Rey D. Iturralde-García[a], Cristina Castañé[a], Francisco J. Wong-Corral[b], Jordi Riudavets[a]*

Biological control of *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in stored dried beans

[a]IRTA, Ctra. Cabrils km 2, E-08348 Cabrils, Barcelona, Spain

[b]*Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Rosales y Luis Encinas S/n, 83000, Hermosillo, Sonora, Mexico*

*Corresponding author: Jordi Riudavets
IRTA
Ctra. Cabrils km 2
Cabrils, Barcelona, Spain E-083240
Phone: 93 750 75 11 (ext. 1250)
E-mail: Jordi.riudavets@irta.cat

ORCID of the authors
Rey D. Iturralde-García: 0000-0001-8027-1529
Cristina Castañé: 0000-0002-1080-1253
Francisco J. Wong-Corral: 0000-0003-1647-8001
Jordi Riudavets: 0000-0002-7839-0323

Biological control of *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in stored dried beans

Abstract

This study assesses the feasibility of using natural enemies for the control of *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae), key pests of stored dried beans, *Phaseolus vulgaris* L. (Fabales: Fabaceae). The predatory mites *Blattisocius tarsalis* Berlese (Acari: Ascidae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) were able to prey on *A. obtectus* eggs, reducing the bruchid population by more than 60% under both controlled and warehouse conditions. Therefore, they show good potential as biological agents for controlling this pest. The larval parasitoids *Anisopteromalus calandrae* Howard and *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) were both moderately effective (34-38% reduction) at suppressing *A. obtectus* populations, but when *A. calandrae* was combined with *B. tarsalis*, a significant improvement in control efficacy (81% reduction in emergence) was observed. Therefore, the release of *A. calandrae* combined with *B. tarsalis* seems to be a promising strategy for controlling *A. obtectus*. Neither *B. tarsalis* nor *A. swirskii* were able to prey on *Z. subfasciatus* eggs. Only the parasitoid *A. calandrae* was moderately effective (39% reduction) at supressing *Z. subfasciatus* populations. Further testing is needed to identify other natural enemies that can complement the action of *A. calandrae* in reducing *Z. subfasciatus* populations.

Key words: bruchid weevils, egg predators, larval parasitoids, legumes, stored products

1. Introduction

Acanthoscelides obtectus Say and *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae) are major pests of stored dried beans *Phaseoulus vulgaris* L. (Fabales: Fabaceae). These pests cause significant damage to the legume during long periods of storage (Hill 1990). *Acanthoscelides obtectus* is neotropical in origin but currently has a cosmopolitan distribution. *Zabrotes subfasciatus* is widespread in several countries in Africa, Asia and the Americas. *Acanthoscelides obtectus* attack dry bean seeds in the field and subsequently infest storage units; *Z. subfasciatus* attack only stored seeds (Cardona 1989; Oliveira et al. 2013; CABI 2019). The larvae of both bruchids are internal feeders; they can bore into seeds. Larvae develop inside the seeds until they reach adulthood, when they emerge from the legume and actively disperse (Subramanyam and Hagstrum 1996; Soares et al. 2015).

Pest management in stored dried beans commonly involves treatments with pesticides (fumigants and/or residual insecticides) (Paul et al. 2009). However, the use of pesticides poses a threat to the health of operators handling the fumigant. In addition, there are significant environmental concerns about the use of pesticides, and consumer demand for residue-free products is increasing. In addition, the number of active substances available is shrinking due to changes in legal approval processes and to the emergence of resistant pest populations (Benhalima et al. 2002; Opit et al. 2012; Pimentel et al. 2008; Clarke et al. 2011). Therefore, there is a demand for alternative, environmentally safe methods of pest control. Among these, the use of natural enemies such as predatory mites and parasitoid wasps stands out. Biological control using beneficial insects and mites is an effective pest management tool that does not induce resistance and involves no risks for operators (Niedermayer and Steidle 2013).

The present study evaluates the effectiveness of two types of natural enemies in the control of these two weevils: four polyphagous predatory mite species that prey on eggs and two polyphagous larval parasitoid species. The predatory mite *Blattisocius tarsalis* Berlese (Acari: Ascidae) is frequently found in storage areas of different commodities (Haines 1981; Riudavets et al. 2002a, b). However, the capacity of the other mite species to control storage pests is unknown. *Amblyseius swirskii* Athias-Henriot, *Neoseiulus cucumeris* Oudemans (Acari, Phytoseiidae) and *Stratiolaelaps scimitus* Berlese (Acari, Laelapidae) are commercially available biological control agents for different pests in many vegetable crops grown in open fields and greenhouses (Jess and

Kilpatrick 2000; Messelink et al. 2006; Gerson and Weintraub 2007). *Amblyseius swirskii* is able to prey on the eggs of stored pests such as *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae) in the laboratory, and the predator *N. cucumeris* is able to consume *E. kuehniella* eggs (Delisle et al. 2015; Riahi et al. 2017; Iturralde-García et al. 2020). The larval parasitoids *Anisopteromalus calandrae* Howard and *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae) often attack coleopteran larvae that develop inside the seeds of different stored commodities (Belda and Riudavets 2013; Castañé and Riudavets 2015; Berger et al. 2017).

The main objective of this study was to evaluate the effectiveness of these biological control agents on these two bruchid populations under controlled and warehouse conditions. We hypothesized that combining parasitoids with predatory mites would control both bruchid populations better than the use of either biological agents alone. Therefore, we first selected predatory mites based on their consumption of bruchid eggs and their ability to survive at different relative humidity levels. Predatory mites and parasitoid wasps were then tested, alone and in combination, on infested dried beans in small cages. Finally, the dispersion capacity of predators and parasitoids was evaluated on a larger scale: in 12-kg containers of beans under controlled conditions and in commercial 25-kg bags of beans under warehouse conditions.

2. Materials and methods

2.1. Insect and mite colonies. Insect colonies were maintained at a constant temperature of 28 ± 2ºC, 75 ± 5% relative humidity (RH) and a photoperiod of 16 hours of light to 8 hours of darkness. Colonies of *A. obtectus*, *A. calandrae* and *L. distinguendus* were initiated with samples collected from warehouses in Spain; a colony of *Z. subfasciatus* was started with samples from the University of Sonora (México). Every week, unsexed adults of *A. obtectus* or *Z. subfasciatus* were placed in plastic containers with dry bean seeds (*P. vulgaris*; cv. Riñón) to obtain weevils of known ages. To obtain eggs of both weevil species, adults were isolated with dry beans for 48 hours in plastic cages. Eggs of *A. obtectus* were collected with a fine hairbrush from the bottom of the cages while for *Z. subfasciatus* seeds with attached eggs were collected. *A. calandrae* was reared by offering *A. obtectus* or *Z. subfasciatus* larvae (aged 15 to 21 days) to newly emerged adults; *L. distinguendus* was reared similarly but was only offered *A. obtectus* larvae. Sugar water (20% sucrose) in a cotton plug was supplied as additional food. After three weeks, a new generation of adult parasitoids was available for experiments. The predatory mites *B. tarsalis*, *A. swirskii*, *N. cucumeris* and *S. scimitus* were supplied by Agrobio SL (Almería, Spain).

2.2. Survival and consumption of bruchid eggs by predatory mites. To assess the ability of the four species of predatory mites to prey on bruchid eggs, 15 two-day-old eggs of *A. obtectus* or *Z. subfasciatus* were offered to one female in a small cage (2.5 cm diameter). Female predatory mites were collected using a fine hairbrush and transferred into the experimental cages. After 24 h, the female mite's survival was assessed and the number of completely or partially damaged eggs recorded. With a stereomicroscope, damaged eggs can be easily distinguished from healthy and turgid eggs. A control treatment using 15 two-day-old eggs of *A. obtectus* or *Z. subfasciatus* but no predatory mites was also done. Ten replicate experiments of each combination of predator and bruchid species and of the control treatment were conducted. Experiments with *A. obtectus* were carried out at 28 ± 2ºC and at two levels of RH, 80% and 75%; *Z. subfasciatus* was kept at 80% RH to test the ability of predatory mites to survive at different environmental humidity. Saturated salt/water solutions were used to maintain a stable RH inside containers where the small cages were deposited during the experiment.

2.3. Effectiveness of predatory mites and larval parasitoids in reducing bruchid populations. To further test the predatory capabilities of *B. tarsalis* and *A. swirskii*, 45 two-day-old eggs of *A. obtectus* or *Z. subfasciatus* were offered to 15 mite females over three weeks as follows: 15 two-day-old eggs per week were offered to three (in the first week), six (in the second week) and six (in the third week) predatory females of each species. The final proportion was one female predatory mite to three bruchid eggs. After four additional weeks, necessary for surviving eggs to reach adulthood, the number of emerged weevils was counted. To test *A. calandrae* and *L. distinguendus*, beans containing 15 two-day-old eggs of *A. obtectus* or *Z. subfasciatus* were introduced weekly for three weeks, and, on the fourth week, three pairs of adults were released, resulting in a total proportion of one female parasitoid to 15 hosts. A plastic tube containing sugar water (20% sucrose) and a cotton plug was also included as additional food in the experimental arena. After three additional weeks, necessary for surviving weevil eggs and parasitoids to reach adulthood, the number of weevils and/or parasitoids was counted until no more emergence was recorded. To test the combined effect of the predatory mites *B. tarsalis* and *A. swirskii* with the parasitoid *A. calandrae* on the populations of *A. obtectus* or *Z. subfasciatus*, a similar test was performed in which female predatory mites were introduced during the first three weeks and parasitoid pairs in the fourth week, resulting in the same proportions of prey to hosts as previously described. A control treatment with 45 two-day-old eggs of *A. obtectus* or *Z. subfasciatus* but without predatory mites or parasitoids was also done. For all experiments, 710-mL plastic containers containing 100 g of beans, including the seeds with bruchid eggs, were used. Ten replicate experiments were conducted for each predatory mite, parasitoid species or combination and for the control treatment. Experiments were carried out in controlled conditions at a temperature of 28 ± 2ºC, 75 ± 5% RH and a photoperiod of 16 h of light to 8 h of darkness.

2.4. Searching ability of predatory mites and *A. calandrae*. To assess the ability of *B. tarsalis* and *A. swirskii* females to locate their prey in a more realistic arena, bruchid eggs were offered in 12-kg containers of beans. Two predator-to-prey ratios were tested, 1:3 and 2:1. A fixed number of prey (45 two-day-old eggs of *A. obtectus*) were offered to 15 or 90 female predatory mites. PVC pipes (40 cm tall, 20 cm diameter) filled with 12 kg of beans were used in this experiment. A stainless-steel screened cage (7 cm high, 5 cm internal diameter) containing 60 g of beans and infested with 45 two-day-old eggs of *A. obtectus* was located at the bottom of the PVC pipe. Next, the appropriate number of predatory mites was released at the top, on the surface of the beans, and pipes were sealed with fabric mesh. After four additional weeks, PVC pipes were poured off, the screened cages were recovered, and the number of weevils that had emerged in the cages was counted.

The combination of the predatory mite *B. tarsalis* with the parasitoid *A. calandrae* was assessed in a similar arena of PVC pipes. A fixed number of prey/hosts (45 two-day-old eggs of *A. obtectus*) was offered to 90 female predatory mites (2:1 predator-to-prey ratio) and to three pairs of *A. calandrae* (1:15 parasitoid-to-host ratio). Predatory mites were released the same week as the bruchid eggs, while the parasitoids were released four weeks later. After three additional weeks, the number of emerging weevils and parasitoids in the screened cages was counted. For the control treatment, 710-mL plastic containers containing 60 g of beans infested with 45 two-day-old eggs of *A. obtectus* were used. Six replicates were conducted of each treatment, including the control. The experiments were carried out at in controlled conditions at a temperature of 28 ± 2ºC, 75 ± 5% RH and a photoperiod of 16 h of light to 8 h of darkness.

To assess the ability of *B. tarsalis* to locate its prey in larger arenas, a similar test was done with commercial woven polypropylene bags (42 x 66 cm containing 25 kg of beans) in experimental rooms (23.5 ± 1.5ºC, 68 ± 10% RH). One infested screened cage containing 45 two-day-old eggs of *A. obtectus* in 60 g of beans was placed at one end of the bag. At the opposite end, 9 ml of commercial diet substrate containing *B. tarsalis* (90-180 individuals) was released. One polypropylene bag was placed on the floor of each empty room (3 x 2 m). After one week, the bags were opened, and the infested chickpeas in screened cages were allowed to develop under controlled conditions (28 ± 2ºC; 75 ± 5% RH; 16 h: 8 h light: dark). Over the following weeks, the emerging *A. obtectus* were counted. The control treatment without predators used 710-mL plastic containers containing 60 g of beans infested with 45 two-day-old eggs of *A. obtectus*. Six replicates were conducted, each in a different room.

2.5. Data analysis. The percentage of mortality was calculated by subtracting the number of surviving weevils from the total number of eggs offered at the start of the experiment. An arcsine square root transformation was performed on the percentage of mortality rates so that these data complied with the normality and homoscedasticity requirements of parametrical tests. The following data sets were analysed using a one-way analysis of variance (ANOVA): a) percentage of mortality of *Z. subfasciatus* and *A. obtectus* eggs after one female predatory mite was released; b) percentage of mortality of *Z. subfasciatus* and *A. obtectus* when predatory mites, parasitoids or a combination of both were released; c) percentage of mortality of *A. obtectus* when two predatory-to-prey ratios of *A. swirskii* and *B. tarsalis* were tested alone, or when *B. tarsalis* was combined with *A. calandrae* in PVC pipes or when 9 ml of commercial *B. tarsalis* were released in a propylene bag. *Post-hoc* comparisons were conducted for all tests using Tukey corrections for multiple comparisons. All statistical analyses were conducted using JMP (JMP 13.1.0, 2016, SAS Institute, Inc).

3. Results

3.1. Survival and consumption of bruchid eggs by predatory mites. All females of *B. tarsalis*, *A. swirskii* and *N. cucumeris* survived at 80% RH; no *S. scimitus* female survived when offered eggs of *Z. subfasciatus*, and only 10% of females survived when offered *A. obtectus* eggs. At 75% RH, most females of *B. tarsalis* and *A. swirskii* survived, but no *N. cucumeris* survived. Only *N. cucumeris* consumed *Z. subfasciatus* eggs, resulting in a significant difference in egg mortality compared to the control (Table 1). However, when *A. obtectus* eggs were offered, all predatory mites tested produced significantly higher mortality than that observed in the control, at both 75% and 80% RH.

3.2. Effectiveness of predatory mites and larval parasitoids in reducing bruchid populations. Neither predatory mites nor the parasitoid *L. distinguendus* were able to reduce the emergence of *Z. subfasciatus* adults compared to the control treatment. Only the parasitoid *A. calandrae* significantly reduced the *Z. subfasciatus* population when interacting for at least one week ($F = 12.17$; df = 6, 79; $P < 0.001$) (Fig. 1a). As expected, when predatory mites were combined with the parasitoid *A. calandrae*, the mortality of *Z. subfasciatus* was not significantly different than that observed with *A. calandrae* alone. However, immature stages of *A. obtectus* were more susceptible to attack by these natural enemies ($F = 64.61$; df = 6, 79; $P < 0.001$). When tested individually, both parasitoids, *A. calandrae* and *L. distinguendus,* and both predatory mites, *B. tarsalis* and *A. swirskii,* significantly impacted the mortality of *A. obtectus* (Fig. 1b). The combination of predatory mites with the parasitoid *A. calandrae* had a synergistic effect, producing a *A. obtectus* mortality between 52% and 65%. The combination of the parasitoid with *B. tarsalis* was significantly more effective than its combination with *A. swirskii* (Fig. 1b). Parasitoid reproduction was low, with less than 0.70 individuals produced in average per host.

3.3. Searching ability of predatory mites and *A. calandrae*. When *A. obtectus* eggs were offered 40 cm deep in a 12-kg pile of beans, both *B. tarsalis* and *A. swirskii* were able to locate and prey upon them. *A. obtectus* mortality was significantly higher than in the control treatment when either predatory mites or a combination with *A. calandrae* were released at the two predator-to-prey ratios tested (Table 2). No significant differences were observed among the predator-to-prey ratios tested; *B. tarsalis* caused *A. obtectus* mortality ranging from 60% to 67% in PVC pipes and polypropylene bags. *Amblyseius swirskii* caused similar bruchid mortality with the high ratio tested in the PVC pipes, but bruchid mortality was significantly lower with a lower ratio. The best results were obtained with the combination of *B. tarsalis* with the parasitoid *A. calandrae,* which resulted in 81% mortality of *A. obtectus* (Table 2).

4. Discussion

Two of the predatory mites tested, *S. scimitus* and *N. cucumeris*, had difficulty surviving at high temperatures and low humidity, typical conditions in storehouses. *Stratiolaelaps scimitus* is used as a biocontrol agent of a number of pest species, including *Frankliniella occidentalis* Pergande and *Thrips tabaci* Lindeman

(Thysanoptera: Thripidae) under greenhouse conditions (16-25ºC and 24-32ºC with 64-98% and 35-78% RH, respectively) (Wu et al. 2014). *Stratiolaelaps scimitus* survive high temperatures for short periods, but when the temperature is constantly high, as in the present experiment, this predator is unable to survive. *Neoseiulus cucumeris* is often used to control thrips and spider mites on horticultural plants, and it can prey on storage pests such as *E. kuehniella* in a laboratory under controlled conditions (25ºC and 60-70% RH) (Sarwar et al. 2009; Delisle et al. 2015). Although *N. cucumeris* can survive high temperatures (25ºC), our study used an even higher temperature (28ºC), making it difficult for this predator to survive. However, both *S. scimitus* and *N. cucumeris* are very sensitive to the environmental humidity and do not thrive at medium or low RH. However, *A. swirskii* is able to survive the high temperatures and low humidity common to orchards in the Middle East from where it is native. It coexists with whiteflies and tolerates low humidity and high temperatures better than nearly all other species of predatory mite (Nomikou et al. 2001). This species is commonly used for the biological control of thrips and whiteflies in greenhouse crops and feeds on *E. kuehniella* eggs under controlled conditions (25ºC and 65% RH) (Fathipour and Maleknia 2016; Riahi et al. 2017). *Blattisocius tarsalis* was also able to survive the tested conditions; this was expected since this predator is spontaneously present in warehouses and food processing facilities (Dizlek et al. 2019).

The predatory mites *A. swirskii* and *B. tarsalis* were unable to prey on *Z. subfasciatus* eggs. This is probably because the eggs are firmly attached to the bean and have a protective coating once they harden (Southgate 1979); this may impede the predator's perforation of the eggshell. However, both predatory mites were able to prey on *A. obtectus* eggs. Unlike *Z. subfasciatus*, this bruchid species lay eggs in the debris of pulses, so predatory mites do not have to deal directly with the seed. According to Jimenez et al. (2017), the bean testa contain toxic compounds that cause paralysis or antixenosis in bruchid adults and antibiosis and antixenosis in larvae. These toxic compounds, which are associated with bean resistance to pests, can also have adverse effects on biological control agents (Velten et al. 2008). This might explain the differences observed in the predatory capacity of the two mite species on *A. obtectus* and *Z. subfasciatus* eggs. Both predatory mites were able to locate and prey on *A. obtectus* eggs in PVC pipes, indicating that they can locate their prey at a distance of 40 cm in a total volume of 12,566 cm^3 of beans. *B. tarsalis* also demonstrated good potential for reducing *A. obtectus* density in infested commercial bags under warehouse conditions, where it effected a similar suppression rate (65% mortality). This good suppression rate in commercial bags indicates a good dispersion capacity as well, since mites were able to locate their prey at a distance of 60 cm in a total volume of 46,653 cm^3 of beans. Thus, the release of either of the predatory mites alone seems to hold promise for the biological control of *A. obtectus* eggs.

The two parasitoids tested were able to locate and, to some extent, to reduce *A. obtectus* populations: *A. calandrae* effected a 38% reduction and *L. distinguendus* a 34% reduction from an initial population of 45 eggs. *A. calandrae* was also able to reduce *Z. subfasciatus* populations by up to 39%. However, these reductions in weevil populations were low compared to the reductions these species have been reported to effect with the same weevils in different pulses and/or with other weevils. *Anisopteromalus calandrae* produced up to 95% *A. obtectus* mortality in the cowpea *Vigna unguiculata* L (Fabales: Fabaceae) (Berger et al. 2017). This legume has a rougher surface than the smooth beans used in the present study. *Phaseolus vulgaris* seeds are a difficult substrate for the movement of parasitoid wasps; their slippery surface does not allow parasitoid females to get a firm grip during drilling and parasitization (Mitsunaga and Fujii 1999; Niedermayer and Steidle 2013). *Anisopteromalus calandrae* was able to reduce *Sitophilus zeamais* (Motschulsky) and *Rhyzopertha dominica* Fabricius populations by more than 95% when released in rice at 28ºC under similar conditions to those used in the present study (Solà et al. 2020). Another factor that may have contributed to the parasitoid's poor performance is the release ratio used in this study. Some authors have argued that weevil control is effective only when parasitoids are introduced in quantities of one parasitoid per ten hosts or higher (Arbogast and Mullen 1990, in corn; Sanon et al. 1998, in cowpeas). However, *A. calandrae* has been used at the same release ratio and under the same environmental conditions as the present study to control *Callosobruchus chinensis* L. in chickpeas, resulting in more than 90% bruchid control (Iturralde-García et al. 2020).

The combination of *A. calandrae* with predatory mites did not improve the parasitoid's efficacy in controlling *Z. subfasciatus.* This is not surprising since the mites were unable to prey on the eggs. However, the combination of *A. calandrae* with *A. swirskii* or *B. tarsalis* improved control of *A. obtectus* (52-65% suppression) when the predators were released in small cages. Control improved even more (81% suppression) when two natural enemies were released in PVC pipes containing 12 kg of beans (Fig. 1a, b; Table 2). It is important to mention that there was no direct competition between the two natural enemies since one (parasitoids) attacked the beetles at a developmental stage located inside the beans and the other (predatory mites) attacked the prey outside the beans. Improved control has previously been observed with the combined release of multiple natural enemies that attack the host at different developmental stages. The larval parasitoid *A. calandrae* and the predator *Xylocoris flavipes* Reuter (Hemiptera: Lyctocoridae) together effected 95% suppression of *A. obtectus* progeny (Berger et al. 2017); the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and the larval parasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) effected 84% reduction of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) populations (Brower and Press 1990). Therefore, the combination of *B. tarsalis* with *A. calandrae* can be recommended for the biological control of *A. obtectus* populations. This method should not be a problem in bulk beans, since the dead bodies of parasitoids and predators are almost imperceptible due to the small size of both natural enemies. Furthermore, they can be separated from the beans using standard cleaning procedures.

In conclusion, the predatory mites *A. swirskii* and *B. tarsalis* were able to prey on *A. obtectus* eggs, reducing the bruchid population by more than 60% under both controlled and warehouse conditions. Therefore, they demonstrate good potential as biological agents for controlling this pest. Both parasitoids were moderately effective (34-39% reduction) in suppressing both bruchid populations, but significant improvement (81% reduction) was observed when *A. calandrae* was combined with *B. tarsalis*. The combined use of both natural enemies to control *A. obtectus* seems to be a promising strategy for containing the growth of the bruchid populations below damaging levels, allowing to reduce the number of pesticide application during the sometimes long storage period. However, further research is needed on the use of other natural enemies in combination with *A. calandrae* to reduce *Z. subfasciatus* populations.

5. References

Arbogast RT, Mullen MA (1990) Interaction of maize weevil (Coleoptera: Curculionidae) and parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in a small bulk of stored corn. J Econ Entomol 83:2462-2468

Belda C, Riudavets J (2013) Natural enemies associated with lepidopteran pests in food and feed processing companies. J Stored Prod Res 53:54-60

Benhalima H, Chaudhry MQ, Mills KA, Price NR (2002) Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J Stored Prod Res 40:241-249

Berger A, Degenkolb T, Vilcinskas A, Schöller M (2017) Evaluating the combination of a parasitoid and a predator for a biological control of seed beetles (Chrysomelidae: Bruchinae) in stored beans. J Stored Prod Res 74:22-26

Brower JH, Press JW (1990) Interaction of *Bracon hebetor* (Hymenoptera: Braconidae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in suppressing stored product moth populations in small inshell peanut storages. J Econ Entomol 83:1096-1101

CABI (Centre for Agriculture and Biosciences International) (2019) Invasive species compendium. https://www.cabi.org/isc/datasheet/57289. Accessed 18 March 2020

Cardona C (1989) Insects and other invertebrate bean pests in Latin America. In: Schwartz HF, Pastor-Corrales MA (eds) Bean production problems in the tropics. CIAT, Cali, pp 505-570

Castañé C, Riudavets J (2015) Sampling arthropod pests and natural enemies in stored barley. J Stored Prod Res 64:54-61

Clarke JH, Wynn SC, Twining SE (2011) Impact of changing pesticide availability. Asp Appl Biol 106:263-267

Delisle JF, Brodeur J, Shipp L (2015) Evaluation of various types of supplemental food for two species of predatory mites, *Amblyseius swirskii* and *Neoseiulues cucumeris* (Acari: Phytoseiidae). Exp Appl Acarol 65:483-494

Dizlek H, Karagoz M, Faraji F, Cakmak I (2019) Mites in dried figs of Turkey: Diversity, species composition and density. Syst Appl Acarol-UK 24(6):992-997

Fathipour Y, Maleknia B (2016) Mite predators. In: Omkar (ed) Ecofriendly pest management for food security. Elsevier Inc., San Diego, pp 329-366

Gerson U, Weintraub PG (2007) Mites for the control of pests in protected cultivation. Pest Manag Sci 63:658-676

Haines CP (1981) Laboratory studies on the roles of an egg predator, *Blattisocius tarsalis* (Berlese) (Acari: Ascidae), in relation to the natural control of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) in warehouses. Bull Entomol Res 71:555-574

Hill DS (1990) Pests of stored products and their control. CRC Press, Boca Raton, Florida

Iturralde-García RD, Castañé C, Riudavets J (2020) Biological control of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored chickpeas through the release of natural enemies. Biol Control 149: 104322

Jiménez JC, de la Fuente M, Ordás B, García Domínguez LE, Malvar RA (2017) Resistance categories to *Acanthoscelides obtectus* (Coleoptera: Bruchidae) in tepary bean (*Phaseolus acutifolius*), new sources of resistance for dry bean (*Phaseolus vulgaris*) breeding. Crop Prot 98:255-266

Jess S, Kilpatrick M (2000) An integrated approach to the control of *Lycoriella solani* (Diptera: Sciaridae) during production of the cultivated mushroom (*Agaricus bisporus*). Pest Manag Sci 56:477-485

JMP. 2016. JMP 13.1.0. SAS Institute Inc., Cary NC, 1998-2014

Messelink GJ, Van Steenpaal SE, Ramakers PM (2006) Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. BioControl 51(6):753-768

Mitsunaga T, Fujii K (1999) An experimental analysis of the relationship between species combination and community persistence. Res Popul Ecol 41:127-134

Niedermayer S, Steidle JLM (2013) The Hohenheimer box – A new way to rear and release *Lariophagus distinguendus* to control stored product pest insects. Biol Control 64:263-269

Nomikou M, Janssen A, Schraag R, Sabelis MW (2001) Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Exp Appl Acarol 25:271-291

Oliveira MRC, Corrêa AS, Souza GAd, Guedes RNC, Oliveira LOd (2013) Mesoamerican origin and pre- and post-Columbian expansions of the ranges of *Acanthoscelides obtectus* Say, a cosmopolitan insect pest of the common bean. PLoS ONE 8(7): e70039

Opit GP, Philipps TW, Aikins MJ, Hasan MM (2012) Phosphine resistance in *Tribolium castaneum* and *Rhyzopertha dominica* from stored wheat in Oklahoma. J Econ Entomol 105:1107-1114

Paul UV, Lossini JS, Edwards PJ, Hilbeck A (2009) Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. J Stored Prod Res 45:97-107

Pimentel MAG, Faroni LRD, Batista MD, da Silva FH (2008) Resistance of stored-product insects to phosphine. Pesqui Agropecu Bras 43:1671-1676

Riahi E, Fathipour Y, Talebi AA, Mehrabadi M (2017) Attempt to develop cost-effective rearing of *Amblyseius swirskii* (Acari: Phytoseiidae): Assessment of different artificial diets. J Econ Entomol 110(4):1525-1532

Riudavets J, Lucas E, Pons MJ (2002a) Insects and mites of stored products in the northeast of Spain. IOBC/WPRS Bull 25(3):41-44

Riudavets J, Maya M, Montserrat M (2002b) Predation by *Blattisocius tarsalis* (Acari: Ascidae) on stored product pests. IOBC/WPRS Bull 25(3):121-126

Sanon A, Ouedraogo AP, Tricault Y, Credland PF, Huignard J (1998) Biological control of bruchids in cowpea stores by release of *Dinarmus basalis* (Hymenoptera: Pteromalidae) adults. Environ Entomol 27:717-725

Sarwar M, Wu K, Xu X (2009) Evaluation of biological aspects of the predacious mite, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) due to prey changes using selected arthropods. Int J Acarol 35(6):503-509

Soares MA, Quintela ED, Mascarin GM, Arthurs SP (2015) Effect of temperature on the development and feeding behavior of *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) on dry bean (*Phaseolus vulgaris* L.). J Stored Prod Res 61:90-96

Solà M, Riudavets J, Castañé C 2020. Control of *Rhyzopertha dominica* and *Sitophilus zeamais* in stored rice with different release rates of the larval parasitoid *Anisopteromalus calandrae.* Entomol Gen, in press. https://doi.org/10.1127/entomologia/2020/0899

Southgate BJ (1979) Biology of the Bruchidae. Annu Rev Entomol 24:449-473

Subramanyam B, Hagstrum DW (1996) Integrated management of insects in stored products. Marcel Dekker Inc., New York

Velten G, Rott AS, Conde Petit BJ, Cardona C, Dorn S (2008) Improved bruchid management through favorable host plant traits and natural enemies. Biol Control 47:133-140

Wu S, Gao Y, Xu X, Wang E, Wang Y, Lei Z (2014) Evaluation of *Stratiolaelaos scimitus* and *Neoseiulus barkeri* for biological control of thrips on greenhouse cucumber. Biocontrol Sci Technol 24(10):1110-1121

Figure Caption

Fig. 1. Percentage of mortality (± SE) of bruchids when predatory mites and parasitoids were released, alone or in combination, in 100-g containers of beans. The predator-to-prey and parasitoid-to-host ratio was 1:15. a) *Z. subfasciatus*; b) *A. obtectus*. Values followed by a different lowercase letter are significantly different (Tukey test, $P < 0.05$).

Table 1. Percentage of survival and egg mortality (± SE) when 15 two-day-old *A. obtectus* or *Z. subfasciatus* eggs were offered to one female mite in a small cage (2.5 cm diameter) for 24 h at 28ºC and 80% or 75% RH.

Predatory mites	*Z. subfasciatus*		*A. obtectus*			
	80% RH		80% RH		75% RH	
	survival	Egg mortality	survival	Egg mortality	survival	Egg mortality
Control	-	25.17 ± 2.42b	-	20.51 ± 4.40b	-	19.58 ± 3.46b
B. tarsalis	100	31.75 ± 1.91ab	100	51.80 ± 2.39a	83.33 ± 16.67	45.73 ± 4.24a
A. swirskii	100	34.35 ± 2.84ab	100	40.51 ± 4.06a	90.00 ± 10.00	42.14 ± 1.85a
N. cucumeris	100	40.32 ± 3.43a	88.89 ± 11.11	40.83 ± 4.18a	0	33.50 ± 3.97a
S. scimitus	0	30.51 ± 3.88ab	10.00 ± 10.00	47.58 ± 1.92a	-	-
		$F_{4, 49} = 3.29$		$F_{4, 49} = 11.31$		$F_{3, 39} = 11.40$
		$P < 0.050$		$P < 0.001$		$P < 0.001$

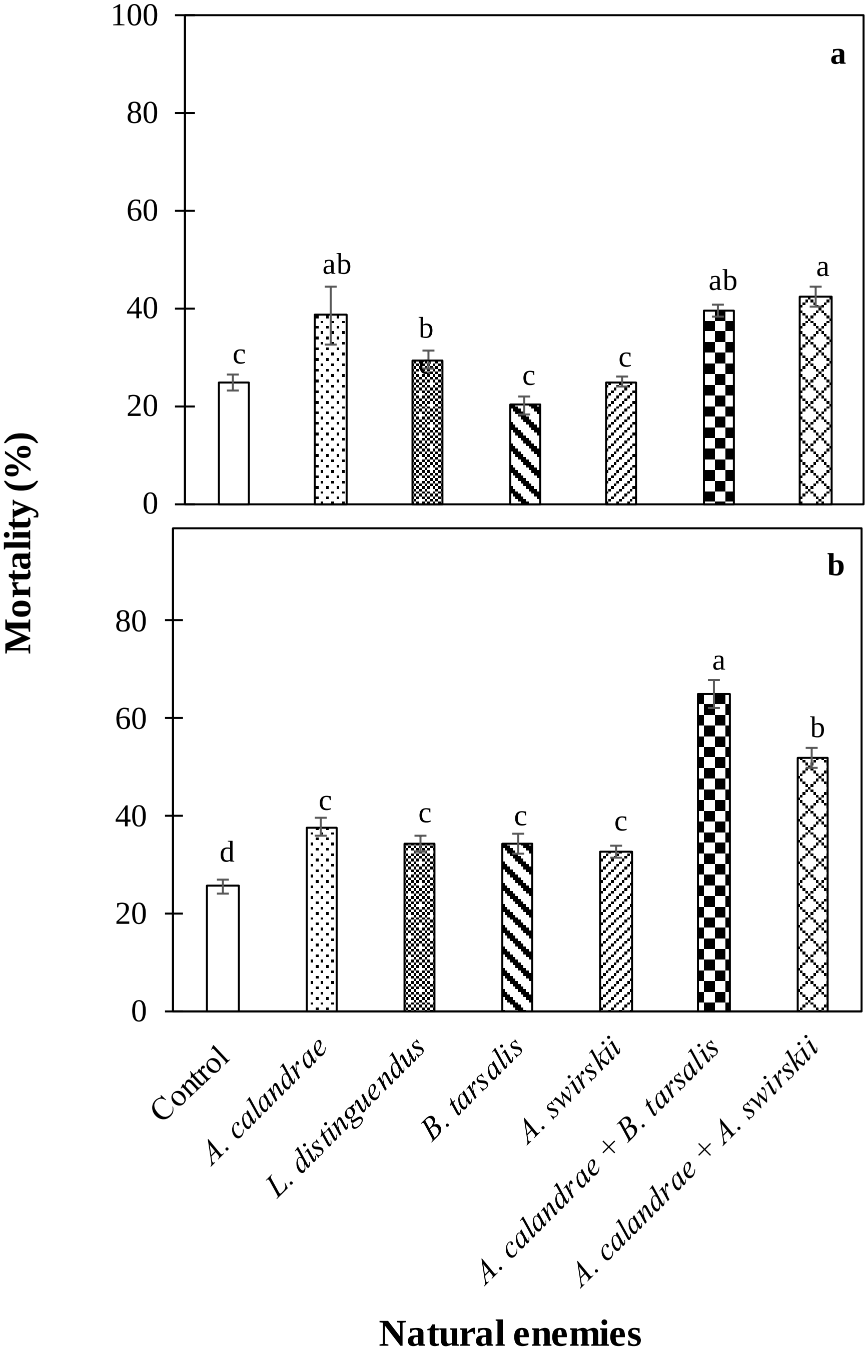
Average values in the same column followed by a different lowercase letter are significantly different (Tukey test, $P < 0.05$).

Table 2. Percentage of mortality (± SE) of *A. obtectus* when *B. tarsalis* or *A. swirskii* were released at two predator-to-prey ratios or when *B. tarsalis* was combined with *A. calandrae* in 40-cm PVC pipes filled with 12 kg of dried beans (28 ± 2ºC, 75 ± 5% RH), or when 9 ml of *B. tarsalis* enriched diet were released (90-180 individuals) in commercial bags containing 25 kg of dried beans (23.5 ± 1.5ºC, 68 ± 10% RH).

Treatments	Natural enemy-to-prey ratios	Experimental arena	Mortality (%)
Control	15:45	PVC pipes	26.11 ± 1.65d
Control	90:45	PVC pipes	25.33 ± 2.52d
Control	90-180:45	Commercial bags	26.29 ± 0.49d
A. swirskii	15:45	PVC pipes	41.55 ± 1.94c
A. swirskii	90:45	PVC pipes	60.77 ± 2.67b
B. tarsalis	15:45	PVC pipes	67.18 ± 3.51b
B. tarsalis	90:45	PVC pipes	65.80 ± 3.59b
B. tarsalis	90-180:45	Commercial bags	65.71 ± 4.15b
B. tarsalis + *A. calandrae*	90:45 + 1:15	PVC pipes	81.08 ± 3.43a
			$F_{8, 53} = 55.58$
			$P < 0.001$

Average values in the last column followed by a different lowercase letter are significantly different (Tukey test, $P < 0.05$)

370



a
b
Mortality (%)
100
80
60
40
20
0
c
ab
b
c
c
ab
a
d
c
c
c
c
a
b
Control
A. calandrae
L. distinguendus
B. tarsalis
A. swirskii
A. calandrae + B. tarsalis
A. calandrae + A. swirskii
Natural enemies

371

1. Acknowledgments

This research was funded by the Instituto Nacional de Investaciones Agrarias (INIA) of the Spanish Ministry of Economy and Competitiveness (grants RTA2014-00006-C02-01 and RTI2018-095526-R-100) and by the CERCA Program (Centres de Recerca de Catalunya, Spain) of the Generalitat de Catalunya. The first author is grateful to the Consejo Nacional de Ciencia y Tecnología (CONACYT México) for his scholarship. We thank the company AGROBIO S.L. for providing the predatory mites used in these experiments.

1. Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

Funding: This research was funded by the Instituto Nacional de Investaciones Agrarias (INIA) of the Spanish Ministry of Economy and Competitiveness (grants RTA2014-00006-C02-01 and RTI2018-095526-R-100) and by the CERCA Program (Centres de Recerca de Catalunya, Spain) of the Generalitat de Catalunya. The first author is grateful to the Consejo Nacional de Ciencia y Tecnología (CONACYT México) for his scholarship. We thank the company AGROBIO S.L. for providing the predatory mites used in these experiments.

This article does not contain any studies involving human participants and animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.