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NOTE

Diversity, rate, and distribution of wheat midge parasitism in the Peace River region of Alberta, Canada

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Abstract
Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is a major pest of wheat (Poaceae) that was first reported in the Peace River region of northwestern Alberta, Canada in 2011. Although parasitism is an important factor of mortality in wheat midge elsewhere, little is known about the prevalence, species, or distribution of wheat midge parasitoids in the Peace River region. To address this knowledge gap, we conducted a survey of wheat midge parasitoids in commercial wheat fields across the region in 2016 and 2017. For a given field, parasitism of wheat midge larvae ranged from 36 to 71%. All but one parasitoid ($n = 2167$) were identified as *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae). The exception was a specimen in the genus *Inostemma* tentatively identified as *I. walkeri* Kieffer (Hymenoptera: Platygasteridae). These findings identify parasitism as an important factor that is suppressing populations of wheat midge in the Peace River region, provide the first report of *Inostemma walkeri* for North America, and provide the first report of this species as a parasitoid of *S. mosellana*.

Wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is an invasive, economically important pest of wheat (*Triticum aestivum* Linnaeus) (Poaceae) and is present in most areas of the world where wheat is grown (Affolter 1990; Olfert *et al.* 2016; European and Mediterranean Plant Protection Organization Global Database 2018; Fauna Europaea 2018). Introduced into North America in the 1800s, it is now a major pest in spring wheat on the Canadian Prairies (Olfert *et al.* 1985; Dexter *et al.* 1987).

In Canada, wheat midge is univoltine and emerges in late June or early July over a five- to six-week period (Olfert *et al.* 1985). Adults lay their eggs on the florets of emerging wheat heads in groups of one to four (Olfert *et al.* 2009). The eggs hatch within four to seven days, and the neonate larvae crawl into the florets, where they feed on the kernel for two to three weeks (Doane and Olfert 2008; Elliott *et al.* 2009). During this time, eggs and larvae may be parasitised by species of wasps (Hymenoptera: Pteromalidae, Platygasteridae) (Affolter 1990; Chavalle *et al.* 2018). At the end of the feeding period and when moist conditions occur, parasitised and nonparasitised larvae drop to the soil from the wheat heads, bury themselves, form an overwintering cocoon, and enter diapause (Lamb *et al.* 1999; Doane and Olfert 2008). Nonparasitised larvae break diapause the following spring and return to the soil surface to pupate (Doane *et al.* 1987; Elliott and Mann 1996). In Canada, larval diapause is terminated

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after 220 degree-days with a base temperature of 3 °C (Doane and Olfert 2008). Inside the bodies of parasitised larvae, immature wasps complete their development the following spring and emerge as adults.

Parasitism can be an important factor in suppressing wheat midge populations. In Europe, reports of parasitism exceeding 60% are common (Basedow and Schuette 1982; Carl and Raps 1992). In the United Kingdom, parasitism has been reported to reach 99% (Barnes 1956). In Canada and the United States of America, reported levels of parasitism range from 22 to 63% (Olfert *et al.* 2003; Shanower 2005; Knodel and Ganehiarachchi 2016).

In the current study, we surveyed commercial wheat fields in the Peace River region of northwestern Alberta, Canada in 2016 and 2017 to determine the percentage parasitism of wheat midge and to characterise the geographic distribution and diversity of the associated parasitoid complex. Wheat midge was first reported in the region in 2011, but it was likely present before this time (Meers 2011, 2013). In 2014, large numbers of the wheat midge parasitoid *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae) were recovered as adults in sweep net samples (Meers 2014). In 2015, three unidentified parasitoids were recovered from seven midge cocoons near Girouxville, Alberta. There is otherwise no information on parasitism of wheat midge in the Peace River region.

Full details of larval collection, overwintering methods, and the subsequent recovery of adult midge and their parasitoids are described in Dufton (2019). In brief, we collected 6000 wheat heads from each of 11 fields in 2016 and 3000 wheat heads from each of an additional 11 fields in 2017 (Table 1). Wheat heads were collected in August of both years from 30 locations within each field along a predetermined collection pattern. Collections were timed to coincide with the development of wheat kernels between early milk and early dough stages, when larvae were anticipated to be in their final larval instar. To increase the likelihood of midge recovery, we selected fields that had been seeded to wheat midge–susceptible cultivars. The collected wheat heads were run through a single-head thresher (Almaco specialised equipment model SV SRE-2; Almaco, Nevada, Iowa, United States of America). Material passing through the thresher was sieved (Brass Laboratory Test Sieves, aperture sizes: 4.75 and 1 mm; Endecotts Ltd., London, United Kingdom) to remove large pieces of wheat chaff (awns and stems). The remaining material, consisting of kernels and smaller fragments of chaff, was examined by hand to recover midge larvae. We recovered fewer than 1.25 larvae per 100 heads in four of the fields sampled in 2016; these fields were omitted from further consideration. Wheat heads from the remaining fields were processed until at least 500 larvae per field were recovered.

From wheat heads collected in 2016 and 2017, we recovered 21 028 and 28 276 midge larvae, respectively. Rearing procedures were adapted from unpublished methods developed and used by Agriculture and Agri-Food Canada technical staff to maintain a wheat midge colony presently held at the University of Manitoba, Winnipeg, Manitoba, Canada (S. Wolfe, personal communication). These larvae were overwintered in autoclaved soil in plastic cups with solid lids (Solo® 59.1 mL, 20–30 g of autoclaved soil) with 11–73 (2016) or 47–153 (2017) larvae per cup. Throughout the study, soil moisture was maintained at 17.5% (Basedow 1977; Olfert *et al.* 2016), and mould was controlled using a 0.1% solution of methyl paraben. Cups were placed inside double insulated boxes. Onset TidbiT, version 2, data loggers (Onset Computer Corporation, Bourne, Massachusetts, United States of America) placed inside the boxes recorded the temperature every 15 minutes. Cups were overwintered in two locations to reduce the potential for larval mortality associated with potential temperature fluctuations due to unusual weather (location 1) or equipment malfunctions (location 2). Emergence rates from both overwintering locations were similar, and results were pooled. Location 1 was out-of-doors in mesh cages insulated with wheat straw and sheltered by spruce trees. Location 2 was a walk-in refrigerator. Larvae collected in 2016 were overwintered from 24 September 2016 to 27 April 2017. Average temperatures inside of the insulated boxes at

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Table 1. Wheat midge larval parasitism in the Peace River region of Alberta and British Columbia, Canada, from which wheat heads were collected in 2016 (August 10–20) and 2017 (August 2–31). Associated values of estimated larval density and larval parasitism are reported as mean standard deviation (± SD).

Year	Nearest town	Latitude	Longitude	Wheat cultivar	Larvae/100 heads	Larval parasitism (%)*
2016	Beaverlodge	55.514	−119.403	Stettler	45.8 ± 22.7	54.3 ± 9.3 ($n = 2$)
	Fort Vermilion	58.340	−116.073	Stettler	79.0 ± 31.2	38.9 ± 6.6 ($n = 5$)
	Girouxville	55.715	−117.287	Stettler	< 1.25	
	Girouxville	55.773	−117.286	Stettler	82.3 ± 54.9	No emergence
	Guy	55.553	−117.183	Roblin	93.2 ± 91.0	46.7 ± 13.3 ($n = 3$)
	Hythe	55.316	−119.463	CDC Go	18.7 ± 11.3	No emergence
	Jean Côté	55.970	−117.394	Stettler	278.2 ± 70.0	63.2 ± 28.8 ($n = 6$)
	La Crête	58.224	−116.133	AC Intrepid	< 1.25	
	McLennan	55.659	−117.053	Superb	71.3 ± 19.2	71.0 ± 3.6 ($n = 2$)
	North Star	55.708	−117.389	Stettler	< 1.25	
	Notikewin	55.970	−117.678	Alsask	< 1.25	
2017	Bonanza	56.053	−119.931	Alsask	14.1 ± 8.0	No emergence
	Fort Vermilion	58.355	−116.041	Stettler	284.8 ± 146.1	71.3 ± 12.3 ($n = 7$)
	Fort Vermilion	58.340	−116.043	Stettler	168.3 ± 103.6	No emergence
	Fort Vermilion	58.307	−115.933	Alsask	41.3 ± 19.5	No emergence
	Girouxville	55.773	−117.286	Stettler	186.0 ± 88.2	45.5 ± 5.2 ($n = 7$)
	Girouxville	55.743	−117.389	Harvest	100.3 ± 47.8	66.6 ± 4.2 ($n = 3$)
	Guy	55.558	−117.206	Roblin	15.2 ± 12.2	65.5 ($n = 1$)
	Jean Côté	55.956	−117.420	Stettler	5.1 ± 3.4	No emergence
	La Crête	58.228	−116.204	AC Intrepid	52.6 ± 27.3	No emergence
	McLennan	55.659	−117.053	Superb	72.2 ± 39.3	35.5 ± 13.4 ($n = 3$)
	Rolla	58.872	−120.192	Thorsby	45.7 ± 24.1	No emergence

*n = total number of samples with emergence following overwintering (emergence ranged from 1 to 223 adult wheat midges + parasitoids/sample).

locations 1 and 2 were –3.3 (± 7.0 standard deviation) °C and 2.5 (± 0.9) °C, respectively. Larvae collected in 2017 were overwintered from 13 December 2017 to 17 April 2018. Average temperatures inside the insulated boxes at locations 1 and 2 were –5.1 (± 7.6) °C and 3.2 (± 1.0) °C, respectively. In 2016, cups were regularly monitored, with water added as needed to maintain soil moisture. In 2017, cups were not monitored for soil moisture or mould during the overwintering period.

Following the overwintering period, the processing of larvae differed between years. For larvae collected in 2016, extra steps were undertaken to assess overwintering mortality. Live larvae recovered after these steps were recombined and retained with larvae from the same field placed in cups of soil (155–500 individuals/cup/field). In some cups, however, we placed only one larva to assess whether multiple parasitoids might emerge from one host. Cups were maintained in the lab under natural ambient light (intensity of two lumens) and temperature (19.7 ± 1.2 °C). Cups were checked daily, with emergent adult wheat midges and parasitoids mouth aspirated and preserved in 95% ethanol.

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For cups containing larvae collected in 2017, their contents (larvae and soil) were combined into larger, clear containers (diameter: 11.5 cm; height: 13 cm) ranging from 300 to 863 larvae after overwintering. Containers were placed into clear plastic bins (35 cm × 82 cm × 49 cm) with lids to maintain humidity. Bins were held in the lab under natural ambient light (intensity of two lumens) and temperature (20.4 ± 1.9 °C). Bins were checked daily, with emergent adult wheat midges and parasitoids mouth aspirated and preserved in 95% ethanol.

We calculated percentage larval parasitism as (emergent parasitoids/(emergent parasitoids + emergent adult midges)) × 100% (Olfert *et al.* 2003). Parasitoids were identified using morphological traits described in Gibson *et al.* (1997) and Doane *et al.* (1989), referenced to voucher specimens held at Agriculture and Agri-Food Canada, Beaverlodge, and by sending material to individuals with appropriate taxonomic expertise.

We recovered from less than 1.25–285 larvae per 100 wheat heads in the commercial fields that we sampled (Table 1). In 2016, the total numbers of midge and parasitoids recovered from cups containing multiple larvae were 518 and 627, respectively. We recovered 16 adult wheat midges and 13 parasitoids from cups seeded with individual larvae. No insects emerged from material collected in fields near Hythe and Girouxville, Alberta (Table 1). For the remaining fields, parasitism ranged from 39 to 71% (Table 1). In 2017, we recovered 1063 adult wheat midges and 1513 adult parasitoids from cups containing multiple larvae. We recovered 10 adult wheat midges and 14 parasitoids from cups seeded with individual larvae. No insects emerged from material collected in fields in Alberta near Bonanza, Fort Vermilion, Jean Côté, and La Crête, nor from the field sampled near Rolla, British Columbia, Canada. For the remaining fields, parasitism ranged from 36 to 71% (Table 1). The recovery of adult wheat midges and parasitoids from field-collected material was unexpectedly low. This may have resulted from a number of factors, including prolonged diapause as a result of insufficient moisture or temperature, mechanical injury during wet-sieving, or potentially high larval mortality from desiccation due to extended duration in wheat heads during processing.

All but one of the parasitoids recovered in 2016 and 2017 were identified as *Macroglenes penetrans*. First reported in the 1950s in Manitoba, *M. penetrans* is believed to have been accidentally introduced to North America from Europe as early as the 1800s (Affolter 1990; Mason *et al.* 2017). The results from our study are similar to and, in some instances, higher than the average rate of parasitism by *M. penetrans* in other parts of North America. In Saskatchewan, Canada, average percentage parasitism of wheat midge by *M. penetrans* has been reported to reach 62% (Olfert *et al.* 2003) and is estimated to annually control an average of 20–45% of the wheat midge population (Olfert *et al.* 2009). Based on soil surveys conducted between 1995 and 2006, average percentage parasitism by *M. penetrans* was reported to be 22% in North Dakota (Knodel and Ganehiarachchi 2016). In Montana, *M. penetrans* constituted 35% of the insects (adult wheat midge + parasitoids) emerging from soil samples (Shanower 2005).

The sole exception in the present study was a wasp reared from material collected in 2017 near Guy, Alberta. The specimen was identified as *Inostemma* sp. by Dr. Lubomir Masner (National Identification Service, Canadian National Collection, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada). The specimen was then sent to Dr. Peter Buhl (IT University of Copenhagen, Copenhagen, Denmark), who tentatively identified the species as *Inostemma walkeri* Kieffer using the Kieffer (1926) and von Szelényi (1938) keys. It is a shiny, black wasp, 0.9 mm in length, with a horn extending from the first abdominal segment forward towards the head (Fig. 1).

The recovery of what tentatively has been identified as *I. walkeri* in the present study appears to be the first report of this species from wheat midge and the first report of this species in North America. It is only the second report of an *Inostemma* species associated with wheat midge in North America. *Inostemma* is a genus with worldwide distribution, and many of its species remain undescribed (Masner and Huggert 1989). This genus is

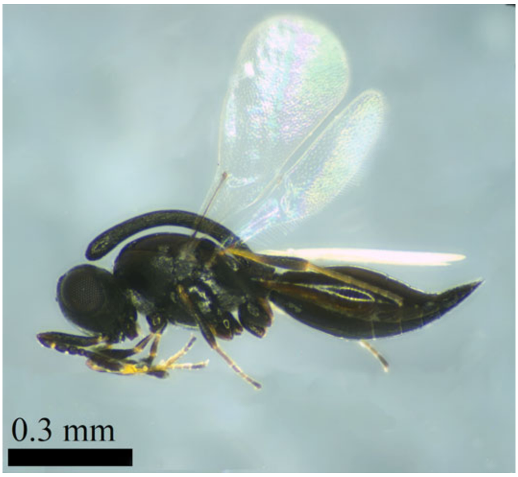

Fig. 1. Female *Inostemma walkeri* that emerged from a wheat midge larvae collected at a commercial wheat field near Guy, Alberta in 2017.
Photo credit: Shelby Dufton (Agriculture and Agri-Food Canada, Beaverlodge).

recognised by the horn-like process projecting dorsally from first abdominal segment in females (Masner and Huggert 1989). Masner and Huggert (1989) suggest that this characteristic horn houses a long ovipositor that allows them to parasitise their hosts in microsites that would otherwise be difficult to reach. Species in this genus are ovo-larval parasitoids of gall midges (Masner and Huggert 1989). *Inostemma walkeri* is an updated name given to a species collected in "grass" and "cerealia" called *Inostemma boscii* by Walker (1836). *Inostemma walkeri* has previously been found in England, Scotland, Ireland, Germany, Poland, Finland, and Israel (Kloet and Hincks 1945; Laborius 1972; Gerson and Neubauer 1976; Czajkowska 1978; Williams 2003; O'Connor *et al.* 2004; Koponen *et al.* 2016). In Israel, *I. walkeri* has been observed to emerge from field-collected specimens of the citrus blossom midge, *Contarinia citri* Barnes (Diptera: Cecidomyiidae), in lemon groves (Gerson and Neubauer 1976). In Germany and Poland, *I. walkeri* has been reported to attack the eggs of the brassica pod midge, *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae) (Laborius 1972; Czajkowska 1978; Williams 2003). These reports suggest that this species is a generalist and not specific to wheat midge.

In Europe, *I. mosellanae* has been identified as a parasitoid of wheat midge (Affolter 1990), but there are no reports of *I. mosellanae* in North America. Additionally, unlike *I. walkeri*, the horn of *I. mosellanae* extends only from its first abdominal segment to its scutellum (Affolter 1990). *Inostemma horni* was observed to seek and oviposit in wheat midge eggs in the state of Washington, United States of America but was not observed to develop in wheat midge (Reeher 1945). In the present study, we used autoclaved soil to which we added midge larvae collected from wheat. This provides indirect evidence that the *I. walkeri* specimen we recovered could have developed only in wheat midge.

Euxestonotus error Fitch and *Platygaster tuberosula* Kieffer (Hymenoptera: Platygastridae) are parasitoids of the wheat midge elsewhere in North America and were anticipated as potentially being present in the Peace River region. First reported in North America in New York state, United States of America in 1861, *E. error* is also now known from Montana, United States of America (Gahan 1933; Echegaray *et al.* 2016). Both species were released as biocontrol agents in Langenburg, Saskatchewan in 1993 and 1994, but only *P. tuberosula* appeared to establish (Doane *et al.* 2001; Olfert *et al.* 2003; Doane *et al.* 2013). In 2015, *P. tuberosula* was introduced and established as a biocontrol agent in Montana. However, neither species was recovered in the current study. This may reflect the recent expansion of wheat midge to the Peace River region. The pest was first reported in the region in 2011, with large numbers

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being reported only starting in 2014 (Meers 2011, 2013; Alberta Agriculture and Forestry 2014). We also note that the Peace River region is separated from the most proximal source for both wheat midge and its parasitoids, central Alberta, by approximately 150 km of boreal forest. This forest may pose a barrier and delay movement of these two parasitoid species into the region. Continued monitoring efforts may yet detect the establishment of one or both of these species within Canada's most northerly major wheat-producing region.

Parasitoids are one tool of many in the multipronged approach for managing wheat midge. No single form of control completely mitigates the impact of this pest and, as such, parasitoids remain an important, environmentally sustainable component. Parasitoids may not prevent outbreaks altogether, but they can often reduce their severity and possibly the frequency of further outbreaks. The rates of parasitism indicated in this study show that *M. penetrans* likely helps to contribute to wheat midge control in the Peace River region. Efforts to promote and preserve parasitoid populations should be made in this area. *Macroglenes penetrans* was recovered from wheat fields throughout most of the Peace River region, including sites along the northern, eastern, and southern boundaries in Alberta and extending about 10 km west into British Columbia near the community of Rolla. The evidence indicates that this parasitoid is well established in the region, with further collections in British Columbia likely to extend its known westernmost distributions. Future studies in the Peace River region should focus on how parasitoid diversity changes over time and assess the role of parasitism as one factor suppressing wheat midge populations within a broader programme of integrated pest management.

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