale cultivars and seeding rates affect wheat stem sawfly survivorship and parasitism by *Bracon cephi*

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## Abstract

Several insects, including the wheat stem sawfly (*Cephus cinctus* Norton, Hymenoptera: Cephidae), interfere with biomass and grain production of triticale (×*Triticosecale*). We conducted a 2-year study in southern Alberta to compare the cultivars Pronghorn and AC Ultima at 200 and 400 seeds/m<sup>2</sup> in terms of wheat stem sawfly damage and incidence of a parasitoid wasp (*Bracon cephi* Gahan, Hymenoptera: Braconidae). Plants of AC Ultima seeded at the high rate were on average less damaged than those of Pronghorn, and had more parasitoids. AC Ultima was characterized by more solid pith development in the lumen, compared with Pronghorn, and it can be considered adequate germplasm for the development of more resistant solid triticale cultivars.

Key words: insect, pest, parasitoid, triticale

## Résumé

Plusieurs insectes dont le cèphe du blé (*Cephus cinctus* Norton, Hyménoptères : Cephidae), réduisent la production de biomasse et de grain par la triticale (×*Triticosecale*). Les auteurs ont entrepris une étude de deux ans, dans le sud de l'Alberta, en vue de comparer les dommages causés par le cèphe et les effets d'une guêpe parasite (*Bracon cephi* Gahan, Hyménoptères : Braconidae) sur les cultivars Pronghorn et AC Ultima semés à raison de 200 ou de 400 graines au mètre carré. En moyenne, les plants d'AC Ultima semés à la densité la plus élevée présentaient moins de dommages que Pronghorn et les parasitoïdes étaient plus nombreux. AC Ultima se caractérise par un endocarpe plus robuste dans le lumen que celui de Pronghorn, aussi pourrait-on envisager l'usage de son plasma germinal pour créer des cultivars de triticale plus résistants. [Traduit par la Rédaction]

Mots-clés : insecte, ravageur, parasitoïde, triticale

## Introduction

Triticale (×Triticosecale) is a man-made cereal crop with agronomic and market traits well suited for the production of ethanol. Triticale was produced from a cross of bread wheat (Triticum aestivum L.) and rye (Secale cereal L.) and it is grown mainly for grain and animal feed. Triticale is characterized by high yield potential, good tolerance to most biotic and abiotic stresses, and limited use for human food. Therefore, it is a crop that can be used for ethanol production (McKenzie et al. 2013) and other industrial applications. In a benchmark study conducted at 36 locations across western Canada, triticale outperformed most cultivars in various wheat classes in terms of grain yield, biomass, and other desirable traits for ethanol feedstock (Beres et al. 2013). Furthermore, triticale can be more resistant to diseases and may compete better against weeds (Oettler 2005). With increased interest in the bioeconomy and the need for feedstocks, land area planted to triticale may expand and experience increased insect pest pressure.

Limited research was done on insect pests of triticale in Canada, but some plot and laboratory work were reported. The assemblage of insect pests that attack triticale is similar to those that attack other cereals, such as wheat and barley. According to a recent field guide, several noctuid cutworms, grasshoppers, greenbug, wheat midge, and two coleopteran pests (wireworms and cereal leaf beetle) attack triticale (Philip et al. 2018). Triticale was one of the crops that supported development of cereal leaf beetle larvae (Oulema melanopus L.) in a laboratory study of host preferences (Kher et al. 2016). The authors concluded that triticale was a secondary host of this insect. Nevertheless, one of the highest levels of infestation and leaf damage observed in the field by this pest was from winter forage triticale near Lethbridge, Alberta (Hector Carcamo, Agriculture and Agri-Food Canada, personal communication, 2013 field observation of 4 out of 10 stems infested with larva). Spring and winter triticale cultivars are not only characterized by agronomic advantages to mitigate abiotic production risks, but they also attract insect pests at various times of the year.

A notable pest omission for triticale in the current field guide of insect pests in the Prairies by Philip et al. (2018) is wheat stem sawfly (Cephus cinctus). The biology and management of wheat stem sawfly was studied for over a century since this native insect was reported to threaten wheat production in the Great Plains of North America (Fletcher 1896; Beres et al. 2011). Damage occurs from reduction of seed weight from larval mining inside the stems and harvest losses from lodging of stems cut at the base when the larva constructs an overwintering chamber (Holmes 1977; Beres et al. 2007). Stem lodging of the triticale cultivar Pronghorn from cutting by this insect can exceed 50% under high pest pressure (Beres et al. 2013), which is comparable to current susceptible wheat cultivars. Furthermore, the relatively thick stem diameters of triticale stands, compared with wheat, can produce wheat stem sawfly populations that are disproportionately females (Carcamo et al. 2020) with the potential to contribute to pest population.

Plant density and cultivar can affect the risk of insect pest damage and populations of their natural enemies. For example, plant density of solid stem wheat (AC Lilian) and hollow stem CDC Go can affect wheat stem sawfly infestation and yield (Beres et al. 2011). In this study (Beres et al. 2011), both of these bread wheat cultivars had the highest sawfly infestation at the lowest plant density; however, the durum cultivar AC Avonlea had low sawfly infestion rates regardless of plant density. In another study, Wu et al. (2013) demonstrated impacts of wheat cultivars on sawfly damage in terms of tunneling lengths by larvae and significant differences in the proportion of wheat stems where the pest was attacked by a parasitoid wasp. More studies comparing variety effects on insect populations, particularly those that include pests and their natural enemies, are warranted for field crops.

Research on wheat stem sawfly in triticale was motivated by the overlapping geographies between this crop and insect in the production areas in the brown soil zones. The objectives of this study were to compare wheat stem sawfly damage between two cultivars of triticale commonly planted in western Canada and to model interaction effects of cultivar × seeding rates. We also quantified levels of sawfly parasitism by a braconid wasp (*Bracon cephi* Gahan, Hymenoptera: Braconidae) in relation to these factors. We hypothesized that cultivars would not differ in terms of sawfly damage, but seeding rates could affect the crop and its interaction with the pest and its parasitoid. We predicted that at high seeding rates the plants may be less attractive to the pest and suffer less cutting damage.

## Materials and methods

The study site in 2008 and 2009 was located about 10 km west of Lethbridge in southern Alberta (lat. 49°42'N, long. 112°50'W), Canada, in the Mixed Moist Grassland Ecoregion of the Prairies Ecozone of Canada. This site serves as a dedicated wheat stem sawfly research site established by staff from Agriculture and Agri-Food Canada since the late 1960s

(Peterson et al. 1968) and later re-inoculated to maintain sawfly populations (Beres et al. 2005). This semiarid region is characterized by cool annual average temperatures around 5 °C and low annual precipitation (<400 mm). Soils are comprised of mostly Orthic Dark Brown Chernozem clay loams. Farming systems at the site consisted of a wheat-herbicide fallow, no tillage rotation. Crop fertility and other standard agronomic practices such as planting in early May were followed to optimize crop yields for the ecoregion and to encourage high sawfly infestation. The cultivars Pronghorn and AC Ultima were selected because they were considered industry standards in 2008 and 2009; they were locally adaptated and characterized by high yield potential. The two seeding rates of 200 seeds/m<sup>2</sup> and 400 seeds/m<sup>2</sup> were selected to fall below and at the recommended rate (Collier et al. 2013). The cultivar by seeding rate treatments were randomly allocated in each of three blocks of four plots (3.7 m  $\times$  58.5 m).

Sampling to assess plant traits, sawfly infestation, parasitoid incidence, and cutting damage occurred at harvest time upon plant maturity in both years of the study. Near the middle of each plot, plants from a 1 m row were dug out, placed in paper bags and stored at 10 °C. In the laboratory, a subsample of 50 stems were randomly selected and dissected and the remaining were classified as cut or uncut by sawfly larvae. Stems not infested by larvae were ranked (1 = empty to 5 = solid) for level of solid pith lumen by internode as described by Carcamo et al. (2020). For infested stems, we recorded status (dead or alive) of sawfly larvae or the parasitoid *B. cephi* and the internode where they were found. Internodes were assigned a number from bottom to top and these were used to estimate the average internode location where a sawfly or parasitoid was found.

The experimental data were split into subsets based on the years of observations and separate models were executed for each year (2008 and 2009). The observed response variables were modeled using the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC) with 1000 iterations at multiple levels of iteration (MAXOPT = 1000 and NLOPTIONS MAX-ITER = 1000). The normal distribution of the response was not assumed and therefore the models were "generalized." The best-fitting distribution was selected for each variable based on the Bayesian information criterion (BIC). The models were "mixed" due to the inclusion of fixed factors (rate, cultivar, and rate  $\times$  cultivar) and the block was a random factor. Models of variance heterogeneity were tested and selected based on the values of the BIC associated with the respective models.

## **Results and discussion**

Data for pith expression by internode and overall stem pith expression were collected only in 2009 (Table 1). Expression of pith in the lumen was generally low (2 or less on a scale of 1 to 5), but varied in the five internodes of a stem and was affected by cultivar but not by seeding rate or their interaction (Table 1). Given the low levels of pith expression in these triticale cultivars, it is unlikely that annual variability is significant as observed in some solid stem bread wheats (Beres et al. 2012). Near the top of the plant, for internodes 4 and 5, the

						Inte	Internode				
			1		2		3		4		5
Cultivar	Rate	nur	se	nui	se	nui	se	nui	se	nui	se
Pronghorn	200	1.2225	0.0963b	1.0565	0.032135a	1.0395	0.02337a	1.512	0.0918a	1.6975	0.1695a
AC Ultima	200	2.1675	0.34355ab	1.3785	0.221a	1.095	0.0614a	1.0295	0.08445b	1.16	0.07405a*
Pronghorn	400	1.156	0.0939b	1.023	0.031555a	1.0195	0.023055a	1.235	0.08305ab	1.5105	0.16285a
AC Ultima	400	2.343	0.2841a5	1.5065	0.1864a	1.0495	0.05105a	1.0315	0.07515b	1.3835	0.0826a*
Note: Bonferroni-grouping is indicated by letters where least squares-means with the same letter are not significantly different. *The Bonferroni-grouping does not reflect the significant difference (Bonferroni-adjusted p = 0.002) of AC Ultima 400 and AC Ultima 200.	ig is indicated b 0 and AC Ultim	y letters where 1a 200.	least squares-means	with the same l	letter are not signific	cantly different.	*The Bonferroni-gr	ouping does not	reflect the significa.	nt difference (Bo	nferroni-adjusted p

Table 1. Least squares-means (mu) and standard errors (se) of rank of solid pith for each internode for each cultivar and seeding rate.

2017).

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mean solidity of pith of Pronghorn was greater than that of AC Ultima ( $F_{[1,5]} = 12.80$ , p = 0.0159), but at lower internodes of Pronghorn plants, mean pith solidity was less than that of AC Ultima. For AC Ultima, the mean pith expression of the first internode ( $\mu \approx 2$  out of 5) was statistically significantly greater than for Pronghorn ( $F_{[1,5]} = 27.20$ , p = 0.0034). Also, the average pith expression for the observed five internodes was greater for AC Ultima than for Pronghorn ( $F_{[1,5]} = 14.91$ , p = 0.0119). Seeding rate did not affect pith expression to a statistically significant degree as observed for wheat elsewhere (Beres et al. 2012).

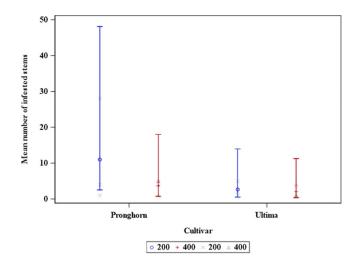
Development of triticale cultivars with solid pith for increased biomass production to feed a developing bioenergy industry (Cantale et al. 2016) may coincide with improved management of wheat stem sawfly. The solidity of pith in the lumen of the experimental cultivars vary and the average solidity of pith from AC Ultima was higher than that from Pronghorn, and the differences were greater for low internodes (i.e., closer to the base of the plant). In the current study, AC Ultima was characterized by solid pith in the first internode, compared to Pronghorn. This may contribute to the control of sawfly because larvae migrate to the base of the plant at the end of the growing season when the stem is drier. Thus, pith solidity in the lower internodes can impede successful movement of the larva that would otherwise make an overwintering chamber, which can induce stem lodging. Higher levels of pith expression, closer to those observed in durum wheat (3.75 or more on a scale of 1–5), are hypothesized to be a factor in the attenuation of sawfly damage (Beres et al.

Wheat stem sawfly infestation and subsequent damage in terms of cut stems was low (less than 10% of stems infested) in 2008 and moderate or high in 2009. The total number of infested stems per sample was not statistically significantly different between the experimental cultivars and experimental seeding rates in 2008 and 2009 and there were no significant interactions (Figs. 1a and 1b). For Pronghorn samples at the experimental low seeding rate, the mean infestation rate was 10% in 2008 and 70% in 2009. The range of observed survivorship of larvae at the end of the summer was 20%–60% and the effects of the cultivar and seeding rate were not statistically significant (data not shown). The proportion of stems cut by the larvae that would topple to the ground (Figs. 2a and 2b) was concordant with infestation (tau = 0.68239, p < 0.0001). In 2008, there were no significant differences among the treatments, but there was much higher variability in cutting among plots planted at 200 seeds/ $m^2$  than those planted at 400 seeds/m<sup>2</sup> (Fig. 2*a*). In 2009, there was no (statistically significant) difference in cutting damage between cultivars, but the seeding rate affected it ( $F_{[1,6]} = 12.53$ , p = 0.0122). The level of cutting was lower at 400 seeds/m<sup>2</sup> than at 200 seeds/m<sup>2</sup> and the difference was especially large for Pronghorn (Fig. 2b). Plant traits such as height and stem diameter can influence sawfly infestation and subsequent damage. Adults preferentially infest taller plants (Buteler et al. 2009) and lay more female eggs on plants with thicker stem diameters (Carcamo et al. 2020). These traits are expected to increase in stands planted at lower seeding rates and may explain the higher level of cutting damage observed in our current study in

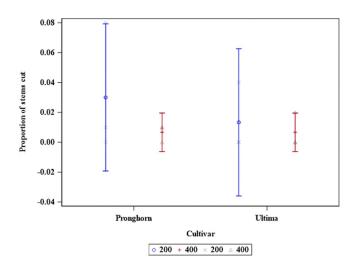
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**Fig. 1.** Number of triticale stems infested by wheat stem sawfly in 2008 (*a*) and in 2009 (*b*) on two triticale cultivars (Pronghorn and AC Ultima) at two different seeding rates (200 and 400 per  $m^2$ ).

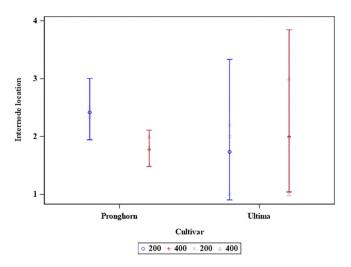


**Fig. 2.** Proportion of triticale stems cut by wheat stem sawfly larvae in 2008 (*a*) and 2009 (*b*) on two triticale cultivars (Pronghorn and AC Ultima) at two different seeding rates (200 and 400 per  $m^2$ ).



the triticale plots that were seeded at 200 seeds/m<sup>2</sup> vs those seeded at 400 seeds/m<sup>2</sup>. The proportion of sawfly larvae parasitized by the wasp *B. cephi* was higher in plots seeded at the higher seeding rate.

The internode location, where the sawfly larva was found before harvest, was affected by seeding rate or cultivar depending on the year (Figs. 3*a* and 3*b*). In 2008, more sawfly were found higher along the stem of Pronghorn planted at 200 seeds/m<sup>2</sup> (mean internode location = 2.41) than in plots planted at 400 seeds/m<sup>2</sup> (mean = 1.77) ( $F_{[1,5]} = 8.17$ , p = 0.0355). In 2009, at the 200 seeding rate, the mean internode position of sawfly larvae in AC Ultima plants (mean = 2.93) was higher than in Pronghorn plants (mean = 2.41) ( $F_{[1,6]} = 11.77$ , p = 0.0140). This information is important for conservation of the key sawfly parasitoid **Fig. 3.** The location (Internode 1–4) of sawfly larvae in 2008 (*a*) and 2009 (*b*) on two triticale cultivars (Pronghorn and AC Ultima) at two different seeding rates (200 and 400 seeds per m<sup>2</sup>).

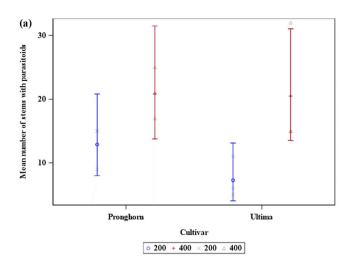


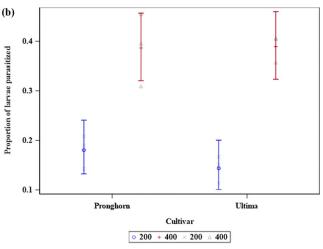
*B. cephi*, which overwinters in cereal stubble and can significantly reduce sawfly populations as discussed below. As suggested by Meers (2005), harvesting heights should be adjusted to leave stubble as high as possible (more than two internodes for these two cultivars) to avoid harming this natural enemy.

The parasitoid wasp B. cephi was scarce in 2008; therefore, only the 2009 results are presented. Seeding rate significantly affected the count of stems with parasitoids ( $F_{[1,6]} = 22.28$ , p = 0.0033) (Fig. 4a). Although cultivar and seeding rate interactions were not statistically significant, AC Ultima triticale seeded at 200 seeds/m<sup>2</sup> had the lowest number of parasitoids, 7 per 100 stems and significantly lower than the higher seeding rate of 400 seeds/m<sup>2</sup> for both cultivars, which averaged just over 20 parasitoids (Fig. 4a, Bonferroni-adjusted p = 0.0323 for the comparison with Pronghorn at 400 and p = 0.0351 for AC Ultima at 400). The proportion of larvae parasitized was concordant ( $\tau = 0.76761$ , p < 0.0001) with the total number of stems with parasitoids (Fig. 4b). For the experimental cultivars, the mean proportion of larvae killed by the parasitoid at the high seeding rate was greater than that at the 200 seeding rate ( $F_{[1,6]} = 73.9$ , p = 0.0001). Parasitoid cocoons (mature larvae of the second generation) were found consistently in the second internode regardless of cultivar or seeding rate. While it is possible to quantify the populations of first generation parasitoids by careful examination of pinsize exit holes from the stems, this was not attempted in this study. Such exit holes would be expected to occur at higher internode locations where younger sawfly larvae were foraging earlier in the summer. Differential expression of solid pith along the internodes could restrict the position of the older sawfly larva and direct the second generation parasitoid towards lower areas of the stem where farmers may increase its chance of survivorship by leaving stems with more internodes.

Natural enemies can interact with agronomic factors to enhance pest management. The wasp *B. cephi* is a key factor that

**Fig. 4.** The mean number of triticale stems with parasitoids, *Bracon cephi*, in 2009 (Fig. 4*a*) and the proportion of sawfly larvae parasitized (Fig. 4*b*) on two triticale cultivars (Pronghorn and AC Ultima) at two different seeding rates (200 and 400 per m<sup>2</sup>).





contributes to wheat stem saw fly mortality, and therefore it can contribute to wheat seed weight, which would otherwise be lost to pest damage (Buteler et al. 2008) and it can reduce wheat stem cutting by wheat stem sawfly (Carcamo et al. 2016). In the current study, there were more parasitoids in the plots seeded at 400 seeds/m<sup>2</sup> than those seeded at 200 seeds/m<sup>2</sup>, and corresponded with lower cutting levels. Planting at higher seeding rates is recommended for hollow stem wheat cultivars to maximize yields (Beres et al. 2012) and reduce sawfly damage. The current results motivate further research to elucidate parasitoid abundance at the current experimental high seeding rate. It is hypothesized that stems are thinner and wasps are more capable to parasitize larvae in more dense stems, thus benefiting foraging by the second generation of parasitoids.

## Conclusion

Triticale is a multipurpose and resilient crop and therefore serves as an attractive feedstock for the production of ethanol, which may lead to expanded acreages and insect pest threats such as wheat stem sawfly. For risk management with this pest, we demonstrated important differences between the two cultivars, Pronghorn and AC Ultima, and the need to seed at high rates. Combining host plant resistance, cultural strategies, and biological control (Carcamo et al. 2016) will remain an important strategy to manage insect pests in a sustainable manner. For triticale, developing host plant resistance in the form of a solid pith in the lumen will likely be a compatible strategy to increase biomass production. Future research should focus on integrating these strategies with *B. cephi*.

## Acknowledgements

We are grateful for the excellent laboratory and field technical support at AAFC's Lethbridge Research and Development Center provided by R. Dyck, S. Simmill, T. Larson, X. Wu, S. Daniels, and C. Herle.

## Article information

#### History dates

Received: 3 December 2021 Accepted: 8 March 2022 Accepted manuscript online: 19 May 2022 Version of record online: 21 September 2022

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Brian Beres served as an Editor in Chief at the time of manuscript review and acceptance; peer review and editorial decisions regarding this manuscript were handled by Tyler Wist.

## **Competing interests**

The authors declare there are no competing interests.

## Funding information

This research was supported by Agriculture and Agri-Food Canada's Agricultural Bioproducts Innovation Program.

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