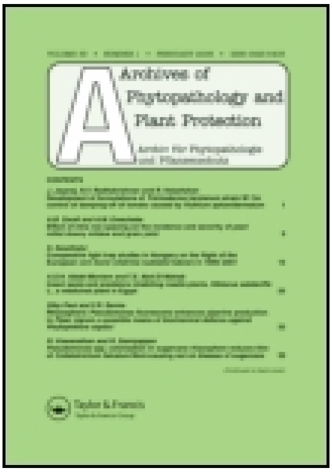

Archives Of Phytopathology And Plant Protection

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gapp20

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Published online: 05 Feb 2014.

To cite this article: Maryam Afiunizadeh & Javad Karimzadeh (2015) Assessment of naturally occurring parasitism of diamondback moth in field using recruitment method, Archives Of Phytopathology And Plant Protection, 48:1, 43-49, DOI: 10.1080/03235408.2014.882113

To link to this article: http://dx.doi.org/10.1080/03235408.2014.882113

Archives of Phytopathology and Plant Protection, 2015
Vol. 48, No. 1, 43–49, http://dx.doi.org/10.1080/03235408.2014.882113

Assessment of naturally occurring parasitism of diamondback moth in field using recruitment method

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(*Received 28 December 2013; accepted 5 January 2014*)

The recent major outbreaks of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), in cruciferous plants in Iran has led to attempts for sustainable pest management strategies, mainly based on the natural enemies. The present study aimed to investigate naturally occurring parasitism of *P. xylostella* in cabbage and cauliflower fields of central Iran. For this purpose, field studies were performed to identify parasitoids of *P. xylostella*, and to evaluate percentage parasitism of *P. xylostella* using the recruitment method in main cabbage-growing areas of Isfahan province in 2009. In present study, seven species of parasitoid wasps (five larval and two pupal parasitoids) and two species of hyperparasitoid wasps were determined. The parasitoids included the braconids *Cotesia vestalis* (Kurdjumov), *Bracon hebetor* Say and *Apanteles* sp., the ichneumonid *Diadegma semiclausum* (Hellen), and the eulophid *Oomyzus sokolowskii* (Kurdjumov) as larval parasitoids, and the ichneumonids *Diadromus collaris* (Gravenhorst) and *Diadromus subtilicornis* (Gravenhorst) as pupal parasitoids. In addition, the pteromalids *Mokrzeckia obscura* Graham and *Pteromalus* sp. were identified as the hyperparasitoids, which in turn parasitise *C. vestalis*. The most predominant species were *C. vestalis* and *D. semiclausum* with the proportional abundance of 0.43 and 0.42, respectively. Percentage parasitism varied significantly between host plants, but not between areas; the parasitised proportion of *P. xylostella* larvae fed on common cabbage was significantly greater than that on cauliflower (0.42 vs. 0.34). The mean percentage parasitism varied between 14.5 and 68.4 for different fields, and accounted for 37.4% of *P. xylostella* population on an average. The greatest parasitism was achieved by *C. vestalis*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5% of field populations of *P. xylostella*, respectively. These findings illustrated the important role of parasitoids for sustainable management of diamondback moth.

Keywords: *Plutella*; parasitoids; parasitism; recruitment; Iran

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera, Plutellidae), is the most destructive and cosmopolitan pest of cruciferous plants (Talekar & Shelton 1993). The overuse of chemical pesticides against this pest has resulted in resistance to all groups of insecticides, including insect-growth regulators (Alizadeh et al. 2012) and *Bacillus thuringiensis* Berliner (Karimzadeh & Sayyed 2011; Gulzar et al. 2012). In addition, the intensive use of insecticides has eliminated effective natural predation of *P. xylostella* in

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field. Previous studies have shown that larval and pupal parasitoids are the most effective natural enemies of *P. xylostella* (Talekar & Shelton 1993; Sarfraz et al. 2005; Karimzadeh & Wright 2008; Alizadeh et al. 2011). In this regard, parasitoids must be the cornerstone of any pest-management programme looking for *P. xylostella* sustainable management (Verkerk & Wright 1996).

Another key biotic factor in regulation of *P. xylostella* populations in the field is host-plant availability (Kfir 1997; Karimzadeh et al. 2004; Soufbaf et al. 2012). Plants may mediate many of the interactions between herbivores and their parasitoids, influencing the preference and performance of parasitoids (Cortesero et al. 2000; Karimzadeh et al. 2013). Generally, in a tritrophic system variation in host-plant characteristics (Soufbaf et al. 2010a, 2010b; Soufbaf et al. 2013) may have differential effects on a herbivore and its associated natural enemies (Karimzadeh & Wright 2008). Many studies revealed that different host-plant species or cultivars have differential effects on *P. xylostella* parasitism success by parasitoids, in particular, *Cotesia vestalis* (Haliday) and *Diadegma semiclausum* (Hellen) (e.g. Talekar & Yang 1991; Verkerk & Wright 1997; Haseeb et al. 2001; Liu & Jiang 2003; Karimzadeh et al. 2004; Karimzadeh & Wright 2008).

In recent years, *P. xylostella* has shown major outbreaks in cabbage and cauliflower fields in Isfahan province (central Iran). To overcome such a serious problem, struggling farmers have used all available synthetic insecticides even up to more than 10 times of recommended doses. Unfortunately, the overuse of pesticides not only had no satisfactory control of the pest, but also has increased the environmental and health concerns. The present study aimed to identify larval and pupal parasitoids of *P. xylostella*, and to assess the natural percentage parasitism of field populations of *P. xylostella* on two different host plants.

Materials and methods

The study sites

The study was carried out in two main cabbage-growing areas of Isfahan province (Iran), which were located in Falavarjan (32°33′N; 51°31′E) and Mobarakeh (between 32°3′N and 32°28′N; between 51°13′ and E51°48′E) counties.

Parasitoid species and their abundance

To identify the larval and pupal parasitoids of *P. xylostella*, in each county two fields of common cabbage (*Brassica oleracea* var. *capitata*) cv. Globe Master and two fields of cauliflower (*Brassica oleracea* var. *botrytis*) cv. Royal were selected. Each field was sampled fortnightly between June (one month after transplantation) and October (harvest) 2009. Sampling was carried out on 10 randomly selected plants within each field, where all *P. xylostella* second, third and fourth instar larvae, prepupae and pupae on each plant were collected and reared under laboratory conditions (25 ± 5 °C, 70 ± 5% RH and L:D 16:8 h). Emerged parasitoids were then identified by Gavin Broad (Department of Entomology, The Natural History Museum, London, UK), Hosseinali Lotfalizadeh (Department of Plant Protection, East Azerbaijan Research Centre for Agriculture and Natural Resources, Iran), Jeno Papp (Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary), John LaSalle (Division of Entomology, Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia), James B. Whitfield (Department of Entomology, School of Integrative Biology, School of Life Sciences, College of Liberal Arts and Sciences, University of Illinois, Urbana, USA), Kees van Achterberg (Department of Terrestrial Zoology, National Museum of Natural History, Leiden, Netherlands) and Mark R. Shaw (National Museums of Scotland, Edinburgh, UK). The number of individuals for each parasitoid species was recorded and used as an index for abundance.

Percentage parasitism

Recruitment method (van Driesche et al. 1991) was used to evaluate natural parasitism of *P. xylostella* larvae. The sampling was carried out during August to October 2009. In each area, two fields of common cabbage and two fields of cauliflower were chosen such that there was a minimum of 5 km distance between the fields. No pesticide was applied in the selected fields from one week before to end of sampling process. Sampling was then carried out on 10 randomly selected plants within each field; all the second, third and fourth instar larvae, prepupae and pupae of *P. xylostella* were collected and transferred to laboratory. In laboratory, only the fourth instar larvae were reared under standard constant conditions (25 ± 5 °C, 70 ± 5% RH and LD 16:8 h) and all other stages were discarded. The larva reared on the related host plant until the moth had pupated or the parasitoid cocoon had formed (here the number of formed parasitoid cocoons was recorded as the parasitism success; Karimzadeh et al. 2004). After 48 h, the same plants were searched and the number of second instar larvae recruited to plants was recorded (as the recruited hosts). The whole process was repeated one week later in the same fields. The rate of parasitism was calculated as the ratio of the parasitism success to the recruited hosts (Verkerk & Wright 1997).

Statistical analyses

Differences in the level of parasitism rate between host-plant types and between regions were analysed using logistic analysis of deviance (binomial errors). All statistical analyses were completed in *R. 2.10.0* (Crawly 2005, 2007).

Results

Larval and pupal parasitoids of P. xylostella *and their abundance*

In present study, seven species of parasitoid wasps (five larval and two pupal parasitoids) and two species of hyperparasitoid wasps were determined (Table 1). Larval parasitoids were three braconids, *C. vestalis* (Haliday), *Apanteles* sp. and *Bracon hebetor* Say, an ichneumonid, *D. semiclausum* (Hellen), and an eulophid, *Oomyzus sokolowskii* (Kurdjumov). Pupal parasitoids were ichneumonids *Diadromus collaris* (Gravenhorst) and *Diadromus subtilicornis* (Gravenhorst). The hyperparasitoids were pteromalids *Mokrzeckia obscura* Graham and *Pteromalus* sp. that act as the parasitoids of *C. vestalis*. The most predominant species were *C. vestalis* and *D. semiclausum* with the proportional abundance of 0.43 and 0.42, respectively (Table 1).

Natural percentage parasitism of P. xylostella

There was a significant difference ($t_{157} = -3.339$, $p < 0.01$) between host plants for the mean percentage parasitism; the mean percentage parasitism of *P. xylostella* larvae fed on common cabbage was significantly greater than on cauliflower (42.8 vs. 33.7).

Table 1. Parasitoids of *P. xylostella* and their abundance in Isfahan province, Iran.

Parasitoid			Occurrence				
					Number[b]		
Species	Family	Type	Time	Plant[a]	*F*	*M*	Total
Cotesia plutellae	Braconidae	Larval	May–Oct.	CA, CO	212	255	467
Apanteles sp.	Braconidae	Larval	Sept.	CA	0	5	5
Bracon hebetor	Braconidae	Larval	Oct.	CO	0	3	3
Diadegma semiclausum	Ichneumonidae	Larval	May–Oct.	CA, CO	176	279	455
Diadromus subtilicornis	Ichneumonidae	Pupal	July–Oct.	CA, CO	5	10	15
Diadromus collaris	Ichneumonidae	Pupal	July–Oct.	CA, CO	8	0	8
Oomyzus sokolowskii	Eulophidae	Larval–pupal	June–Oct.	CA, CO	86	39	125
Mokrzeckia obscura	Pteromalidae	Hyperparasitoid	Sept.	CA	0	2	2
Pteromalus sp.	Pteromalidae	Hyperparasitoid	Sept.	CA	0	1	1

[a]Host-plant type: CA = cauliflower, CO = common cabbage.
[b]County: *F* = Falavarjan, *M* = Mobarakeh.

However, no significant difference was observed between areas ($t_{156} = 0.797$, $p = 0.43$; Table 2). In addition, comparison of sampling times showed that mean percentage parasitism in second sampling time was significantly greater ($t_{157} = 3.810$, $p < 0.001$) than that in first one (42.8 vs. 32.3). The mean percentage parasitism varied between 14.5 and 68.4 for different fields, and accounted for 37.4% of *P. xylostella* population on average (Table 2). The greatest parasitism was achieved by *C. vestalis*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5% of field populations of *P. xylostella*, respectively.

Table 2. Natural parasitism of *P. xylostella* by larval parasitoids in Isfahan province, Iran.

Sampling				Percentage parasitism (Mean ± SE)		
County	Host plant	Field	Time			
Falavarjan	Common cabbage	1	1st	29.4 ± 6.7		
			2nd	51.5 ± 8.3		
		2	1st	49.5 ± 10.7		
			2nd	30.4 ± 6.1	40.2 ± 4.3	
	Cauliflower	1	1st	14.5 ± 4.8		
			2nd	41.1 ± 8.2		
		2	1st	32.5 ± 5.5		
			2nd	43.2 ± 9.1	31.2 ± 4.0	34.9 ± 2.9
Mobarakeh	Common cabbage	1	1st	37.8 ± 4.1		
			2nd	68.4 ± 8.8		
		2	1st	36.1 ± 5.8		
			2nd	36.8 ± 4.3	42.8 ± 3.6	
	Cauliflower	1	1st	31.9 ± 4.8		
			2nd	42.4 ± 3.4		
		2	1st	29.3 ± 3.2		
			2nd	28.2 ± 3.8	35.3 ± 2.1	38.9 ± 2.2

Discussion

The results presented here show that despite numerous applications of insecticides against diamondback moth in cabbage fields of Isfahan province, the diversity and performance of parasitoids are noticeable. The level of natural parasitism under current situation (high pressure of pesticides and no support for biological control agents) is fascinating, implying that biological control plays a key role in suppressing *P. xylostella* populations.

In this regard, it is necessary to support naturally occurring parasitism in fields by limiting pesticide use. Furthermore, mass rearing and release of effective parasitoids such as *C. vestalis*, *D. semiclausum* and *Diadromus* spp. accompanied with more environmentally friendly pesticides such as *B. thuringiensis* might be complementary to natural check by parasitoids. It also is essential to evaluate parasitism level accurately during several subsequent growing seasons in each area to have a better understanding of natural check by parasitoids.

Both of the host plants used in this study have shown to be partially resistant to attack by *P. xylostella* (Jafary et al. 2010, 2012). The observed differences in parasitism between these two host plants, therefore, might be due to biochemical differences between host plants. The induced volatiles from infested plants vary between plant species or cultivars; such volatiles can cause special behaviour in parasitoids (Vet & Dicke 1992). Several studies have demonstrated that two specialist larval parasitoids of diamondback moth, *C. vestalis* and *D. semiclausum*, have different responses to various cruciferous species or cultivars (Talekar & Yang 1991; Verkerk & Wright 1997; Liu & Jiang 2003; Karimzadeh et al. 2004; Rossbach et al. 2006). Apart from the mechanisms underpinning the plant-mediated differences in natural parasitism of *P. xylostella* (Bukovinszki et al. 2005; Kahuthia-Gathu et al. 2008; Karimzadeh & Wright 2008), such difference can be useful for manipulating crop–pest–parasitoid system in order to enhance the effects of parasitoids.

Here, it is documented that Isfahan province has a high potential for biological control of *P. xylostella*. Naturally occurring parasitism must be supported and improved. This cannot be practicable unless the pressure of chemical pesticides is limited and studies focus on sustainable management strategies based on native parasitoids.

Acknowledgements

We thank G. Broad, H. Lotfalizadeh, J. Papp, J. LaSalle, J. B. Whitfield, K. van Achterberg and M. R. Shaw for determination of species.

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