

## Invited Review Article

**Parasitoid (*Bracon cephi*) effects on grain yield of selected genotypes of wheat infested by *Cephus cinctus***X. Wu<sup>1,2</sup>, H. Cárcamo<sup>2</sup>, B. Beres<sup>2</sup>, B. Pang<sup>1,2\*</sup><sup>1</sup>Laboratory of Entomology, College of Agriculture, Inner Mongolia Agricultural University, Inner Mongolia, China 010019<sup>2</sup>Lethbridge Research Centre, AAFC, Lethbridge, Alberta, Canada

\*Corresponding author: baopingpang@hotmail.com

**Abstract**

The wheat stem sawfly has been a major pest of spring wheat in the southern prairies of Canada and the adjoining parts of the United States for several decades. *Bracon cephi* (Gahan) is an important endemic ectoparasitoid of the wheat stem sawfly that can reach very high levels of parasitism. The objectives of this study were to determine the effect of *B. cephi* on the feeding damage (stem mining) caused by the sawfly and consequences on plant fitness in terms of grain yield in various hollow- and solid-stemmed wheat genotypes. This study was conducted at Coalhurst, west of Lethbridge, Alberta in 2003-2005, and 2008. Uninfested stems had lighter grain heads than those infested by the sawfly. There was no consistent difference in grain head weights between uninfested stems, sawfly infested (but dead), and those parasitized by *B. cephi*. In 2008, the length of the feeding tunnel was significantly shorter in parasitized stems than those cut or with dead sawfly larvae; in other years the difference between stems with dead larva and those parasitized by *B. cephi* were not significant. We conclude that although *B. cephi* reduced stem mining by the wheat stem sawfly it did not affect the stem seed weight. Nevertheless, reduction in stem lodging during the growing season and lower sawfly populations in following years are important reasons to conserve this parasitoid.

**Keywords:** tritrophic interactions; wheat stem sawfly .**Abbreviations:** AC - Agriculture and Agri-Food Canada.**Introduction**

Over the last two decades, considerable attention has been paid to tritrophic interactions between plants, herbivores and their natural enemies (Vet and Dicke, 1992; Turlings & Wäckers, 2004; Ode, 2006). Gomez & Zamora (1994) studied the guild of three parasitoid species and its benefits to the plant through their effects on the weevil herbivore. Plants that had parasitized weevils produced a higher average number of seeds than those without parasitoids. In a greenhouse experiment, Smallegange et al. (2008) investigated the relationship between the gregarious parasitoid, *Cotesia glomerata*, their host *Pieris brassicae* L. and the plant host *Brassica nigra* L. They suggested that plant fitness was higher when the gregarious parasitoid deposited a single brood into its herbivorous host *Pieris brassicae* L. than those plants with unparasitized caterpillars feeding on them. In a similar study, Van loon et al. (2000) came to the conclusion that parasitization of *Pieris rapae* by *Cotesia rubecula* potentially conferred considerable fitness to *Arabidopsis thaliana* in terms of higher seed production. Under field conditions, maize plants attacked by *Spodoptera littoralis* larvae suffered much more feeding damage and produced about 30% less seeds at maturity, if the larvae were

not parasitized by *Cotesia marginiventris* (Fritzsche-Hoballah & Turlings, 2001). Buteler et al. (2008) demonstrated that mean seed weight per fertile spikelet was higher in wheat plants where the sawfly herbivore was parasitized by *Bracon cephi* and *Bracon lissogaster*. Not all studies have shown that plants benefit indirectly from parasitism of herbivorous larvae. In some cases parasitism can lead to greater leaf consumption rate by the herbivores (Parker & Pinnell, 1973). This was implied by Coleman et al. (1999) who observed significantly higher weights for parasitized larvae of *Pieris brassicae* L. compared to those not parasitized by *Cotesia glomerata* L. Similar reports were found in other studies (Rahman, 1970; Smith & Smilowitz, 1976; Slansky, 1978, 1986). Therefore, it seems that parasitoid effects on plant fitness are variable. Impacts of the parasitoid on plant fitness may depend on the specific nature of the tri-trophic relationship and the timing of the attack.

Wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a native insect that initially fed on wide-stemmed grasses in the northern Great Plains of North America. The wheat stem sawfly produces one generation annually, adults emerge from the previous year's stubble in

**Table 1.** Stem lumen, wheat class, and literature reference for wheat genotypes used in the 2003-2005 study of *Bracon cephi* and sawfly effects on wheat yield.

Cultivar	Stem type	Class	Reference
<i>Coalhurst site</i>			
AC Navigator	Hollow	Canada Western Amber Durum (CWAD)	Clarke et al. 2000
Kyle	Hollow	CWAD	Townley-Smith et al. 1987
AC Barrie	Hollow	Canada Western Red Spring (CWRS)	McCraig et al. 1996
AC Cadillac	Hollow	CWRS	DePauw et al. 1998
McKenzie	Hollow	CWRS	Graf et al. 2003
AC Abbey	Solid	CWRS	DePauw et al. 2000
AC Eatonia	Solid	CWRS	DePauw et al. 1994
B9973B03&AC4AW	Solid	Experimental line	Clarke et al. 1998
B9973B03&AG2AT	Solid	Experimental line	Clarke et al. 1998
G9608B1-L12J11BF02	Solid	Experimental line	Clarke et al. 1998

late spring and move into fields of spring wheat. Yield loss results from larval mining and the un-recovered toppled stems. Seamans (1945) and McNeal et al (1955) concluded that larval mining reduced wheat weight loss by 6-10% while Wallace and McNeal (1966) estimated the losses at 5 to 30 %. Beres et al (2007) suggested grain losses from cutting damage ranged from 10 to 15%. Based on the distribution of sawfly damage in the Canadian prairies, there are potentially more than 4 million ha of wheat that sustain between 1 - 10% damage (Beres et al., 2007). *Bracon cephi* (Gahan) is an idiobiont ectoparasitoid and the only abundant parasitoid of wheat stem sawfly in cultivated fields in Canada (Holmes, 1963). *Bracon cephi* overwinters as mature larva and adults emerge in early summer at the same time as the sawfly but they do not attack the larvae until they are in the late instar (Criddle, 1923, Nelson & Farstad, 1953). The new generation of parasitoids emerges late in the summer and can attack the sawfly larvae after it has cut their host stems but its success rate is low (2.5%) (Holmes et al., 1963). Cause of variations in effectiveness of parasitoids greatly depends on sawfly density, variety of host plant, date of plant maturity and weather conditions (Holmes et al., 1963). The objective of this work was to improve our understanding of the role of *B. cephi* in preventing stem mining damage and protecting wheat yields in areas prone to attack in various wheat genotypes. Specifically, we tested the hypothesis that *Bracon cephi* had greater potential to prevent sawfly damage in hollow-stemmed wheat than in wheat with solid pith and more resistant to larval sawfly damage.

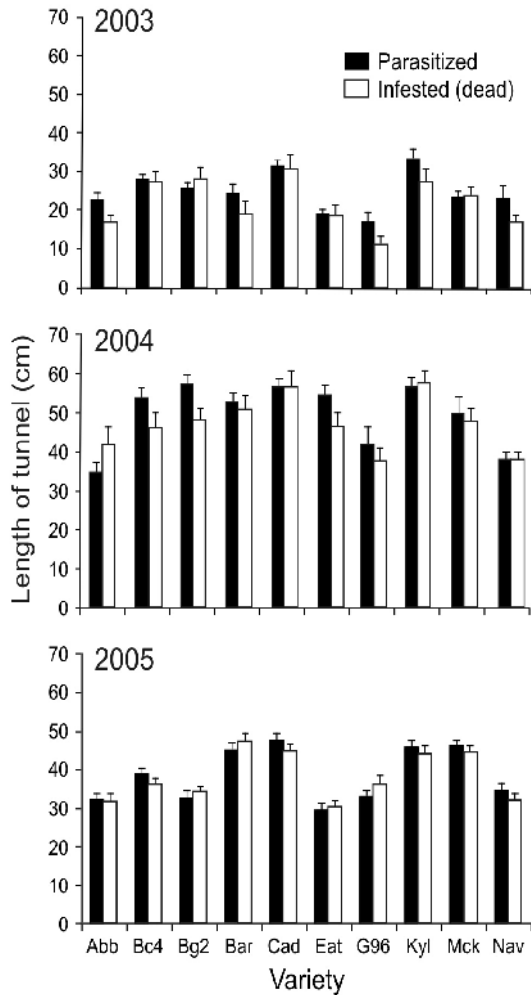
## Materials and methods

These studies were conducted at the Agriculture and Agri-Food Canada wheat stem sawfly research nursery (Beres et al. 2005) 10 km west of Lethbridge. For the main study conducted from 2003 to 2005, ten wheat genotypes (Table 1) were planted and included 3 hollow-stemmed susceptible hard red spring bread wheat cultivars (AC Barrie, AC Cadillac, McKenzie), 2 hollow-stemmed amber durum cultivars (AC Navigator, Kyle), 2 solid-stemmed hard red spring bread wheat cultivars (AC Abbey, AC Eatonia), and 3 solid or partially solid-stemmed, experimental synthetic hexaploid wheat lines (B9973B03&AC4AW, B9973B03&AG2AT, G9608B1-L12J11BF02) with genetic material from a solid durum wheat and a hard red spring bread wheat (Clarke et al.1998). In 2009, a smaller study was conducted by sub-sampling wheat stems from the hollowed stem cultivar CDC Go (hard red spring bread wheat

([http://agbio.usask.ca/uploads/gjg533/cdc\\_go.pdf](http://agbio.usask.ca/uploads/gjg533/cdc_go.pdf))) that had been planted in 2008 as part of a related agronomic study at the same site. For the first study, a self-propelled, 6 row precision cone seeder was used to sow plots to an area of 2m x 3m, a depth of 25-30 mm, and at a density of 200 seeds/ m<sup>2</sup>. Seeding dates were 17 May 2003, 26 April 2004 and 2 May 2005. Around mid to late June each year, the herbicides Target at 1.5 L/ha mixed with Horizon at 0.23 L/ha were applied to control weeds. For the second data set processed in 2009, plots were arranged in experimental units of 3m x 10m, and planted on 25 April 2008 at densities of 150, 250 and 350 seeds/ m<sup>2</sup> using a Conserva Pak air drill with a 23cm row spacing. The herbicides Achieve Liquid Gold (1.5 L/ha) and Buctril (0.5 L/ha) were applied on 4 June 2008.

In early September, a sample of wheat stems was collected near the middle of each plot by excavating approximately 30 cm of plant material from 3 adjacent rows. Plants were placed in large paper bags and stored at 10°C until the stems could be dissected to determine sawfly damage and presence of the parasitoid. Outside stem diameter for each main stem and tiller of a plant was measured about 1cm below the second node at approximately 0, 45 and 90° angles with a digital caliper. The plant collection protocol was similar in 2008 except that two samples of 0.5 m were collected per plot. Stems collected in 2008 and processed in 2009 were divided into four categories depending on sawfly or parasitoid presence.

Uninfested stems were those without sawfly and no evidence of tunnelling; infested-dead (henceforth referred to as “infested”) were those stems with sawfly damage and a dead larva found in the stem but killed by factors other than the parasitoid; “parasitized” were those stems where *B. cephi* was found in the stem at some developmental stage; cut stem were only sampled in 2008 and included those stems cut by sawfly. Only the 3 first categories were processed in the first data set from 2003-2005 when our objective was only to relate tunnel length to presence of parasitoid and yield relative to infested and uninfested stems. The relationship between tunnel length and yield was explored through simple correlation analysis. Statistical analysis to compare stem categories was performed with SAS version 9.1.3. Differences among stem treatments were compared using the Proc Mixed and running an ANCOVA by variety within each year with stem treatment and the stem diameter covariate as the fixed factors and replicate as the random factor. Stem diameter was used as a covariate to remove the known effect of this variable on ovi position choices of the wheat stem sawfly and expected



**Fig 1.** Length of tunnel bored by the larvae of wheat stem sawfly for stems that were infested (dead sawfly larva) and stems with larva parasitized by *Bracon cephi* in 2003, 2004, and 2005. Abbreviations: Abb=AC Abbey, Bc4=B997-C4AW, Bg2=B997-G2AT, Bar= AC Barrie, Cad= AC Cadillac, Eat= AC Eatonia, G96= G960-BF02, Kyl= Kyle, Mck= McKenzie, Nav= AC Navigator.

yield differences between thinner and thicker stemmed plants. Furthermore, we repeated the analysis using only main stems, without the diameter covariate, but do not report the results because they were similar to those using all stems and did not change the conclusions. Differences among means when the Proc Mixed ANCOVA yielded significant differences were tested using Fisher's Least Significant Differences with a probability of 0.05.

## Results and discussion

### Tunnel length.

The solid stem genotypes G960-BFO2, AC Eatonia, and the hollow-stemmed durum cultivar AC Navigator, had shorter tunnel lengths than other genotypes regardless of stem treatment in all years. The hollow stem genotypes Kyle and AC Cadillac had similar and consistently longer tunnel lengths than the other genotypes; no consistent differences

between stems infested with larvae parasitized by *Bracon cephi* and those where the larva died from other factors (infested-dead) were observed. AC Abbey, B997-C4AW, B997-G2AT, AC Barrie and McKenzie had intermediate tunnel lengths in all of three years (Fig.1-3). There was no consistent significant relationship between tunnel length and yield for the genotypes in any year (range of  $R^2$  values = 0.0097 - 0.04). In 2008, parasitized stems from CDC Go had significantly shorter tunnel lengths than stems infested with dead larvae or cut stems (Table.2). The solid-stemmed genotypes were expected to have shorter tunnel length in stems initially infested by sawfly but eventually killed by the host than in those stems where the larvae was killed by the parasitoid. Stems with solid pith are known to kill eggs and early-instar larvae through mechanical crushing (Holmes and Peterson, 1961, 1962). However, the mortality usually occurs when the egg was laid in or the larvae move into the solid portion of the stem (Holmes, 1979). In our study, infestation levels ranged from 58% to 87%. At these infestation levels, the effect of the parasitoid may be diminished because cannibalism among sawfly larvae results in higher mortality of *B. cephi* which may be consumed by un-parasitized sawfly larvae (D. Weaver personal comm. with HC). We did not assess stems for pith expression within the lumen of the stem in this study. However, it is known from other studies that pith solidness varies and usually it is the lower internodes that are the most solid. If this was the case in our study, then it would further explain the lack of consistent differences in tunnel lengths between infested and parasitized stems.

### Grain Yield.

In 15 comparisons of hollow stems, only AC Cadillac and AC Navigator in 2003 had significantly higher seed weights in stems with parasitoids than in those where the sawfly from other factors (Table.3). In 2008, there was also no parasitoid effect on grain yield (Table 2). For stems with solid pith, G960-BF02 was the only genotype with consistently higher seed weights in stems with larvae parasitized by *B. cephi* than those with dead larvae or not infested in all 3 years (Table 3). Our results did not provide strong support for the hypothesis that parasitoids could provide benefits to plant hosts infested by sawfly, and there was no strong pattern for influence of solid pith on the parasitoid-sawfly interaction. Buteler et al (2008) reported higher stem yield in hollow-stemmed wheat where the sawfly larvae were parasitized by *Bracon* species than those without it, but they did not include solid-stemmed genotypes in their study. The disagreements between these two studies may be related to the different parasitoid complexes. *Bracon lissogaster*, which is common within 100 km from the Alberta border (Weaver pers comm.) it is rare in Canadian agroecosystems. It may be possible that this species is more effective at killing sawfly larvae earlier in their life cycle before they cause sufficient plant damage. Alternatively, the more northern climate in Canada delays the attack of parasitoids, regardless of potential species, to the point where damage is already too high. This was suggested from our tunnel data in 3 of the 4 years; there was no major difference in tunnel lengths. Our study also suggested that tunnel length was not tied to wheat yield. It appears that as long as there is some damage inside the stem, the length of the tunnel is of little consequence for yield. Perhaps the feeding by the larvae destroys important phloem and xylem tissue even if it occurs at an early stage of the plant and larva. By the time the parasitoids attack the larvae, grain fill and some important plant fitness components such as seed weight could already

**Table 2.** Number of fertile spikelets per spike (mean ± SE), number of seeds, seed weight and length of tunnel in different infestation classes of CDC Go wheat stems in 2008

Infestation class	Number of fertile spikelets/ear	Number of seeds/ear	Seed weight/fertile spikelets (mg)	Tunnel length (mm)
<i>B.cephi</i>	12.9±0.17 a	27.4±0.60 ab	1099.4±26.94 a	360.7±15.76 b
Cut	13.0±0.23 a	28.0±0.83 a	1108.6±34.28 a	479.7±19.39 a
Infested (dead)	12.7±0.21 a	26.9±0.75 ab	1069.9±34.89 a	400.3±24.31 ab
Uninfested	12.3±0.10 a	24.4±0.32 b	1001.0±14.91 a	—

Means within columns not sharing letters are significantly different (p<0.05, LSD).

**Table 3.** Seed weight per spike in wheat stems by infestation category in 2003, 2004 and 2005

Variety	Infestation category	Seed weight/ear (mg)					
		2003		2004		2005	
		Mean	SE	Mean	SE	Mean	SE
AC Abbey	Parasitized	711.4a	48.39	662.2a	40.53	764.4a	31.33
	Infested (dead)	695.1a	59.60	769.2a	42.95	830.3a	40.01
	Uninfested	447.4a	22.81	596.1a	23.91	688.8a	151.44
B997-C4AW	Parasitized	853.9a	32.90	681.8a	48.00	735.2a	32.20
	Infested (dead)	896.4a	39.00	565.8a	68.50	778.7a	40.67
	Uninfested	750.9a	69.91	596.3a	37.80	759.2a	150.73
B997-G2AT	Parasitized	1019.9a	43.92	600.8a	78.65	835.9a	63.27
	Infested (dead)	1013.2a	80.47	621.5a	67.23	1090.8a	51.92
	Uninfested	930.1a	77.68	654.4a	40.86	945.4a	88.17
AC Barrie	Parasitized	556.3ab	32.87	605.4a	32.23	545.6a	36.28
	Infested (dead)	598.9a	56.51	630.3a	40.76	603.1b	57.64
	Uninfested	397.8b	16.18	646.4a	30.30	502.1ab	234.53
AC Cadillac	Parasitized	752.7a	27.86	624.9a	43.73	533.3a	44.73
	Infested (dead)	684.4b	60.34	730.7a	55.09	484.1a	65.28
	Uninfested	466.6b	34.14	515.3a	27.30	510.9a	130.78
AC Eatonia	Parasitized	745.3a	30.13	594.9ab	36.91	463.7a	57.57
	Infested (dead)	761.6a	38.35	688.0a	23.64	586.8a	55.18
	Uninfested	609.1a	19.20	549.4b	20.58	580.9a	80.61
G960-BF02	Parasitized	661.9a	35.89	667.4a	40.27	445.5a	31.73
	Infested (dead)	468.7b	37.73	616.5a	26.87	327.7b	37.86
	Uninfested	369.4b	14.81	498.1b	15.39	285.4b	58.41
Kyle	Parasitized	957.6a	57.64	1031.1a	61.27	1317.7a	66.67
	Infested (dead)	870.6a	85.73	1073.9a	70.83	1332.8a	62.09
	Uninfested	742.5a	73.30	1123.4a	57.70	1221.7a	126.16
McKenzie	Parasitized	652.6a	28.26	643.2a	53.10	771.9a	34.61
	Infested (dead)	660.7a	44.45	691.3a	54.14	802.5a	37.88
	Uninfested	504.9a	43.08	669.9a	31.24	815.0a	149.62
AC Navigator	Parasitized	1322.9a	67.43	1370.1a	70.17	1450.4a	65.67
	Infested (dead)	1019.4b	61.32	1285.2a	90.43	1442.9a	66.17
	Uninfested	898.5b	37.47	1203.2a	38.83	1319.8a	53.98

Means within columns for each variety not sharing letters are significantly different (p<0.05, LSD).

be affected (Bruckner & Frohberg, 1987), or larvae have already infested across most stem internodes (Nansen et al., 2005). This was suggested by similar tunnel lengths between the stems parasitized and the infested-dead stems. According to Morrill et al. 1994, the loss of head weight increases with the number of damaged nodes. In our study, the number of damaged nodes was 2.4-3.8 in the stems parasitized and 2.4-4 in infested-dead stems. The exception was G960-BF02 which had a mean of 1.8 nodes bored. There were no significant differences between treatments by varieties. In our studies, despite using stem diameter as covariate, in many instances uninfested stems had consistently lower grain yield than those infested or parasitized. It appears that yield potential of tillers is so much lower than main stems (usually infested by sawfly), that its feeding is not sufficient to reduce

the differences. Alternatively, though unlikely, sawfly feeding stimulates the plant to overcompensate its grain yield. We conclude that in our study *B. cephi* does not have a consistent indirect effect on grain weight of stems attacked by wheat stem sawfly. It appears that such stems have much higher yield potential than uninfested plants and the impact of the parasitoid on the mature larvae at the late growth stage is too small relative to the magnitude of the yield potential in these stems. Nevertheless, the most severe form of loss from sawfly comes from the stems that are girdled and topple to the ground (Holmes, 1982), and are not harvested. Thus, reduction in stem lodging during the growing season and lower sawfly populations in following years are important reasons to conserve this parasitoid.

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