How host larval age, and nutrition and density of the parasitoid *Dinarmus basalis* (Hymenoptera: Pteromalidae) influence control of *Acanthoscelides obtectus* (Coleoptera: Bruchidae)

I. Schmale[1], F.L. Wäckers[2], C. Cardona[3] and S. Dorn[1]*

[1]Swiss Federal Institute of Technology (ETH), Institute of Plant Sciences, Applied Entomology, 8092 Zürich, Switzerland: [2]Netherlands Institute of Ecology, Center for Terrestrial Ecology, PO Box 40, 6666 ZG Heteren, Netherlands: [3]Centro Internacional de Agricultura Tropical, Cali, Colombia

Abstract

Choice of the targeted host developmental stage, regulation of parasitoid numbers released and introduction of food supplements are operational factors with a potential to influence the level of biological control. In a closed laboratory storage system maintained over two generations of the host, the impact of these three parameters on the control potential of the parasitoid *Dinarmus basalis* Rondani was investigated for high populations of larvae of *Acanthoscelides obtectus* (Say) feeding inside dry common bean seeds *Phaseolus vulgaris*. The beans were already infested with immature bruchids at the beginning of the storage period to simulate harvest conditions, characterized in a previous study. Treatments resulted in a reduction of 48–75% of the bruchid population within 16 weeks of storage. The best timing of parasitoid release was at the simulated harvest, as later releases reduced the bruchid population only by about half this percentage. Host feeding is postulated to be the key factor involved in the observed difference. The effect of increasing the number of parasitoids strongly depended on host age and food supplement. Addition of vials with honey had no direct effect on the bruchid population or on the parasitoid progeny. The ecological significance of these findings and implications for biological control are discussed.

Introduction

Biological pest control is receiving increased attention as a safe alternative to the sole dependence on pesticides. Doubts regarding the effectiveness of biological control in post-harvest systems (e.g. Southgate, 1978) have been dispelled by studies showing that parasitoid releases in storage systems can effectively reduce damage by storage pests (Parrella *et al.*, 1992; Schmale *et al.*, 2003). For example, egg parasitoids (van Huis *et al.*, 1998, 2002) and larval-pupal

parasitoids (Islam & Kabir, 1995; Sanon *et al.*, 1998; Ndoutoume *et al.*, 2000) showed great potential to reduce damage by the cowpea bruchid beetles, *Callosobruchus* spp. (Coleoptera: Bruchidae) in traditional storage system in the tropic belt. As user safety is a key requirement in on-farm post-harvest treatments, integrated pest management programmes based on natural resources including parasitoids may be a promising option for small-scale farms in developing countries (Dorn, 1998; Raja *et al.*, 2001; Babu *et al.*, 2003).

Parasitoids may induce mortality of their herbivore host through oviposition, host-feeding or wounding without subsequently using the host (Jervis & Kidd, 1986; Heimpel & Collier, 1996). Inoculative biological control aims at a sustainable establishment of a parasitoid population, and factors of influence include a high number of ovipositions by

*Author for correspondence
Fax: +41 1 632 39 21
E-mail: silvia.dorn@ipw.agrl.ethz.ch

the parasitoid. In contrast, inundative release of a parasitoid aims at maximum pest destruction within a short period of time, and a combination of host feeding and oviposition might be an optimal approach towards this target. This is exemplified by host-feeding and parasitism of an egg parasitoid, *Trichogramma turkestanica* Meyer (Hymenoptera: Trichogrammatidae), of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), (Hansen & Jensen, 2002).

In many parasitoid species, females are able to assess the quality of a host larva and to decide whether to use it for future reproduction by host feeding, or for immediate reproduction by oviposition (e.g. Heimpel & Rosenheim, 1995; Gauthier *et al.*, 1997; Tran & Takasu, 2000). Various parameters, such as parasitoid density, nutrition, host larval age, host density, and encounter rate can affect this decision (Jervis & Kidd, 1986; Heimpel & Collier, 1996; Jervis *et al.*, 1996; Bertschy *et al.*, 2000). The first three parameters are operational factors as they can be fully or largely controlled by the use of the biological control agent. Regulation of parasitoid density can be achieved by releasing a distinct number of wasps into the system, regulation of nutrition by offering an accessible food source (Schmale *et al.*, 2001; Wäckers, 2001), and choice of the targeted host developmental stage by an appropriate timing of parasitoid release into a system with a known prevailing host age (Schmale *et al.*, 2002, 2003). In the current study, the prevailing age of the immature bruchids was seven days after oviposition, at which time the young larvae had already bored into the beans, where they then develop until the adults emerge from the seeds.

In this work, the influence of the three key parameters, host larval age, parasitoid nutrition and density, on post-harvest management of the bruchid *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) in dry beans by the parasitoid *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae) was investigated. Mainly known as a larval-pupal parasitoid of *Callosobruchus* spp. (Islam & Kabir, 1995; Sanon *et al.*, 1998), this synovigenic ectoparasitoid also attacks larvae of *A. obtectus*, and rearing on beans containing fourth instar larvae of this host has proved to be successful (Schmale *et al.*, 2001). The bruchid *A. obtectus* can infest beans prior to harvest, and as many as 90% of the samples taken over three years in Valle de Cauca, Colombia, were infested by immature stages of *A. obtectus* at harvest. The relatively narrow time period of subsequent adult emergence in the samples held under standardized laboratory conditions indicated that beans at harvest contained a relatively synchronous population of young larvae (Schmale *et al.*, 2002). Hence, the system was suitable for the objectives of this study.

Material and methods

Beans

The experiment was carried out with the widely cultivated susceptible common bean *Phaseolus vulgaris* L. var. Diacol-Calima (Fabaceae), subsequently named 'Calima'. Seeds were obtained from 'La Providencia' farm in Restrepo, Valle de Cauca, Colombia. They were harvested a year before the experiments took place. Following harvest, they were kept in a storeroom at 5°C for six months. Pilot studies showed that after this period no new insects emerged from

the seeds when they were brought to and maintained at 26°C and 80% RH (I. Schmale, personal observation).

Herbivore

The host herbivore *A. obtectus* was used in all experiments. This species was reared from a locally collected strain at the Bean Entomology Laboratory at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia on *P. vulgaris* var. 'Calima'. Cultures were maintained at 26°C, 80% RH using the methods described by van Schoonhoven *et al.* (1983).

Parasitoid

Dinarmus basalis was collected from *A. obtectus* feeding on beans cv. 'Calima' in a legume grain store in Buga, Valle de Cauca, Colombia. Subsequently, the parasitoid was reared on the same host-bean system at the Bean Entomology Laboratory in CIAT. Prior to being used in the experiment, newly emerged adult parasitoids were removed from the culture and kept separately for a maximum of one week. A drop of honey was placed on the inner surface of the glass jar close to the lid. A previous study showed that parasitoids reared in this manner have a mean lifespan of 64 days and a mean lifetime progeny production of 28 individuals (Schmale *et al.*, 2001).

Experimental procedure

Experiments were carried out at $22(\pm 1)$°C, 70% RH and 24 D, which corresponds to the storage conditions in Colombian bean growing areas. Glass jars (height 26.5 cm, diameter 16 cm) were each filled with 3 kg of bean seeds. To simulate a high-level field infestation at the time of harvest (Schmale *et al.*, 2002), beans were infested consecutively 14, 7 and 0 days before the start of the experiment with 60, 120 and 30 eggs of *A. obtectus*, respectively. Hence, 1 kg of seeds was infested with a total of 70 eggs. One day before each new infestation the beans were carefully mixed to achieve a random distribution of host larvae. One male and one female *D. basalis*, 1–7 days old, were introduced into each jar either at the beginning of the experiment, i.e. at the time of the simulated harvest ('early'), or 11 days later ('delayed'). To test the influence of parasitoid nutrition, one treatment contained a food supplement (0.1 ml honey) placed inside a plastic tube (length 5 cm, diameter 2 cm) that was covered with gauze (1 mm² mesh size) on both sides. The tubes were placed in the centre of the glass jars. The mesh size of the gauze allowed parasitoids to pass through and feed on the honey, while excluding the larger bruchids.

Treatments

The following treatments were carried out in ten replicates each: (i) five *D. basalis* pairs per kg beans, introduced early, with honey (E5h); (ii) five *D. basalis* pairs per kg beans, introduced early, without honey (no food) (E5nf); (iii) two *D. basalis* pairs per kg beans, introduced early, with honey (E2h); (iv) two *D. basalis* pairs per kg beans, introduced early, without honey (no food) (E2nf); (v) five *D. basalis* pairs per kg beans, delayed introduction, with honey (D5h); (vi) five *D. basalis* pairs per kg beans, delayed introduction, without honey (no food) (D5nf); (vii) control treatment without

parasitoids and without honey (control). The following treatment was carried out in five replicates: (viii) *D. basalis* pairs per kg beans, delayed introduction, with honey (D10h).

Population dynamics

To obtain information on the phenology of both insect species without disturbing the system, the number of live *A. obtectus* and *D. basalis* were counted weekly in a 10.5 cm × 14.7 cm area equal to the size of a DIN A6 postcard drawn laterally on the bean jar. When insect densities were too high to allow accurate counting, one-third of the area (3.5 cm × 14.7 cm) was assessed and the value obtained was subsequently multiplied by three.

Insect population augmentation and bean damage

After 16 weeks, all live and dead adults were counted. Bean damage was assessed by counting all the beans with a visible emergence hole (defined as 'damaged beans') and those without any visible emergence hole (defined as 'undamaged beans').

Data analysis

For statistical analysis one-way ANOVA was used. To test for differences between the mean values Fisher's PLSD (protected least significant difference) was used.

Results

Population dynamics

Visual inspection of the glass jars with the bruchid infested beans and the parasitoids showed that a storage period of 16 weeks allowed *A. obtectus* to produce two generations. The first generation of adult bruchids was visible 4 to 8 weeks, the second and larger generation 11 to 16 weeks after the beginning of the experiment.

Dinarmus basalis developed at least two generations which were visible during the first half of the experimental period. The first generation emerged in weeks 3 and 4, the second generation in weeks 6 to 8. In weeks 9 to 16 only an occasional living parasitoid was observed, but no further emergence peak could be determined.

Insect population augmentation and bean damage

The introduction of parasitoids resulted in a significant reduction in the number of bruchids compared to the control without parasitoids (F = 23.8, df = 7, 32, $P < 0.0001$) (fig. 1). Calculated over the total storage period, the parasitoids reduced bruchid numbers by 48–75% relative to the control (fig. 1), resulting in a 33–70% reduction in bean damage.

Each of the subsequent factorial experiments focuses on one key parameter and its effect on parasitoid efficacy.

Effect of host larval age

When parasitoids were released among populations of young host larvae, i.e. early parasitoid introduction corresponding to the time of and situation at (simulated) harvest, bruchid suppression was significantly higher (by approximately 50%) compared to the delayed parasitoid introduction 11 days later ($P < 0.05$ without food supplement; $P < 0.005$ with food supplement) (fig. 2). In contrast to the high reduction in bruchid numbers, parasitoid progeny production was low in treatments with early parasitoid release. Mean parasitoid progeny production per female was significantly higher when parasitoids were released later, to populations of older host larvae ($P > 0.0001$, df = 6, F = 18.7) (fig. 2).

Effect of parasitoid density

Increasing the number of introduced parasitoids improved bruchid population reduction significantly in treatments without food supplement ($P < 0.05$) or with older
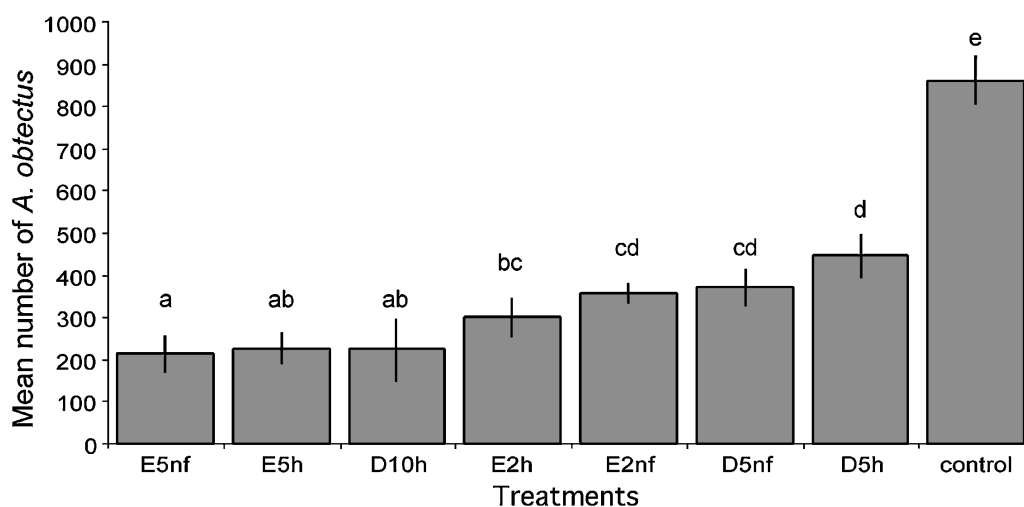

Fig. 1. Numbers of *Acanthoscelides obtectus* adults after a 16-week storage period, per kg bean seeds. Data are expressed as average and standard error of the mean. Treatment abbreviations are: E, early parasitoid introduction (day 0); D, delayed parasitoid introduction (day 11); 2, 5, 10, number of introduced parasitoid pairs; h, honey given as food supplement; nf, no food supplement. Columns capped with different letters were significantly different according to Fisher's PLSD ($P < 0.05$).
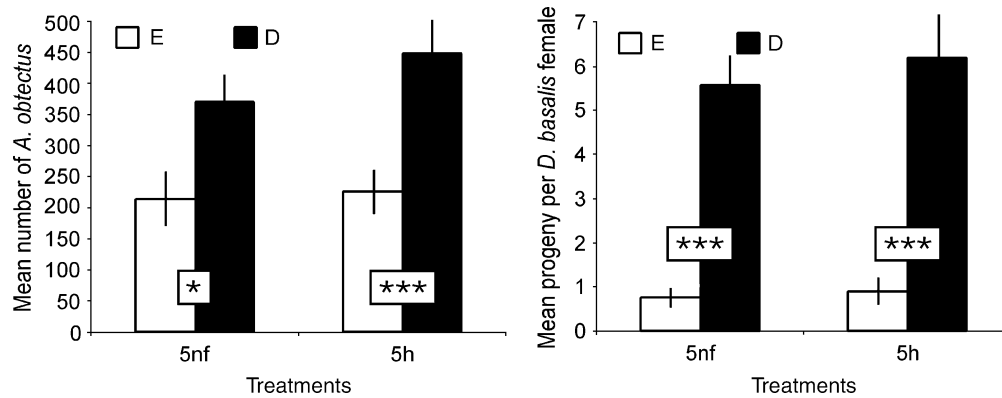
Fig. 2. Influence of timing of parasitoid introduction: numbers of *Acanthoscelides obtectus* adults (per kg seed) and *Dinarmus basalis* progeny per female after a 16-week storage period. Data are expressed as average and standard error of the mean. For treatment abbreviations see fig. 1. Asterisks indicate a significant difference between the two columns (Fisher's PLSD, *** $P < 0.0001$, * $P < 0.05$, n.s; not significant).

host larvae ($P < 0.05$) (fig. 3). In the treatment with early release and supply of honey, no significant differences in the reduction of bruchid numbers or in parasitoid progeny production were found between the introduction of two and of five parasitoid pairs.

Effect of food supplements

The addition of honey had no direct effect on the bruchid population or on parasitoid progeny production (fig. 4). Under conditions of low initial parasitoid density, the supply of honey resulted in a reduced mean number of bruchids, though the effect was not significant (fig. 1).

Discussion

This study shows that *D. basalis* is a promising control agent for *A. obtectus*. High bruchid populations in treatments with parasitoid introduction were reduced by 50–75% compared to the control treatments without parasitoids. Eradication was not achieved by any treatment given this

high level of bruchid infestation of 70 individuals per kg of beans. These results were similar to on-farm experiments with a high initial infestation and a comparable number of parasitoids released (I. Schmale, F.L. Wäckers, C. Cardona and S. Dorn, unpublished). However, successful eradication was achieved in that field study when initial infestation did not exceed 20 to 30 bruchids per kg, indicating a good control efficacy of *D. basalis* under actual storage conditions at low levels of *A. obtectus* infestation.

Timing of introduction

In the laboratory studies, bruchid suppression was most successful when parasitoids were introduced to young host larval instars. Early introduction resulted in a two-fold lower number of emerging bruchids compared to a late introduction. Despite this higher attack rate the number of parasitoid progeny in the treatment with early introduction was significantly lower. This inverse relationship of bruchid reduction and parasitoid offspring production can be explained by selective host use by the parasitoid. Various
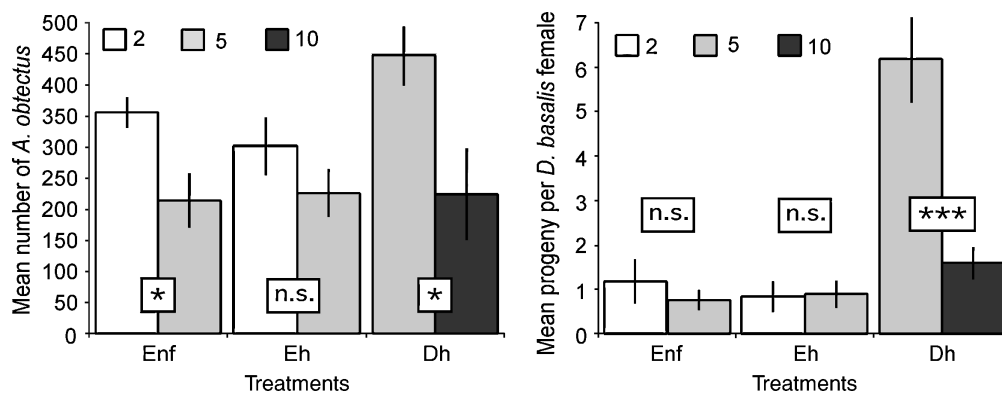

Fig. 3. Comparison of treatments with different numbers of parasitoids introduced: numbers of *Acanthoscelides obtectus* adults (per kg seed) and *Dinarmus basalis* progeny per female after a 16 weeks storage period. Data are expressed as average and standard error of the mean. For treatment abbreviations see fig. 1. Asterisks indicate significant difference between the two columns (Fisher's PLSD, *** $P < 0.0001$, * $P < 0.05$, n.s; not significant).
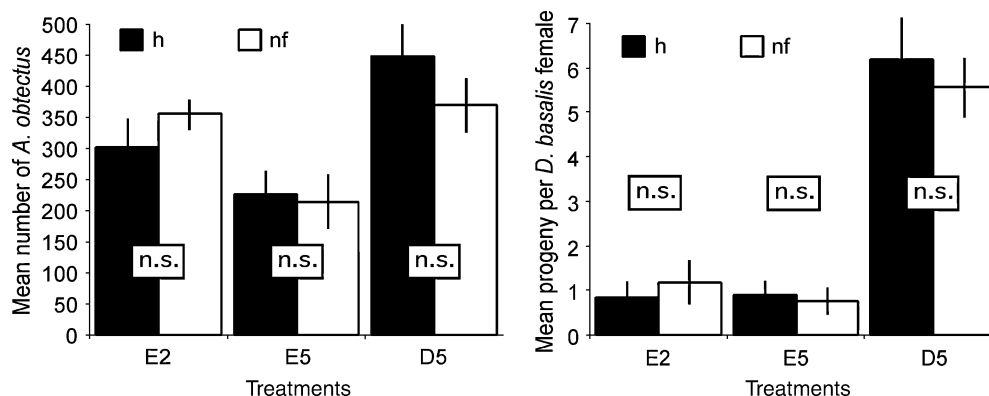
Fig. 4. Comparison of treatments with and without a food supplement: numbers of *Acanthoscelides obtectus* adults (per kg seed) and *Dinarmus basalis* progeny per female after a 16-week storage period. Data are expressed as average and standard error of the mean. For treatment abbreviations see fig. 1. Asterisks indicate significant difference between the two columns (Fisher's PLSD, n.s; not significant).

parasitoids are known to use small larvae either for host-feeding (DeBach, 1943; Bartlett, 1964; Kidd & Jervis, 1991; Heimpel & Rosenheim, 1998), or deposition of male eggs, contributing to a male-biased sex ratio (Gauthier *et al.*, 1997; Bertschy *et al.*, 2000). Large host larvae, on the other hand, are preferentially used for deposition of female eggs (Heimpel & Rosenheim, 1998). Thus, early introduction of parasitoids is likely to favour host-feeding, resulting in the observed high level of bruchid reduction. Their introduction to older host stages resulted in higher numbers of parasitoid progeny, but lower levels of bruchid reduction. There were no indications that the differences in parasitoid progeny production were based on host limitation, since the bruchid population was never eradicated and the ratio between emerging bruchids and parasitoid progeny remained high. Host feeding might have contributed to the best results obtained. In a previous study, access to bruchid infested beans increased the mean longevity of the parasitoid two-fold compared to a control with access to uninfested beans only, clearly indicating that feeding on the host's haemo-lymph acted as a source of additional energy (Schmale *et al.*, 2001).

Parasitoid density

Parasitoid density influenced the suppression of the bruchid population in treatments without a food supplement or with delayed parasitoid introduction. Similar results in bruchid control were reported by Islam & Kabir (1995) who found a 70% reduction of *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Bruchidae) on red lentils when five *D. basalis* pairs were introduced, while 30–50 parasitoid pairs were needed for complete bruchid eradication. As a comparable level of bruchid infestation was used in this study, and only one-third of the parasitoids, it is likely that the number of parasitoids used was too small to achieve full bruchid eradication.

Delayed introduction of parasitoids resulted in attacks on older host larvae and, in this situation, an increase in para-sitoid density resulted in a decrease in parasitoid progeny production. This effect was confirmed by related studies with *C. chinensis* on red lentils (Islam & Kabir, 1995), and with *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae) on

Vigna unguiculata (Walp.) (Gauthier *et al.*, 1997). In both studies more progeny per female were produced at lower parasitoid densities. This finding can be explained by inter-ference between females, either due to interruptions during host searching or oviposition (Gauthier *et al.*, 1997), or due to host feeding on previously parasitized hosts (Ueno, 1999). In populations of younger host larvae no effect of parasitoid density on progeny production could be demonstrated, as progeny production was low in both cases.

Food supplements

Food supplements can prolong the lifespan and enhance offspring production in *D. basalis* (Schmale *et al.*, 2001). Therefore a considerable impact of food supplements on parasitoid and bruchid population dynamics was to be expected. However, the present experiments failed to show a significant difference between treatments with or without food supplements. It is possible that *D. basalis* was able to obtain its entire nutritional needs through host feeding, as sufficient hosts were available. The impact of non-host nutritional sources is likely to be more substantial at lower host densities.

Conclusions

It is concluded that the early introduction of a relatively large number of parasitoids into a storage system offers real potential for significantly reducing field populations of *A. obtectus*, while host feeding is expected to accelerate control, particularly at elevated infestation levels.

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