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Augmentative on-farm delivery methods for the parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) to control the millet head miner *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae) in Burkina Faso and Niger

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Abstract Augmentative on-farm delivery methods for the parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) to control the millet head miner (MHM) *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae) were investigated in Burkina Faso from 2011 to 2012 and in Niger in 2012. Our findings indicate that 7 cm × 10 cm jute bags containing 50 g of millet grains, 30 g of millet flour, 25 *Corcyra cephalonica* larvae and two mated *H. hebetor* females are the most effective option for on-farm delivery of the parasitoid. The parasitoid progeny started emerging from the bags eight days after confinement and 57–71 parasitoid adults emerged from each bag. Using the methods we developed, over 90 % parasitism of MHM larvae was achieved in millet farms. The implications of these findings for a large extension of MHM biocontrol program are discussed.

Keywords Millet head miner · Biological control · *Habrobracon hebetor* · *Corcyra cephalonica*

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Introduction

In the Sahelian region of West Africa, pearl millet, *Pennisetum glaucum* (L.) R. Br., is a major cereal food crop. It is the only cereal crop adapted to this arid region. In addition to the extreme climatic conditions, the millet crop suffers from many constraints, including insect pests (Nwanze and Harris 1992). The millet head miner (MHM), *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae), is the key insect pest of pearl millet in the Sahel region (Gahukar 1984). Damage to the crop is due to larvae that feed on the panicle and prevent grain formation (Ndoye 1991; Nwanze and Harris 1992). Typical yield losses range from 40 to 85 % (Gahukar et al. 1986; Nwanze and Sivakumar 1990; Krall et al. 1995; Youm and Owusu 1998).

Different approaches, including cultural management, host plant resistance and use of pesticides, have been tested (Gahukar 1989, 1990a, b, 1992; Nwanze

and Sivakumar 1990) with limited success and applicability (Nwanze and Harris 1992).

Biological control using the indigenous parasitoid wasp *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) appears promising (Gahukar et al. 1986; Bhatnagar 1987; Youm and Gilstrap 1993). In the earlier 1980s a natural parasitism of 64–95 % due to *H. hebetor* Say (Hymenoptera: Braconidae) was reported in Senegal and Niger (Gahukar et al. 1986; Bhatnagar 1987; Nwanze and Harris 1992). *H. hebetor* has been intensively studied because of its suitability as a biological control agent of stored product moths (Benson 1973; Rotary and Gerling 1973; Nikam and Pawar 1993; Antolin et al. 1995; Yu et al. 1999; Eliopoulos and Stathas 2008; Dabhi et al. 2011; Farag et al. 2012). Later on *H. hebetor* was known to occur as two biologically distinct (warehouse strain and field strain) but morphologically inseparable siblings (Heimpel et al. 1997).

The first experimental augmentative releases of *H. hebetor* for controlling the MHM were attempted in 1985 in Senegal (Bhatnagar 1989) followed by Niger in the early 2000s (Garba and Gaoh 2008). More recently, augmentative releases of *H. hebetor* were successfully carried out in Burkina Faso, Mali and Niger (Payne et al. 2011; Ba et al. 2013; Baoua et al. 2013). The parasitoids were released in 15 cm × 25 cm jute bags containing 200 g of millet grains and 200 g of millet flour, together with 25 larvae of the rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and two mated *H. hebetor* females (Ba et al. 2013). The jute bags were suspended to the ceiling of traditional straw granaries and emerging parasitoids were able to escape through the jute mesh and straw granaries and disperse to parasitize MHM larvae in millet fields (Ba et al. 2013).

Although this biological control program was effective, there are still some limitations with regard to large-scale application, especially (i) place of deployment of jute bag for communities where granaries are made of clay, which could not enable parasitoids to escape, and (ii) in the quantity of millet flour/grain needed for formulating the parasitoid bags. Thus, we investigated the effects of placing jute bags directly within millet fields on the parasitism of MHM. Finally, we investigated the effects of reducing the jute bag size, and thus the content, on the emergence of offspring parasitoids from the bags and their parasitism of MHM in the field.

Materials and methods

Insect cultures

Since *H. albipunctella* is a univoltine species diapausing from October to June (Gahukar et al. 1986), a colony of *H. hebetor* was established and maintained in the laboratory on an alternate host, the rice moth *C. cephalonica*. Both insects were reared in the laboratory under ambient conditions. Both insect colonies were established from wild insects collected in 2011 from each of the countries. *H. hebetor* were collected from the field and *C. cephalonica* from stored products. *C. cephalonica* rearing technique was adapted from that developed by Bal et al. (2002). Wooden cages (20 × 20 × 13 cm) with muslin cloth on three lateral sides and wood at the bottom were used for *C. cephalonica* mass rearing. A mixture of 1.2 kg of millet flour and 1.8 kg of millet grains was introduced into the cages and inoculated with approximately 3,000 *C. cephalonica* eggs. Subsequent generations were regularly obtained after 30 days at room temperature (average 26 °C). Third and fourth instar *C. cephalonica* larvae were used for the mass rearing of *H. hebetor*. For this purpose, 25 *C. cephalonica* larvae were confined within a Petri dish for 48 h with two mated *H. hebetor* females. The subsequent generation of *H. hebetor* emerged 7–14 days after confinement.

Effect of bag size on parasitoid emergence

This experiment was carried out in Burkina Faso and Niger. In Burkina Faso, the experiment was conducted in the laboratory of "Institut de l'Environemment et de Recherche Agricole" in Dori, under a fluctuating temperature of 24–35 °C with 57–77 % relative humidity. In Niger, the experiment was performed in the laboratory of "Institut National de Recherche Agricole du Niger" in Maradi under a fluctuating temperature of 30–37 °C with 50–70 % relative humidity.

The experiment was set with four replicates and two treatments as follows: (i) Treatment 1: Jute bags of 15 cm × 25 cm containing 200 g of millet grains, 100 g of millet flour, 25 *C. cephalonica* larvae (a mixture of third and fourth instar larvae) and two mated *H. hebetor* females. (ii) Treatment 2: Jute bags of 7 cm × 10 cm containing 50 g of millet grains,

30 g of millet flour, 25 *C. cephalonica* larvae (mixture of third and fourth instar larvae) and two mated *H. hebetor* females. All the bags were covered with muslin cloth to trap the emerging parasitoids. For each treatment, a set of 20 bags was used and each batch of five bags represented one replicate. The bags were kept in the lab for one month and emerging parasitoids were sexed, counted and removed daily. After one month, the bags were emptied and any living or dead insects were recorded.

Effect of deploying parasitoid bags within millet fields on parasitism

This experiment was performed in 2011 and 2012 in Northern Burkina Faso. The research sites belong to the Sahel agroecological zone, which has an unimodal rainfall pattern, and the rainy season lasts from June to September. Total rainfalls of 446 and 593 mm were recorded in 2011 and 2012, respectively. This experiment was carried out with jute bags of 15 cm × 25 cm containing 200 g of millet grains, 100 g of millet flour, 25 *C. cephalonica* larvae (a mixture of third and fourth instar larvae) and two mated *H. hebetor* females. Parasitoid bags were deployed within the millet farm and attached to the top of a 2-m-tall wooden stick fixed in the soil and roofed with a calabash (one half of a dried shell of gourd) to avoid direct rainfall. Three bags were attached together per farm at the centre of the farm. In each village the bags were evenly distributed within five millet farms (3 bags per farm), one in the centre of the village and one in each direction (E, W, N and S). Typical villages have a diameter of 1 km, each of E, W, N and S farm was 500 m away from the farm in the centre of the village. Selected villages have endemic occurrences of the MHM with over 60 % infested panicles (Ba et al. 2010; Baoua et al. 2013). Six villages were selected every year for the experiment. New villages different from 2011 were selected in 2012. The selected villages were separated by 5 km based on preliminary studies demonstrating that the parasitoid *H. hebetor* can travel up to 5 km from the release point (Garba and Gaoh 2008). However, control villages, where no parasitoid releases were made, were at least 10 km away from villages receiving the parasitoids. The experimental design includes two treatments: (i) three villages each supplied with 15 parasitoid bags and ii) three control villages that did not receive any parasitoid bags.

Data on MMH parasitism by *H. hebetor* were recorded 30 days after releases. For this purpose, 500 millet panicles were randomly selected in each village from five millet farms (100 panicles per farm) and dissected. The number of living (not parasitized) and parasitized larvae was recorded. The larvae parasitized by *H. hebetor* are easily distinguished by the presence of cocoons (Garba and Gaoh 2008).

Effect of bag size on field parasitism

This experiment was performed during 2012 in Burkina Faso and Niger. In both countries, research sites belong to the same above described Sahel agroecological zone. In 2012, total rainfalls of 623 and 593 mm were recorded at the research sites in Burkina Faso and Niger, respectively. In both countries, the experimental design included three treatments: (i) two villages each supplied with 15 small jute bags (7 cm × 10 cm); (ii) two villages each supplied with 15 large jute bags (15 cm × 25 cm); and (iii) two control villages that did not receive any parasitoid bags. As describe above the villages receiving parasitoids were separated by 5 km and control villages, where no parasitoid releases were made, were at least 10 km away from villages receiving the parasitoids. For villages receiving the parasitoids bags, the bags were deployed within the millet farm and attached to the top of a 2-m-tall wooden stick fixed in the soil. The same arrangement as above was used for deployment of bags within villages and individual farms.

Data on MMH parasitism by *H. hebetor* were recorded 30 days after releases. For this purpose, 500 millet panicles were randomly selected in each village from five millet farms (100 panicles per farm) and dissected. The number of living (not parasitized) and parasitized larvae was recorded.

Data analysis

Field data were subjected to ANOVA (PROC GLM) using SAS software version 8 (SAS 2001). When ANOVAs were significant, means were separated by the Student–Newman–Keuls test at the 5 % level. Laboratory data were subjected to a *t* test to compare emerging parasitoids from the two sizes of jute bags.

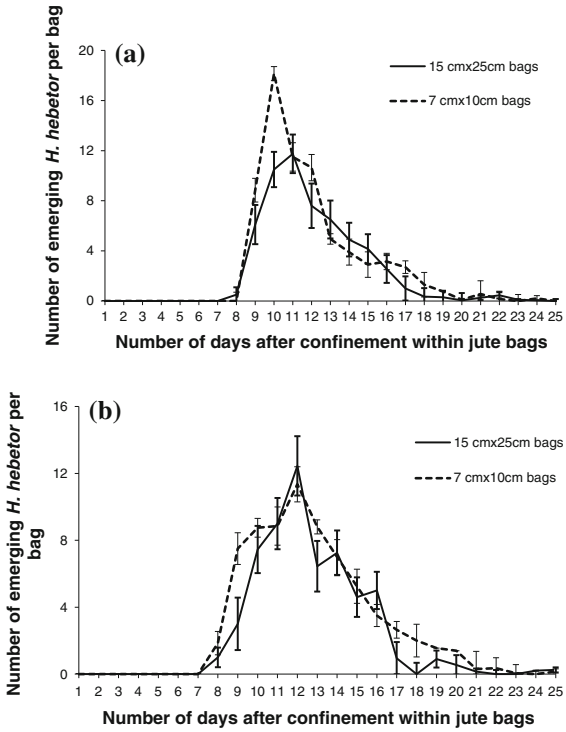

Fig. 1 Daily emergence of *Habrobracon hebetor* (Say) progeny (Mean ± SE) after confinement within different sizes of jute bags in the laboratory in Burkina Faso (**a**) and Niger (**b**)

Percentages of parasitism data were arcsine transformed prior to statistical tests.

Results

Effect of bag size on parasitoid emergence

Parasitoid progeny began emerging from the bags eight days after confinement and greater than 91 % of the parasitoids emerged within 16 days in Burkina Faso (Fig. 1a) and Niger (Fig. 1b). *H. hebetor* developed within less than 13 days on average (Table 1). Regardless of the country, the total number of emerging parasitoids did not significantly differ between the two sizes of jute bags (Table 1). In both countries, a maximum of almost 70 parasitoid offspring emerged from the bags (Table 1). The sex ratio of the emerging *H. hebetor* parasitoids was male-biased regardless of the jute bag's size (Table 1). *H. hebetor* females parasitized up to 95 % of the *C. cephalonica* larvae within the jute bags (Table 1).

Effect of deploying parasitoid bags within millet fields on parasitism

The releases of the parasitoids in millet field significantly increased the natural parasitism of MHM (Fig. 2). In both years, the parasitism by *H. hebetor* was significantly higher in villages where the parasitoid bags were placed than the control villages (2011: $F = 15.30$; df $= 1, 28$; $P = 0.0007$; 2012: $F = 54.32$; df $= 1, 28$, $P = 0.0003$).

Effect of bag size on field parasitism

Regardless of the size of jute bag, the release of the parasitoids significantly increased the natural parasitism of MHM (Fig. 3). In both countries, the parasitism by *H. hebetor* was significantly higher in villages where the 7 × 10 cm parasitoid bags were placed (Burkina Faso: $F = 221.71$; df $= 2, 28$; $P < 0.0001$; Niger: $F = 131.59$; df $= 2, 28$; $P < 0.0001$).

Discussion

Our findings clearly demonstrate that the 7 × 10 cm jute bags delivered the same number of parasitoids as the 15× 25 cm bags. With the small bag 40 % of millet grain/flour is saved per bag. The two *H. hebetor* females parasitized more than 80 % of *C. cephalonica* larvae within the jute bags. This is much higher than reported by Magro and Parra (2001) with *C. cephalonica* and *H. hebetor*. The results suggested by the former authors were achieved with a ratio of one *H. hebetor* female for 40 host larvae whereas our study used 12 host larvae. As reported by Schöller (2000) differences may be explained by the host-parasitoid ratio.

In general, the parasitoids began emerging eight - days after confinement of *H. hebetor* females with *C. cephalonica* larvae. This is in line with findings of Garba and Gaoh (2008). Over 91 % of the parasitoid offspring emerged within 16 days. The average development time was around 12 days and this is consistent with previous findings with *C. cephalonica* larvae (Magro and Parra 2001). Considering the parasitoids reproductive potential at least 1,000 parasitoids can be released in each village within 12 days when using 15 jute bags per village. With the rapid development time of *H. hebetor* combined with the

Table 1 Number of emerging *Habrobracon hebetor* (Say) parasitoids, their sex ratio, and the percentage (%) of *Corcyra cephalonica* (Stainton) larvae parasitized in relation to the size of the jute bags

Countries	Bag size	Number parasitoids emerged (Mean ± SE)	*H. hebetor* proportion (% female ± SE)	% Parasitism *C. cephalonica* (Mean ± SE)	*H. hebetor* development time (Mean ± SE)
Burkina	25× 15 cm	57.10 ± 5.01	38.58 ± 4.64	80.77 ± 0.17	12.15 ± 0.34
Faso	10× 7 cm	69.80 ± 5.57	41.08 ± 3.18	87.19 ± 0.08	11.64 ± 0.31
		$t = -1.69$;	$t = -0.44$;	$t = -1.72$;	$t = -1.08$
		df = 38;	df = 38;	df = 38;	df = 118
		$P = 0.10$	$P = 0.66$	$P = 0.09$	$P = 0.27$
Niger	25 × 15 cm	59.20 ± 8.98	30.07 ± 2.72	95.36 ± 0.16	12.63 ± 0.32
	10× 7 cm	71.30 ± 6.26	24.11 ± 2.58	78.14 ± 0.17	12.80 ± 0.36
		$t = -1.10$;	$t = -1.59$;	$t = 4.60$;	$t = 0.34$
		df = 38;	df = 38;	df = 38;	df = 127
		$P = 0.27$	$P = 0.12$	$P < 0.0001$	$P = 0.73$

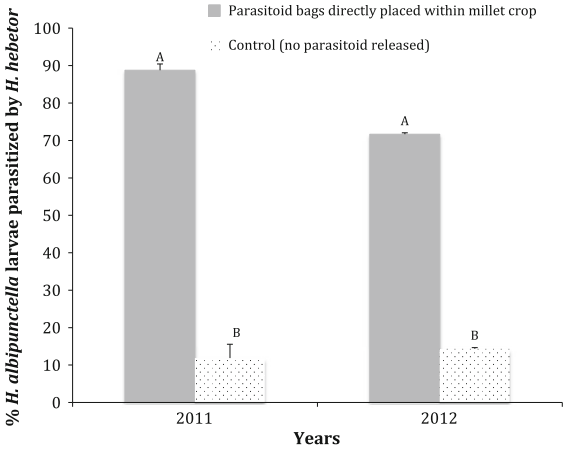

Fig. 2 Parasitized larvae of *H. albinpuctella* (% Mean + SE) due to *H. hebetor*, in villages covered by direct releases of *H. hebetor* within millet farms and control villages in Burkina Faso in 2011 and 2012. (For each year, means were compared by a Student–Newman–Keuls test at the 5 % level, with different alphabetic letters indicating significant differences)

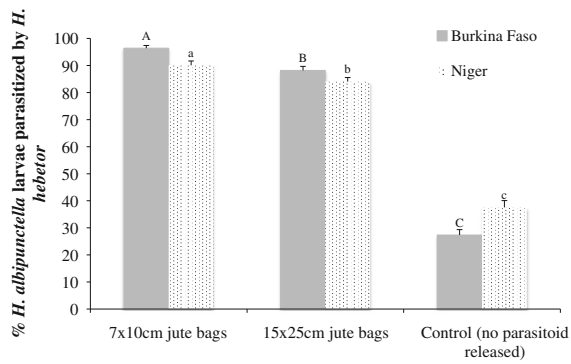

Fig. 3 *Heliocheilus albipunctella* (de Joannis) larvae (% Mean + SE) parasitized by *Habrobracon hebetor* (Say) in villages in Burkina Faso and Niger participating in augmentative releases of *H. hebetor* in 2012 using different sizes of jute bags and control villages (Means were compared by a Student–Newman–Keuls test at the 5 % level with different alphabetic letters indicating significant differences. Upper-case letters were used for Burkina Faso and lower-case letters were used for Niger)

high rate of population growth (Youm and Gilstrap 1993; Nikam and Pawar 1993), we estimate that the set of 15 parasitoid bags per village can lead to parasitoid population buildup of up to several thousand parasitoids within four weeks.

The emerging offspring was male-biased regardless of bag size. Contrasting findings have been reported regarding the *H. hebetor* sex ratio with some studies reporting a male-biased sex ratio (Nikam and Pawar 1993; Gündüz and Gülel 2005; Eliopoulos and Stathas 2008; Landge et al. 2009) and others a female-biased sex ratio (Yu et al. 1999; Ghimire and Phillips 2010; Farag et al. 2012). These conflicting reports on *H. hebetor* progeny sex ratio are likely due to differences in the host larvae used, host larval stages, host-parasitoid ratio and environmental conditions (Whiting and Anderson 1932; Benson 1973; Rotary and Gerling 1973; King 1987; Ode et al. 1996; Yu et al. 2003; Dabhi et al. 2011). Likewise the male-biased sex ratio could be due to unfavourable rearing conditions. As demonstrated by Heimpel et al. (1997) the storage-strain of *H. hebetor* performed poorly on the field

insect pest. In our case we reared the "field strain" of *H. hebetor* on larvae of *C. cephalonica*, a storage pest which may actually have been unfavorable and resulted in a male-biased sex ratio in the bags. Also as hypothesized by Antolin et al. (1995) *H. hebetor* male-biased sex ratios are probably linked to single-locus complementary sex determination caused by a combination of diploid male viability and low allele diversity at the sex locus.

On-farm findings clearly suggest that the deployment of parasitoid bags within millet fields is effective for controlling MHM with up to 85 % larvae parasitized. This is in line with previous augmentative releases of *H. hebetor* from straw granaries (Ba et al. 2013). In addition the small parasitoid bag is more effective than the big bag leading to over 90 % of MHM larvae parasitized. Moreover, the smaller bag requires 40 % less millet grains and flour per bag. This is a clear indication of use of small bags for biological control program.

Despite the already substantial natural parasitism due to *H. hebetor* (up to 38 % in control villages), the augmentative release of *H. hebetor* significantly increased the mortality of MHM. The *H. hebetor* stored-product strain has been commercially used in Germany, Austria and the USA since the late 1990s for the biocontrol of warehouse insects (Brower et al. 1996; Schöller 2001). Outdoor releases of field strain of *H. hebetor* have been effective for controlling the cotton bollworm *Helicoverpa armigera* in Iran (Navaei et al. 2002). In India mass releases of *H. hebetor* have been successful for suppression of coconut head caterpillar *Opisina arenosella* (Desai et al. 2007).

In the Sahel MHM typically infested over 60 % of millet panicle and leads to 53 % yield losses (Baoua et al. 2013). Our findings confirm again the effectiveness of augmentative releases of *H. hebetor* for controlling MHM (Ba et al. 2013; Baoua et al. 2013) and as recently demonstrated effective parasitism by *H. hebetor* reduced grain losses from infested panicles by 34 % (Baoua et al. 2013). Although releases of *H. hebetor* give a good control of the MHM, the parasitoid only attacks 3rd and later instar larvae of MHM when some damage to millet panicles has already occurred. Since indigenous egg parasitoids *Trichogramma* spp. are observed parasitizing MHM eggs (Ndoye and Gahukar 1995; Bal 1993) they could be tested for a more effective/early control of MHM.

Even though the effective augmentative release of braconid parasitoids has been demonstrated elsewhere (Sivinski et al. 1996; Montoya et al. 2000; Wang et al. 2013), the wide use of this approach is still challenging (van Lenteren 2012). Particularly in the Sahel region, the parasitoid has a very limited chance of survival during the long dry season because of the scarcity of vegetation and alternate hosts. Thus, the biological control program will involve repeated releases of parasitoids every year. The numbers required are beyond the production capacity and mandates of research laboratories. To safeguard the sustainability of biological control program, it will be useful to investigate the feasibility of establishing a cottage industry to produce parasitoids. In that regard, we recommend that a feasibility study be conducted to assess economic viability of such an industry.

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