Biological control of *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) in stored chickpeas through the release of natural enemies

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Abstract

In this study, two predatory mites and two parasitoid wasps were evaluated for their effectiveness in controlling *Callosobruchus chinensis* (Coleoptera: Chrysomelidae), a common pest in stored chickpeas. The predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) preyed on the bruchid's eggs but did not consume a large amount; the mite *Blattisocius tarsalis* (Acari: Ascidae) did not consume *C. chinensis* eggs. However, the larval parasitoids *Anisopteromalus calandrae* and *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) were effective at reducing the bruchid's larval population, producing mortality rates above 90% in controlled conditions (28 ± 2ºC, 75 ± 5% relative humidity [RH]). In tubes of 20-cm diameter filled with 9 to 35 kg of chickpeas, both parasitoids were able to parasitize the host at depths of 40, 100, and 150 cm, even when larvae were offered simultaneously at all depths. This indicates that parasitoids will probably be able to locate hosts at least at 150 cm of distance in a storage facility. *A. calandrae* was similarly effective at reducing the bruchid population at different parasitoid-to-host ratios (1:7, 1:15, 1:30, and 1:60). Moreover, *A. calandrae* efficiently reduced *C. chinensis* populations when released in 25-kg commercial polypropylene bags of chickpeas in simulated warehouse conditions (27 ± 2ºC and 65 ± 4% RH). This is the first time that *A. calandrae* and *L. distinguendus* are shown to be effective biological control agents for the integrated management of *C. chinensis* in stored chickpeas and can be an alternative to the application of pesticides for maintaining low bruchid population levels.

Key words: bruchids; predatory mites; larval parasitoids; legumes; stored products.

1. Introduction

The adzuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae), is a primary pest of stored chickpeas (USDA, 2004). Adults attach eggs to the surface of the pulse, and larvae develop concealed inside the pulse. *C. chinensis* can build significant populations within a short period of time (Islam and Kabir, 1995) and can inflict serious damage on chickpeas, including loss of mass, product contamination, and decreased seed germination. Currently, a limited number of highly toxic pesticides are used to control this pest during storage (Iturralde-García et al., 2016; Daglish et al., 2018). However, interest in sustainable alternative control methods is increasing, and biological control could offer an effective alternative for preventing insect populations from reaching pest status (Riudavets, 2018). Natural enemies such as parasitoids and predators have many advantages over chemical control. They leave no toxic residues on the stored commodities, populations cannot develop resistance to them, they are safe for workers and the environment, and their use has been proven to be economically feasible for controlling several pest species (van Lenteren et al., 2020; Riudavets et al. 2020). In addition, storage facilities are suited to the use of biological control since they are closed environments from which natural enemies cannot escape (Schöller and Flinn, 2000; Riudavets, 2018). The environmental conditions in storage facilities are also more stable than those in open fields or greenhouses, where biological control has been widely adopted. The present study evaluated the effectiveness of two types of natural enemies for controlling *C. chinensis*. Two species of predatory mites that prey on eggs, *Blattisocius tarsalis* (Berlese) (Acari: Ascidae) and *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae), were evaluated, along with two larval parasitoids, *Anisopteromalus calandrae* Howard and *Lariophagus distinguendus* Förster (Hymenoptera: Pteromalidae).

Many predatory mites are easy to raise, cheap to purchase, and highly voracious; they are widely used in augmentative biological control strategies (Van Lenteren, 2012; Riudavets et al., 2020). *Blattisocius tarsalis* is a cosmopolitan species that is commonly found in food-storage habitats; it can feed on several Acari, Psocoptera, Lepidoptera, and Coleoptera species (Haines, 1974, 1981a, 1981b; Nielsen, 1999; Riudavets et al., 2002; Stejskal et al., 2005;Thind and Ford, 2006; Gallego et al., 2020). *Amblyseius swirskii* is effective for control of different horticultural pests, including whiteflies, thrips, eriophyid mites, spider mites, and broad mites (Gerson and Weintraub, 2007; Chow et al., 2010; Park et al., 2011; Onzo et al., 2012; Buitenhuis et al., 2014; Soleymani et al., 2016). In addition, *A. swirskii* preys on the eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Riahi et al., 2017). *A. calandrae* and *L. distinguendus* are solitary ectoparasitoids that attack the larvae and pupae of stored-product pests that develop concealed within the host substrate (Menon et al., 2002; Ghimire and Phillips, 2007; Niedermayer et al., 2016). These parasitoids are cosmopolitan; they commonly attack several coleopteran species such as *Sitophilus oryzae* (L.), *S. granarius* (L.), *S. zeamais* (Motschulsky) and *Rhyzopertha dominica* (F.) (van den Assen et al., 1984; Wen and Brower, 1994; Steidle and Scöller, 2002; Adarkwah et al., 2012; Belda and Riudavets, 2013; Castañé and Riudavets, 2015). Both parasitoids have been reported to attack the larvae and pupae of *Callosobruchus* species (Onodera et al., 2002; Ghimire and Philips, 2007).

The general objective of this study was to test the effectiveness of biological control for maintaining a low *C. chinensis* population. We hypothesized that the release of natural enemies would maintain the bruchid population below 10% that of the control. We therefore evaluated the efficacy of four natural enemies in reducing the pest's population growth and the ability of the two parasitoids to penetrate a pile of chickpeas to different depths. We also tested different parasitoid-to-host ratios with *A. calandrae*, and we evaluated the efficacy of *A. calandrae* in controlling pest populations in commercial polypropylene bags of chickpeas.

2. Materials and Methods

2.1. Insect and mite colonies. Colonies were maintained and experiments performed at a constant temperature of 28 ± 2ºC, 75 ± 5% RH and a photoperiod of 16 hours of light to 8 hours of darkness. Stock colonies of all insects were initiated with samples collected in warehouses located in Spain. To raise *C. chinensis*, 100 unsexed adults were placed in 710 ml plastic containers with 200 g of chickpeas every week (cv. Blanco Lechoso) to obtain weevils of known ages. *Anisopteromalus calandrae* and *L. distinguendus* colonies were started with individuals collected from stored products companies in the north-east of Spain. Parasitoids were reared by offering *C. chinensis* larvae (aged 8 to 14 days) to newly emerged adults. Two plastic tubes, each containing sugary water and a cotton plug, were supplied as additional food. After three weeks, a new generation of adults was available for experiments. Predatory mites, *B. tarsalis* and *A. swirskii*, were supplied by Agrobio SL (Almería, Spain).

2.2. Effectiveness of predatory mites and larval parasitoids in suppressing *C. chinensis*. To test the predatory capabilities of *B. tarsalis* and *A. swirskii* simulating a continuous infestation of the bruchid, a total of 45 *C. chinensis* eggs were offered to 15 females over three weeks as follows: chickpeas with 15 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 for every three *C. chinensis* eggs, since a consumption rate of 1-3 eggs per female per day was documented in Riudavets et al. (2002). To test *A. calandrae* and *L. distinguendus*, chickpeas containing *C. chinensis* eggs were introduced each week for three weeks; the developing larvae were offered to three pairs of adults that were released on the third week. The development of bruchid instar larvae were determined according to Hosamani et al. (2018). A total of 45 individuals was introduced with a proportion of one female parasitoid (0 to 7 days old) for 15 hosts. A plastic tube containing sugary water and a cotton plug was also used to provide additional food. After three additional weeks, the number of weevils and/or parasitoids was evaluated weekly. Plastic 710-mL containers containing 100 g of chickpeas were used for all experiments. Ten replicates were conducted for each predatory mite and parasitoid species and for the untreated control.

2.3. Dispersal ability of the parasitoids at different depths. To assess the ability of *A. calandrae* and *L. distinguendus* to locate its host in a vertical arena, *C. chinensis* larvae were offered at the bottom of polyvinyl chloride (PVC) pipes measuring 40, 100, and 150 cm high (20 cm internal diameter). These pipes were filled to the top with chickpeas (containing a total of 9.3, 23.2, and 34.9 kg of chickpeas, respectively). A stainless steel screened cage (7 cm high, 5 cm internal diameter) containing 60 g of chickpeas infested with 15 two-day-old eggs, 15 first-instar larvae, and 15 third-instar larvae of *C. chinensis* (a total of 45 individuals) was located at the bottom of each PVC pipe. An additional treatment was tested with the tallest pipe (150 cm); three infested cages were placed in that pipe at depths of 40, 100, and 150 cm. Each cage contained 60 g of chickpeas infested with

5 two-day-old eggs, 5 first-instar larvae, and 5 third-instar larvae for a total of 15 individual *C. chinensis* instars per cage and 45 individual *C. chinensis* instars per pipe. Next, three pairs of *A. calandrae* or *L. distinguendus* adults (0 to 7 days old) were released on the surface of the grain. A tube containing sugary water was also placed on the surface, and the pipes were sealed with fabric mesh. After a week, the PVC pipes were poured off; the parasitoids were removed, and the screened cages containing infested chickpeas were isolated in plastic containers. The emergence of adult *C. chinensis* and/or parasitoids was then recorded. Six replicates were conducted for each parasitoid species and pipe height. For the control group, plastic 710-mL containers containing 60 g of chickpeas infested with 15 two-day-old eggs, 15 first-instar larvae, and 15 third-instar larvae of *C. chinensis* (a total 45 individuals) were located outside the PVC pipes. The experiment was replicated six times.

2.4. Effectiveness of *A. calandrae* at different host ratios. To assess the efficacy of *A. calandrae* at different host ratios, an increasing number of hosts were offered to a fixed number of parasitoids. Forty-centimeter PVC pipes were used for this experiment, which followed a similar methodology as that described in the previous section: Infested screened cages were deposited at the bottom of the pipe. The parasitoid-to-host ratios were tested. Hosts were offered at ratios of 1:7 (7 eggs, 7 first-instar larvae, and 7 third-instar larvae), 1:30 (30 eggs, 30 first-instar larvae, and 30 third-instar larvae) and 1:60 (60 eggs, 60 first-instar larvae, and 60 third-instar larvae), for a total 21, 90, and 180 *C. chinensis* individuals offered at each ratio. Three pairs of *A. calandrae* were released at the surface of the grain. Six replicates were conducted with each ratio; for the control group, plastic 710-mL containers containing 60 g of chickpeas infested with a total 21, 90, and 180 *C. chinensis* individuals (eggs, first-instar larvae, and third-instar larvae) were located outside the PVC pipes. Six replicates were also conducted. The results of the previous experiment with 40-cm PVC pipes (single depth) and a 1:15 parasitoid-to-host ratio were also compared to those of this experiment. Fifty *C. chinensis* individuals and 50 *A. calandrae* individuals (with three replicates) were weighed to estimate the individual mass for each species; this number was multiplied by the number of adults that emerged to determinate the biomass of both species in each replicate.

2.5. Effectiveness of *A. calandrae* in commercial chickpeas bags. To assess the ability of *A. calandrae* to locate its host in commercial woven polypropylene bags (42 x 66 cm, 25 kg of chickpeas) a test was done in an experimental storage facility at ambient temperature and humidity (27 ± 2ºC and 65 ± 4% RH). One infested screened cage, similar to those previously described, was placed at one end of a bag. The cage contained 30 two-day-old eggs, 30 first-instar larvae, and 30 third-instar larvae of *C. chinensis* in 60 g of chickpeas. At the opposite end of the bag, three pairs of *A. calandrae* (1:30 parasitoid: host ratio) were released; one tube of sugary water was also placed there. One polypropylene bag was placed on the floor of each empty room (3 x 2 m). After one week, the bags were opened, and screened cages containing the infested chickpeas were placed in a climatic chamber (28 ± 2ºC; 75 ± 5% RH; 16 h: 8 h light: dark) to develop. Over the following weeks, the number of *C. chinensis* and *A. calandrae* that emerged were counted. Six replicates were conducted, each in a different room. A control treatment with plastic 710-mL containers containing 60 g of chickpeas infested with a total 90 individuals of *C. chinensis* but without parasitoids was also conducted. Six replicates were done.

2.6. Data analysis. Data normality was analyzed using the Shapiro–Wilk test. The following data sets were analyzed using a one-way analysis of variance (ANOVA): a) the number of *C. chinensis* and parasitoid progeny obtained when predatory mites or parasitoids were released; b) the percentage by which the number of *C. chinensis* and *A. calandrae* were reduced and the biomass of these species at different parasitoid-to-host ratios; and c) the number of *C. chinensis* that emerged when *A. calandrae* were released in commercial bags. Two-way ANOVAs were conducted to analyze the number of *C. chinensis* and parasitoids that emerged in the experiments testing the parasitoids' ability to find the host at different depths. Two-way ANOVAs were also conducted to analyze the number and biomass of *C. chinensis* and the parasitoids at different parasitoid-to-host ratios. Post-hoc comparisons were carried out with Tukey correction for multiple comparisons. The proportions of *A. calandrae* females emerging from *C. chinensis* at different depths, at different parasitoid-to-host ratios, and from commercial bags were determined by a Student's t-test. All statistical analyses were conducted with JMP (JMP 8.0.1, 2009, SAS Institute, Inc).

3. Results

3.1. Effectiveness of predatory mites and larval parasitoids in suppressing *C. chinensis*. The predatory mite *A. swirskii* and the parasitoids *A. calandrae* and *L. distinguendus* significantly reduced the progeny of *C. chinensis* compared to the control treatment. Only the treatment with the predatory mite *B. tarsalis* did not show any significant differences with the control treatment (Table 1). Both parasitoid species were much more effective than the predatory mites in reducing the number of emerged *C. chinensis* adults; a mean number of zero or nearly zero *C. chinensis* emerged in the treatments with parasitoids; with the mite treatments, many more emerged. Of the 30 parasitoid pairs introduced in the ten replicates, most were recovered alive after one week of exposure (around 92% for both parasitoid species). *A. calandrae* and *L. distinguendus* were similarly effective; the mean number of emerged *C. chinensis* did not differ significantly between them (Table 1). There were no significant differences in the proportion of *A. calandrae* females and males that emerged ($t = 0.36$, $P = 0.727$); however, for *L. distinguendus*, significantly more females emerged ($t = 12.54$, $P < 0.001$) (Table 1).

3.2. <u>Dispersal ability of the parasitoids at different depths.</u> A mean of 41.3 ± 0.8 *C. chinensis* emerged in the control treatment, indicating natural mortality of 8.2%. Both parasitoids were able to reduce the emergence of *C. chinensis* at all depths tested, leading to total mortality over 90%. *A. calandrae* and *L. distinguendus* were similarly effective at locating hosts at the three depths tested; there were no significant differences among the numbers of *C. chinensis* that emerged from the samples at different depths (Tables 2 and 3). Furthermore, both parasitoids were similarly effective at suppressing *C. chinensis* whether the same number of hosts was offered at a specific depth or spread across three different depths, indicating that the adult parasitoids had no difficulty finding the hosts under 1.5 m of chickpeas.

Significantly more *A. calandrae* adults than *L. distinguendus* adults emerged at the three depths levels tested, but not at a mixed depth. In addition, more female *A. calandrae* progeny emerged at one depth and in the mixed combination of depths; more female *L. distinguendus* progeny emerged only at one depth (Tables 2 and 3).

Successful parasitism, the emergence of adult parasitoids, accounted only for 12.6% to 36.0% host mortality with both parasitoid species. Other causes, such as natural host mortality, host feeding by adult parasitoids, immature mortality of parasitoid progeny, or a combination of these, also contributed to the total reduction in host emergence.

3.3. <u>Effectiveness of *A. calandrae* at different host ratios.</u> *A. calandrae* was able to reduce the emergence of *C. chinensis* adults and to reproduce in all tested parasitoid-to-host ratios (Table 4). As expected, the mean number of *C. chinensis* that emerged in the control groups significantly increased as the initial number of hosts released increased, ranging from 17.0 to 148.7 adults for the 1:7 to 1:60 ratio treatments. When *A. calandrae* was released, the total number of both *C. chinensis* and *A. calandrae* that emerged increased significantly as the parasitoid-to-host ratios were reduced (Figure 1A). In the parasitoid treatment, *C. chinensis* emergence ranged from 0.7 to 17.8 adults, and *A. calandrae* emergence ranged from 1.8 to 47.8 at the 1:7 and 1:60 ratios, respectively. Furthermore, as the parasitoid-to-host ratio decreased, the bias toward female progeny increased (Table 5).

The emergence of adult parasitoids from a commodity can be perceived as a contaminant similar to the pest, even though the parasitoid control strategy resulted in a significant reduction in the total number of insects (pests plus parasitoids) contaminating the commodity compared to the untreated control. However, parasitoids are usually smaller than their hosts, resulting in a smaller total insect biomass. As expected, there were significant differences in the biomass of emerged adults in the control and in groups in which the parasitoid was released at different parasitoid-to-host ratios (Table 4, Figure 1B). The total biomass reductions in the treated groups were significantly greater than the reductions in the number of emerging adults achieved by decreasing the parasitoid-to-host ratios (Table 6).

3.4. Effectiveness of *A. calandrae* in commercial chickpeas bags. Significantly fewer *C. chinensis* and *A. calandrae* emerged in total when *A. calandrae* was released than in the control (76.2 ± 1.97 versus 29.5 ± 1.34) ($F = 383.56$; df = 1, 11; $P < 0.001$). When contamination is expressed in terms of insect biomass rather than the number of insects, contamination was significantly lower with the parasitoid treatment than in the control (67.0 ± 1.73 mg versus 9.4 ± 1.09 mg) ($F = 789.80$; df = 1, 11; $P < 0.001$) (Figure 2). The parasitoid progeny were female-biased (75.4 ± 3.46%; $t = 7.36$, $P < 0.001$), as already observed in the parasitoid-to-host ratios experiment.

4. Discussion

Neither *B. tarsalis* nor *A. swirskii*, the two predatory mites tested here, have been previously reported to attack *C. chinensis* eggs. Although *B. tarsalis* did not effectively reduce *C. chinensis* in our experiment, it has been reported to prey on other bruchid eggs such as *Acanthoscelides obtectus* (Say) (Riudavets et al., 2002). While *A. swirskii* was able to prey on *C. chinensis* to some extent, its control efficacy was low. The low or null effectiveness of predatory mites may be because *C. chinensis* attach their eggs to the surface of the chickpeas instead of leaving them loose to mix with the debris at the bottom of the container like other storage pests. Nevertheless, the predatory mite *Cheyletus eruditus* (Schrank) prey on eggs of *C. maculatus* (Fab.) that are also attached to the pulse (CABI, 2020). For this reason, further studies may determine whether these predatory mites can still positively contribute to the biological control of this pest species.

The two parasitoids tested were very effective at controlling *C. chinensis* populations: *A. calandrae* eliminated the weevil population, and *L. distinguendus* eliminated a mean of 44.7 individuals from an initial population of 45 individuals (from first-instar larvae to pupae) in our first experiment. A single *A. calandrae* female can kill about 20 *C. maculatus* final-instar larvae during a 24-hour period (Ghimire and Phillips, 2007); a pair of *L. distinguendus* can kill between 12 to 20 *C. chinensis* individuals or 10 to 15 *C. maculatus*, depending on the developmental stage, over 24 hours (Bellows, 1985). We released three pairs of *A. calandrae* or *L. distinguendus* for a longer period (at least one week), which explains the effectiveness we observed.

The sex ratios of *A. calandrae* progeny were balanced, but those of *L. distinguendus* were female-biased in the experiment with small arenas of 100g of chickpeas. This could be because the former exhausted the available hosts while the latter did not. Females of both species use large larvae to oviposit female eggs and smaller larvae to oviposit male eggs and for feeding (Choi et al., 2001; Lebreton et al., 2009). Therefore, the higher activity of *A. calandrae* females may have superparasitized large larvae with female eggs from which only one adult would emerge (Benkhellat et al., 2015); with *L. distinguendus*, large larvae may have been less superparasitized (Wen and Brower 1995). Nevertheless, in the experiment with larger arenas (9 to 45 kg of chickpeas) the progeny of both parasitoids had approximately similar sex ratios.

Both parasitoids were similarly effective (96-99% host mortality) at penetrating a chickpea column 40, 100, or 150 cm deep to find host larvae, even when larvae were offered at mixed depths (Table 2). Both parasitoids seemed to be able to move freely within the chickpea pile, probably because the interstitial spaces between pulses are larger than those in smaller grains such as wheat and maize. Odours emited by the hosts will be more diluted when infested pulses are stored in a flat room than in the larger pipes of this study (with a volume of 0.047 m^3). However, the results of the woven propylene bags confirmed those of the pipes, and in that case host odor was not confined. Therefore, parasitoids will probably be able to locate hosts at least at 150 cm of distance in a storage facility. *A. calandrae* can locate and parasitize *S. oryzae* in a wheat column 220 cm deep, but they have low downward mobility, and, in a previous study, it was necessary to increase the number of parasitoids to adequately suppress *S. oryzae* (Press, 1988). *L. distinguendus* can penetrate 100 cm of maize or 4 m of wheat to locate *S. zeamais* and *S. granarius*, respectively. However, suppression is reduced from 74% to 34% at depths deeper than 90 cm (Steidle and Schöller, 2002; Adarkwah et al., 2012). These authors concluded that the density of parasitoids per unit volume of grain might significantly affect how deeply the parasitoids penetrate the grain.

In the present study, *A. calandrae* effectively controlled *C. chinensis* very well at all parasitoid-to-host ratios tested, achieving more than 90% reduction of host emergence at a ratio of one parasitoid to 60 hosts, the lowest ratio tested. These results are in line with other studies. For example, *A. calandrae* effected 90% suppression of *S. oryzae* in wheat at parasitoid-to-host ratios of 1:15 and 1:20 (Press et al., 1984), and the parasitoid *Dinarmus basalis* (Rond.) achieved 78% suppression of *C. chinensis* in red lentils at a parasitoid-to-host ratio of 1:60 (Islam and Kabir, 1995). While F1 progeny of *A. calandrae* were low at the highest ratio tested (1:7) and had a male-biased sex ratio, progeny increased at the lowest ratio (1:60) and were female-biased. A similar trend has been reported for *D. basalis* with *C. chinensis* (Islam and Kabir, 1995). These differences in reproduction could be attributed to superparasitism that is documented occurs at high ratios (Wen and Brower, 1995).

Although *A. calandrae* females recognize hosts that they have parasitized, they cannot identify hosts parasitized by other females (Benkhellat et al., 2015), and the probability that a female will lay eggs on a host parasitized by another female increases with elevated parasitoid-to-host ratios. When several eggs are deposited on a single host larvae, no more than one parasitoid reaches maturity, resulting in a lower total number of offspring (Choi et al., 2008). Therefore, when the parasitoid-to-host ratio is high, either because of low host availability or a high number of parasitoids, competition among female parasitoids leads to high host mortality and decreased parasitoid offspring (Vinson and Iwantsch, 1980). When the females also feed on hosts, a behavior common to many parasitoids, this further reduces the number of hosts available for reproduction (Bellows, 1985).

While the number of *A. calandrae* progeny increased when the parasitoid-to-host ratios were reduced, the emergence of *C. chinensis* decreased, both in terms of number of individuals and of biomass. However, this reduction in host emergence was significantly higher when expressed as biomass than when expressed as the number of individuals, particularly at low parasitoid-to-host ratios (Table 6, Figure 1A and B). This indicates that merely counting the number of individuals in the grain would lead to an overestimation of the insect contamination at the end of this process. Although adult parasitoids can also be a source of grain contamination, the total insect biomass is significantly reduced by parasitoid treatment.

This parasitoid demonstrated good potential for controlling *C. chinensis* in warehouse conditions since we observed similarly low final contamination (in terms of insect biomass) under warehouse conditions as in PVC pipes with the same parasitoid-to-host ratios but under controlled conditions. *A. calandrae* suppression of *C. chinensis* was high (93%) in woven propylene bags, even though the parasitoid’s movement is more restricted in packaged commodities than in bulk grain (Press and Mullen, 1992).

In summary, under the conditions tested, parasitoid wasps alone show great potential for controlling *C. chinensis*; our hypothesis was confirmed as the bruchid population in the experimental groups was maintained at less than 10% of that in the control groups. These results indicate that *A. calandrae* and *L. distinguendus* are effective biological control agents for the integrated management of *C. chinensis* in stored chickpeas and can represent an alternative to pesticides for maintaining low bruchid population levels. Our study also suggests that the predatory mite *A. swirskii* could play a role in the control of *C. chinensis*, alone or in combination with other natural enemies, and deserves further investigation.

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Authors declare that they have No competing interests.

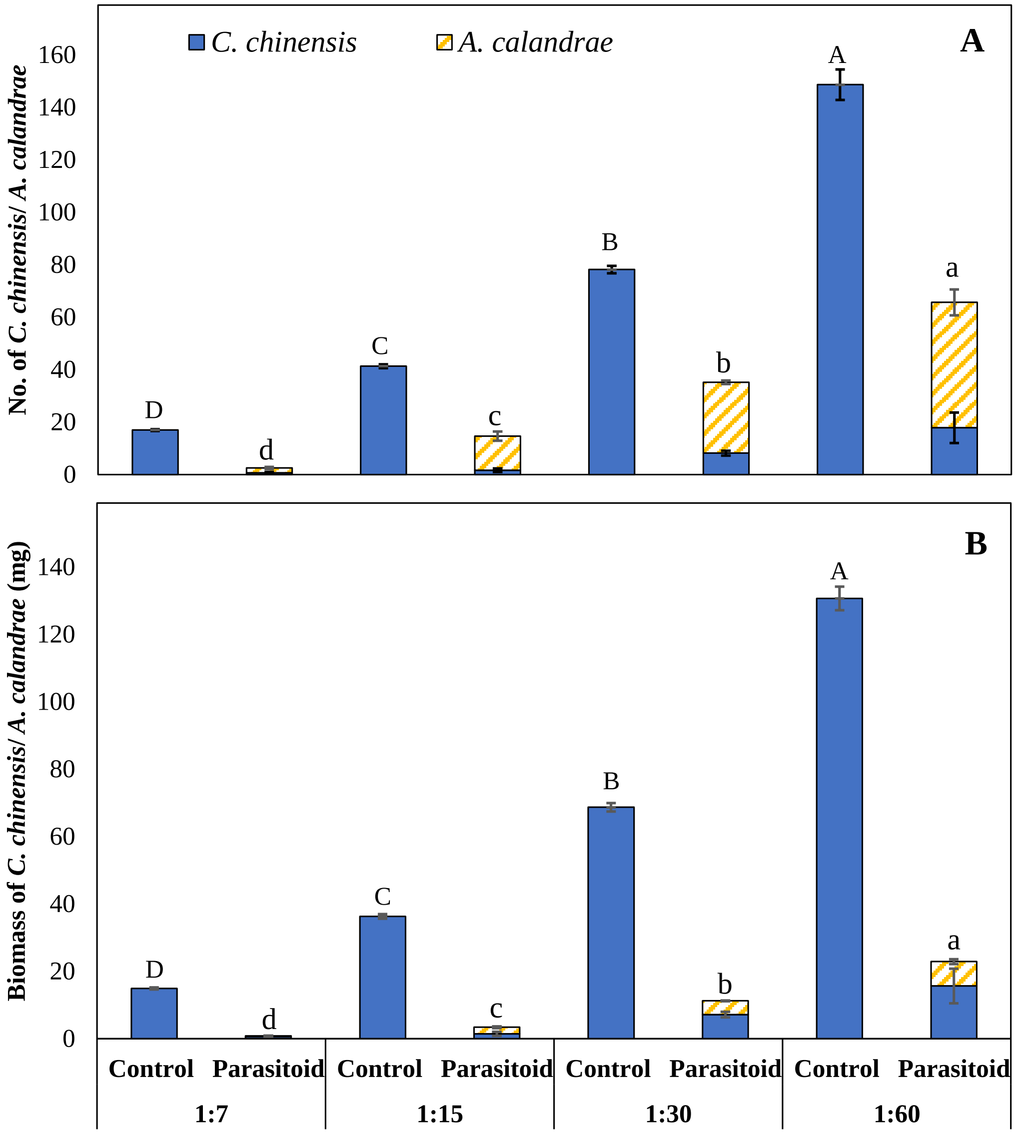

Figure 1. Mean (± SEM) number (A) and biomass (B) of *C. chinensis* in the control and of *C. chinensis* and *A. calandrae* in the treatment in which the parasitoid was released at different parasitoid-to-host ratios in 40-cm PVC pipes filled with chickpeas (n = 6). The number and biomass of emerged *C. chinensis* at different parasitoid-to-host ratios in the control followed by the same uppercase letter do not differ significantly. The number and biomass of emerged *C. chinensis* and *A. calandrae* at different parasitoid-to-host ratios followed by the same lowercase letter do not differ significantly (Tuckey test, $P < 0.05$).

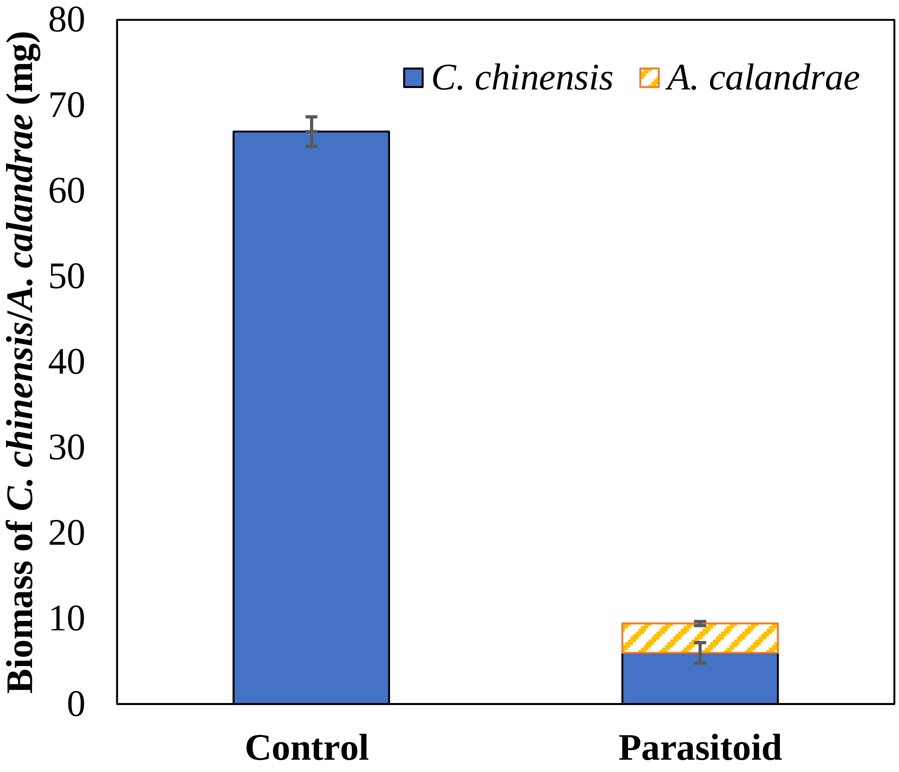

Figure 2. Mean (± SEM) biomass (mg) of C. chinensis in the control and of C. chinensis and A. calandrae when the parasitoid was released into commercial bags of chickpeas (n = 6). There were significant differences between the parasitoid treatment and the control (Student's t-test).

Table 1. Mean (± SEM) number of *C. chinensis*, *A. calandrae*, or *L. distinguendus* that emerged in the treatments with predatory mites, with larval parasitoids, and in the control (no predators or parasitoids were released). The sex ratio of the emerged parasitoids is also shown (n = 10).

Treatments	No. of emerged adults		
	C. chinensis	Parasitoids	Female parasitoids (%)
Control	36.3 ± 0.70 a	-	-
B. tarsalis	33.2 ± 1.15 ab	-	-
A. swirskii	30.1 ± 1.07 b	-	-
A. calandrae	0 c	12.2 ± 1.09 a	52.4 ± 6.71
L. distinguendus	0.3 ± 0.21 c	14.6 ± 0.75 a	76.9 ± 2.15 *
	$F_{4,\,49} = 552.75$; $P < 0.001$	$F_{1,\,19} = 3.28$; $P = 0.087$	

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

* Denotes significant deviation from 50% (Student's t-test)

Table 2. Statistical parameters of the two-way analysis of variance (ANOVA) for the variables “number of hosts” and “number of parasitoids” that emerged when *C. chinensis* larvae were offered to adult *A. calandrae* or *L. distinguendus* (factor one) at three depths (40, 100, and 150 cm) (factor two) in a pile of chickpeas.

Factors	No. of emerged *C .chinensis*		No. of emerged parasitoids	
	F	*P*	*F*	*P*
Parasitoid species	3.79	0.059	18.91	< 0.001
Depth	2.44	0.079	1.38	0.261
Parasitoid species x Depth	0.43	0.732	2.43	0.080

Degrees of freedom: Parasitoid species: 1; Depth: 3; Parasitoid species x Depth: 3

Table 3. Mean (± SEM) number of adult *C. chinensis*, *A. calandrae*, and *L. distinguendus* that emerged, with parasitoid sex ratios, when host larvae were offered at three depth levels (40, 100, and 150 cm) in a pile of chickpeas (n = 6).

Treatments		No. of emerged adults		Female parasitoids (%)	Student's t-test	
		C. chinensis	Parasitoids		*t*	*P*
A. calandrae	40 cm	1.7 ± 0.71	13.0 ± 1.75	62.8 ± 5.39	2.38	0.063
	100 cm	3.0 ± 1.10	16.2 ± 2.52	72.4 ± 4.27*	5.26	< 0.050
	150 cm	2.5 ± 0.81	16.2 ± 1.01	60.7 ± 8.57	1.25	0.268
	Mixed depths	4.3 ± 0.65	12.2 ± 0.87	66.4 ± 5.35*	3.06	< 0.050
L. distinguendus	40 cm	1.3 ± 1.45	5.7 ± 1.54	47.5 ± 12.21	-0.21	0.844
	100 cm	0.5 ± 0.61	7.0 ± 3.31	74.2 ± 6.26*	3.87	< 0.050
	150 cm	0.8 ± 0.22	6.8 ± 1.76	60.4 ± 13.42	0.78	0.472
	Mixed depths	3.3 ± 1.63	12.3 ± 0.55	60.7 ± 5.55	1.93	0.112

* Denotes significant deviation from 50% (Student's t-test)

Table 4. Statistical parameters of the two-way analysis of variances (ANOVA) for the variables “number of emerging adults (host and parasitoids)” and their “biomass”; Comparison of control and parasitoid treatments (factor one) when *A. calandrae* was released at four parasitoid-to-host ratios (factor two) in a 40-cm pile of chickpeas.

Factors	No. of emerged adults		Biomass	
	F	*P*	*F*	*P*
Treatment	793.51	< 0.001	1245.33	< 0.001
Parasitoid: host ratio	820.20	< 0.001	402.43	< 0.001
Treat x Par: host ratio	101.21	< 0.001	182.11	< 0.001

Degrees of freedom: Treatment: 1; Parasitoid-to-host ratio: 3; Treatment x Parasitoid-to-host ratio: 3

Table 5. Mean percentage (± SEM) of *A. calandrae* female progeny when it was released at four parasitoid-to-host ratios in 40-cm PVC pipes filled with chickpeas.

Host ratio	Females (%)	Student's t-test	
		t	*P*
1:7	36.1 ± 11.72	-1.18	0.289
1:15	62.8 ± 5.39	2.38	0.063
1:30	64.3 ± 3.35*	4.28	< 0.010
1:60	73.2 ± 1.93*	12.02	< 0.001

* Denotes significant deviation from 50% (Student's t-test)

Table 6. Mean (± SEM) percentage of reductions in the total number of emerged adults (*C. chinensis* + *A. calandrae*) and in the total biomass compared to the control, when the parasitoid was released in four parasitoid-to-host ratios in 40-cm PVC pipes filled with chickpeas (n = 6).

Parasitoid: host ratio	Percentage of reduction		Statistical parameters	
	No. of emerged adults	Biomass	*F*	*P*
1:7	85.3 ± 3.64	94.2 ± 2.16	4.45	0.061
1:15	64.5 ± 4.13	90.6 ± 1.69	34.11	< 0.001
1:30	65.5 ± 2.14	83.6 ± 3.19	134.26	< 0.001
1:60	55.83 ± 2.26	82.5 ± 3.47	41.36	< 0.001

Degrees of freedom: 1