Naturally-occurring parasitism of diamondback moth in central Iran

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ABSTRACT
The recent major outbreaks of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), in crucifers plants in Iran has led to attempts for sustainable pest management strategies, mainly based on 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 this study, seven species of parasitoid wasps (five larval and two pupal parasitoids) and two species of hyperparasitoid wasps were determined. The parasitoids were included the braconids *Cotesia plutellae* (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, parasitize *C. plutellae*. The most predominant species were *C. plutellae* and *D. semiclausum* with the proportional abundance of 0.43 and 0.42, respectively. The species *M. obscura*, *D. collaris* and *Apanteles* sp. are new records from Iran. Percentage parasitism varied significantly between host plants, but not between areas; the parasitized proportion of *P. xylostella* larvae fed on common cabbage was significantly greater than 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 average. The greatest parasitism was achieved by *C. plutellae*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5 percent 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 and Shelton 1993). The overuse of chemical pesticides against this pest has resulted in resistance to all groups of insecticides, including insect growth regulators and *Bacillus thuringiensis* Berliner. In addition, the intensive use of insecticides has eliminated effective natural predation of *P. xylostella* in field. Previous studies have shown that larval and pupal parasitoids are the most effective natural enemies of *P. xylostella* (Talekar and Shelton 1993; Sarfraz et al. 2005; Karimzadeh and Wright 2008). In this regard, parasitoids must be the cornerstone of any pest management program looking for *P. xylostella* sustainable management (Verkerk and Wright 1996).

Another key biotic factor in regulation of *P. xylostella* populations in the field is host-plant availability (Kfir 1997). Plants may mediate many of the interactions between herbivores and their parasitoids, influencing the preference and performance of parasitoids (Cortesero et al. 2000). Generally, in a tritrophic system variation in host-plant characteristics may have differential effects on a herbivore and its associated natural enemies (Karimzadeh and 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 plutellae* (Kurdjumov) and *Diadegma semiclausum* (Hellen) (e.g., Talekar and Yang 1991; Verkerke and Wright 1997; Haseeb et al. 2001; Liu and Jiang 2003; Karimzadeh et al. 2004; Karimzadeh and 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 up to more than 10 times of recommended doses. Unfortunately, the overuse of pesticides offers no satisfactory control of the pest, and has increased environmental and health concerns. This 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 E 51° 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 (*B. 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 ten randomly selected plants within each field, where all *P. xylostella* 2nd, 3rd and 4th 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 Center 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 Organization (CSIRO), 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 ten randomly selected plants within each field; all the 2nd, 3rd and 4th instar larvae, prepupae and pupae of *P. xylostella* were collected and transferred to the laboratory. In the laboratory, only the 4th 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. A 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 parasitism success (Karimzadeh et al. 2004). After 48 h, the same plants were searched and the number of 2nd 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 and Wright 1997).

Statistical analyses

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

RESULTS AND DISCUSSION

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. plutellae* (Kurdjumov), *Apanteles* sp. and *Bracon hebetor* Say, an ichneumonid, *D. semiclausum* (Hellen), and a eulophid, *Oomyzus sokolowskii* (Kurdjumov). Pupal parasitoids were ichneumonids *Diadromus collaris* (Gravenhorst) and *D. subtilicornis* (Gravenhorst). The hyperparasitoids were pteromalids *Mokrzeckia obscura* Graham and *Pteromalus* sp. that act as the parasitoids of *C. plutellae*. This is the first record of *M. obscura*, *D. collaris* and *Apanteles* sp. on *P. xylostella* in Iran. The most predominant species were *C. plutellae* 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). 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 percent of *P. xylostella* population on average (Table 2). The greatest parasitism was achieved by *C. plutellae*, *D. semiclausum* and *O. sokolowskii*, with a parasitism of 21.0, 12.9 and 3.5 percent of field populations of *P. xylostella*, respectively.

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

Parasitoid			Occurrence				
					Area (number)‡		
species	Family	Type	Time	Plant †	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

* **The species is recorded for first time from Iran.**
† **Host-plant type: CA = cauliflower, CO = common cabbage.**
‡ **County: F = Falavarjan, M = Mobarakeh.**

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

The results indicate 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. plutellae*, *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). 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 behavior in parasitoids (Vet and Dicke 1992).

Several studies have demonstrated that two specialist larval parasitoids of diamondback moth, *C. plutellae* and *D. semiclausum*, have different responses to various crucifer species or cultivars (Talekar and Yang 1991; Verkerke and Wright 1997; Liu and 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 and Wright 2008), such difference can be useful for manipulating crop-pest-parasitoid system to enhance the effects of parasitoids.

CONCLUSION

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.

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