A study to assess the parasitism of insect pests in winter oilseed rape in Belgium: preliminary results

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Abstract: A survey of the parasitoids found in commercial winter oilseed rape was initiated in 2012 and 2013 in the South part of Belgium, using both aerial sampling techniques and soil analysis. Fourteen fields located in two distinct areas and with two different tillage regime (normal and reduced or no tillage) were selected for before and just after flowering. Adult parasitoid hymenoptera were weekly sampled over 8 weeks using sweep net. Pollen beetle larvae, *Meligethes aeneus* (F.) (Col.; Nitidulidae), were collected and their parasitism rate assessed. Samples of soil were taken from 4 fields in 2012 and 8 in 2013 to collect brassica pod midge cocoon, *Dasineura brassicae* (Winnertz) (Dip.; Cecidomyiidae), and to assess their parasitism. The soils were gently washed into sieves and cocoons were isolated in Petri dishes until midge or parasitoid emergence.

The main parasitoid wasps found in the sweep net samples belong to the Tersilochinae family. However, though adults of this family were regularly collected in large numbers, and were synchronized with their host, the parasitism level of the pollen beetle larvae remained low, with many of the fields below 10-15% parasitism. Preliminary analysis shows that there were no apparent differences between the two distinct areas and between the two different tillage regimes. The main explanation of this low parasitism rate could be the high occurrence of the insecticide applications, as most of the farmers regularly applied one or two insecticides during the season: the first to control pollen beetle before flowering and the second to control other insects later (e.g. seed weevil, brassica pod midge). The highest level of parasitism of pollen beetle larvae (43%) was found in an untreated field. The identification of the species is in progress.

The analysis of brassica pod midge cocoons showed that the parasitism rate was low in 2012 (0-5%). However, these results were probably underestimated due to a high mortality of the cocoons during the rearing process. If the parasitism rates were expressed on the basis of rearing success (brassica pod midge or adult parasitoid emerged), the parasitism rate reached up to 59.6% in one specific site, with 58.8% due to 4 Ceraphronidae species and 48.6% due to one species, *Ceraphron serraticornis* Kieffer. In 2013, the parasitism rate was low (0-3.0%), despite a high success in the cocoon rearing process.

These results have shown that several species of parasitoid Hymenoptera are present in Belgium, causing in some cases high parasitism levels. A better use of these parasitoid wasps in the biological or integrated control of several oilseed rape pests is possible, but there is a need to focus on improving understanding of the factors that could explain the variability of the parasitism between sites and the actions that could promote the activity of these beneficial insects and protect their existing populations.

Key words: Pollen beetle, Brassica pod midge, parasitism, Tersilochinae, Pteromalidae, Ceraphronidae

Introduction

Winter oilseed rape has become an important crop in the Walloon area, in the south part of Belgium. This crop can be severely attacked by a set of insects and the systematic use of 2 or 3 insecticides during the season is the common practice, with at least one application before

flowering to control the main insect problem, the pollen beetle *Meligethes aeneus* (Col.; Nitidulidae). However, this insect has developed across Europe resistance against several insecticides (Hansen, 2003; Heimbach *et al.*, 2006, Thieme *et al.*, 2010) and local Belgian populations were not an exception (De Proft, 2008). If new insecticides compounds were developed and registered to replace the old ones, this is only a short-term solution as new resistance could rapidly appear. Furthermore, the systematic use of insecticides against the pollen beetle is suspected to promote other pests as weevils and the brassica pod midge by impacting their natural enemies. Insecticides applied during the flowering period are also at risk for pollination and honeybees. Therefore, other solutions than the classical insecticide protection are searched.

European research projects (BORIS, 1997-2000 and MASTER, 2001-2005) have put in evidence the high importance of several parasitoid hymenoptera in the biological control of oilseed rape pests and their possible uses in the context of IPM and sustainable agriculture. By example, for the pollen beetle, larval parasitism rates of up to 90% have already been observed in Europe and levels higher than 50% are not uncommon (Williams, 2006; Hokkanen, 2006; Buchi, 1991; Krauss and Krompt, 2002).

The parasitoids of insect pests in winter oilseed rape have been poorly studied in Belgium. By example, larval pollen beetle parasitism (levels, species, etc.) are not known. A new research program was recently initiated to try to fill these gaps, in order to adapt the current practice and reduce insecticide dependence. This is a long term study and the researches are planned for several years. The first results of a prospective campaign carried out in spring 2013 are presented here. In addition to this research, results obtained on the parasitism of the brassica pod midge, *Dasineura brassicae* (Dip.; Cecidomyiidae), in the context of a program based on several Cecidomyiidae in several crops, are also included.

Material and methods

Field sites

The parasitoids of the pollen beetle and the other pest were followed in 2013 in 14 commercial fields. Approximately half of the fields were located in an area of intensive agriculture, with a low landscape diversity ("undiversified landscape" – UL) and the second half were located in an area with a more diversified landscape and an higher proportion of woods, pasture and not cultivated area surroundings the fields ("Diversified Landscape" – DL). Several of these fields, in both areas, were managed by farmers using simplified tillage or direct drilling methods ("Reduced") in at least a part of their fields and the other ones followed a traditional system with a normal tillage ("Normal"). Brassica pod midge parasitoids were sampled in commercial winter wheat fields seeded directly after oilseed rape and with a high brassica pod midge pressure, located in the Walloon area.

Sampling

The adult pollen beetles were monitored weekly from bud stage to the end of the flowering period using beating methods. The beatings were performed by shaking 5 x 10 terminal parts of plants just above a plastic tray. One sample was performed in the field margins, the four other inside the field. The insects that fell on the trays (mainly pollen beetle) were directly identified and counted. When the first larvae of pollen beetle were detected, this method was replaced by "funnel beatings", that allowed to harvest pollen beetle larvae. 5 x 10 terminal part of plants (stem, flowers and flower bud) randomly selected into each plots were shaken just above a plastic funnel (\emptyset 30 cm) placed under a plastic bottle (250 ml). The insects that

fell on the funnel were rinsed with water (+ commercial soap) to collect them on the plastic bottle that were brought back to the laboratory for counting, identification and determination of the parasitism rates for the pollen beetle larvae.

For parasitoid hymenoptera sampling, reinforced nets (\emptyset 35 cm) were used, with 2 x 10 go-back moves (sampling of around 2 x 3-4 m²). The sweep nets were emptied under a funnel, identical to those used for the funnel samplings. One sampling was performed in the field margins, the other one inside the field.

For the brassica pod midge parasitoids, soil samples were taken into 4 fields in 2012 and 8 fields in 2013 in Belgium at the end of the winter period. The previous crop of these 12 fields was winter oilseed rape. Soil sample in one field consisted of 20 sub-sample collected using a bulb planter on 10 cm deep. For extraction of *D. brassicae* cocoons, each soil sample was placed in a 5 l bucket filled with water and then kneaded until its full dispersion. Water containing soil was poured on three successive sieves with mesh sizes of 2.8 mm, 1 mm and 300 μ m. This operation was repeated until the entire sample was filtered. Only the sieve of 300 μ m retains the cocoons. The retained cocoons were collected, identified and counted under a binocular magnifier. Cocoons were isolated on a wet filter in Petri dishes and were kept till midge or parasitoid emergence that occurred approximately from 2 weeks to 2 months later.

Insect identification and interpretation of results

The parasitism of the larvae of pollen beetle harvested was detected under a binocular. The parasitism was confirmed by the dissection for the first samples. The adult of the parasitoid wasps collected with the sweep net were identified at the family or subfamily level. Identification to the species level for the most important families is planned. The brassica pod midge parasitoids (Ceraphronidae) were identified to the species level by Peter N. Buhl (Swedish Museum of Natural History).

Results

Pollen beetle parasitism

The results of the monitoring of the pollen beetle (adults, larvae with their parasitism rate) and the parasitoids (adults of Tersilochinae) are listed in Table 1 for the 14 fields monitored. The adult and larvae of pollen beetle first detection, the sampling of the first adults of Tersilochinae and the detection of first parasitized larvae were more or less similar in all the fields, with sometimes one week delay between fields. Adults of pollen beetle arrived first, followed by the parasitoid wasps that were present in all the fields when the first pollen beetle larvae were found.

Parasitism was detected in all fields but the levels remains low with a mean of 12.2% during all the season and a lot of fields around or below 10.0% (Table 2). There were no differences between the two main parameters used to characterize the fields: the diversity of the landscape surrounding the fields and the tillage regime. If higher parasitism rates for fields located on area with a diversified landscape and for fields cropped by farmers with simplified reduced tillage systems were observed, this was only a general trend and the differences were not significant.

			Apri	1			Ma	у				June	
			3	10	17	24	1	8	15	22	29	5	12
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	Pollen beetle	Adult							-	_		_	
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	Pollen beetle	Adult									-		
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	Tersilochinae	Adult			_	_		<u>((()))</u>		111			_
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Р	ollen beetle larvae	e/sample		1-10		10-50	50	-100	>10	00			
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				< J 70		5-1070	10	-2370	25	50/0	> 50	/0	

Table 1. Presence of adult of pollen beetle and Tersilochinae and of pollen beetle larvae, parasitized and unparasitized at the different dates in 2013.

	A	Tillage	Pollen be	Pollen beetle larvae (all sampling dates)					
	Area	system	Total	Parasitized	% parasitism				
Waulsort	DL	Reduced	54	14	25.9%				
Onhaye	DL	Reduced	59	14	23.7%				
Freyr	DL	Reduced	350	102	29.1%				
Ernage	UDL	Normal	149	6	4.0%				
Temploux	UDL	Reduced	284	30	10.6%				
Balatre 1	DL	Normal	477	52	10.9%				
Velaine	DL	Normal	482	36	7.5%				
Lamalle	DL	Reduced	200	5	2.5%				
Couthuin	UDL	Reduced	300	24	8.0%				
Bas-Oha	UDL	Normal	310	36	11.6%				
Wanze	UDL	Normal	336	37	11.0%				
Heron 1	UDL	Reduced	120	5	4.2%				
Landenne	UDL	Reduced	99	9	9.1%				
Heron 2	UDL	Normal	245	29	11.8%				
means		All 12.1	1%						
		Reduced	14.1% a	Div. landscape (DL)	16.6% a				
		Normal	9.5% a	Undiv. landscape (UDL)	8.8% a				

Table 2. Parasitism percentage of the pollen beetle larvae collected during the season in the different fields and means by area and tillage system.

Binomial GLM, likelihood ratio test (p = 0.05). Results followed by the same letter are not different.

The occurrence of the different parasitic hymenoptera sampled with the sweep nets in the different fields are listed in Table 3. A total of 951 adult parasitoids were collected. The main families were Tersilochinae (69.1%) and Pteromalidae (23.6%), with a peak population beginning of May for the first family and beginning of June for the second family. These peaks corresponded to the presence in abundance of pollen beetle larvae in May and seed weevil larvae in June. Even if the specimens were not identified to the species levels, it was assumed that most of the Tersilochinae found were linked to the pollen beetle and that most of the Pteromalidae were linked to the seed weevils, that was particularly abundant in 2013.

The results of the rearing of the brassica pod midge cocoons sampled into the soil to determine their parasitism rates are given in Table 4 for the 3 fields where parasitoids were found. In the 9 other fields, brassica pod midges were harvested (mean of 53 cocoons/field, range 19-85) but no parasitoids were found, while adult midges were obtained with a similar rearing success than the fields with parasitoids. The rearing success of the cocoons (adult of brassica pod midge or parasitoid) was low in 2012 (mean of 27.6%, range 8.4-42.4%) but the method was updated in 2013 (sampling later in the season, management of the humidity when reared, etc.) and the rearing success were increased (mean of 62.7%, range of 19.8-93.7%). A species previously not known to attack the brassica pod midge, *Ceraphron bispinosus* (Hym.; Ceraphronidae), was recorded for the first time during this study, with a presence in the three different locations. In one field, Buzet, the parasitism was particularly high (59.7%) with one species, *Ceraphron serraticornis*, responsible of more than 80% of this parasitism rate. This species was however only found in this field.

	1-	8-	15-	22-	29-	5-	12-		Tatal
	May	May	May	May	May	June	June	Total	
Tersilochinae	45	379	63	52	53	49	15	656	69.1%
Pteromalidae	1	6	3	18	29	118	49	224	23.6%
Braconidae	1	1	-	-	3	20	13	38	4.0%
Platygastridae	-	-	-	-	1	4	-	5	0.5%
Eulophidae	-	-	-	-	2	-	1	3	0.3%
Ichneumonidae (others)	-	-	1	-	-	1	1	3	0.3%
Cynipidae	-	-	-	-	-	1	-	1	0.1%
Not identified	-	5	-	-	3	9	2	19	2.0%
Total	47	391	67	70	91	202	81	951	100.0%

Table 3. Occurrence of the different family of parasitoid hymenoptera sampled in oilseed rape with sweep net in the 14 fields in 2013.

Table 4. Adults parasitoids obtained from brassica pod midge sampled with soils in winter wheat fields that followed oilseed rape in 2012 and 2013.

		Assesse (2012)	Buzet (2012)	Ermeton (2013)
Cocoons harvested	1	1260	3030	101
Rearing success		455 (36%)	255 (8.4%)	61 (60.4%)
Unparasitized (Da	sineura brassicae)	429	103	64
Parasitized		26 (5.1%)	152 (59.7%)	3 (4.7%)
Platygastridae	Platygaster subuliformis	6 (7.7%)	2 (1.3%)	0
Ceraphronidae	Aphanogmus abdominalis	9 (34.6%)	23 (15.1%)	1 (33.3%)
	Ceraphron serraticornis	0	124 (81.6%)	0
	Ceraphron insularis	1 (3.8%)	0	0
	Ceraphron bispinosus	7 (26.9%)	3 (2.0%)	1 (33.3%)
	Total	17 (65.4%)	150 (98.7%)	2 (66.7%)
Eulophidae	Not identified	3 (3.8%)	0 (0.0%)	1 (33.3%)

Discussion

The first results of the research recently initiated in winter oilseed rape in Belgium have highlighted the presence and the diversity of parasitoid hymenoptera. In some cases, high parasitism rates were obtained (e.g. pollen beetle larvae > 50% in several fields at several occasion, brassica pod midges in one field, etc.) and new species were found, indicating that this subject has been poorly studied. Parasitoid hymenoptera could be potentially a possibility for IPM in oilseed rape. There was however a great variability between fields and in most of the case, the parasitism levels were low.

A first rough analysis of two parameters that are known to have an impact on pollen beetle parasitism, the landscape diversity and the tillage systems, did not put in evidence differences between objects, but the analysis was only performed on a limited set of data. This study will be continued and further data will be obtained the next years.

The highest larval pollen beetle parasitism level was observed in a field that did not received insecticides in spring (Freyr) and the lowest parasitism rates were in fields that received one or two insecticides (Ernage, Temploux). Even if the data provided by the farmers have not been entirely compiled and analyzed, it seemed that the insecticides regimes (products, timing of the application) could be the most or one of the most parameter interfering with the parasitoid hymenoptera, by limiting their activity.

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