



Effectiveness of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) in the control of *Sitophilus zeamais* and *Rhyzopertha dominica* in paddy rice

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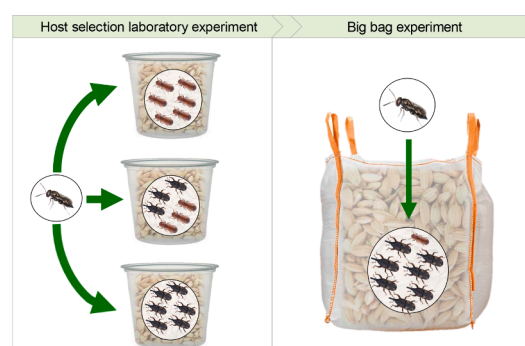
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HIGHLIGHTS

- *A. calandrae* preferred *S. zeamais* to *R. dominica* larvae when both pests were mixed.
- *R. dominica* did not interfere in *A. calandrae* control efficacy of *S. zeamais*.
- *A. calandrae* could suppress *S. zeamais* in the bottom of 500 kg bags of paddy rice.

GRAPHICAL ABSTRACT



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ABSTRACT

Rice is an important cereal crop in Spain that is frequently attacked by pests that cause significant quantitative and qualitative losses. Among them, the maize weevil, *Sitophilus zeamais*, is the key pest, and the lesser grain borer, *Rhyzopertha dominica*, is a serious pest found later in the season. The cosmopolitan ectoparasitoid, *Anisopteromalus calandrae*, which attacks late-instar larvae of coleopterans that develop inside grain kernels, has been reported as an efficient natural enemy. In this study, we evaluated whether the control efficacy of this parasitoid on *S. zeamais* was affected by the presence of the alternative host, *R. dominica*. Our laboratory experiment demonstrated the parasitoid preference for *S. zeamais* larvae when mixed with *R. dominica* larvae at different proportions. This preference for the maize weevil larvae allowed us to maintain a similar control efficacy of *S. zeamais* when it was offered alone than when it was offered together with *R. dominica* larvae. In our second experiment, done in 500 kg bags of paddy rice, similar results were obtained, since the presence of *R. dominica* larvae did not alter the control efficacy of *A. calandrae* on *S. zeamais* larvae. Therefore, our results

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confirmed a good perspective for implementing the use of this natural enemy as a control tool in big bags of paddy rice.

1. Introduction

Pest management to control stored-product insects currently relies on the widespread use of conventional insecticides, particularly in warmer climates. The few authorized synthetic insecticides and their excessive use have led to an increase in pesticide resistance, resulting in a lack of efficacy (Corrêa et al., 2011; Kavallieratos et al., 2015). In addition, there is growing social concern about environmental pollution and human health problems from insecticides on the grains. Therefore, it is necessary to develop adequate alternative control measures, among which biological control is a suitable option. The use of natural enemies has been reported as an effective method to control and prevent insect populations from reaching pest status, especially in closed environments, such as storage facilities (Riudavets, 2018; Schöller and Flinn, 2000).

Rice is an important cereal crop in Spain, with around 800,000 tons produced in recent years (FAOSTATS, 2020). When stored, it is frequently attacked by a range of insect pests that cause important quantitative and qualitative losses. Among them, the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is the most concerning species present in rice facilities in the northeast of Spain, as it happens in the whole Mediterranean region (Adler et al. 2022; Carvalho et al., 2013; Pascual-Villalobos et al., 2006; Riudavets et al., 2002; Trematerra et al., 2004). The damage produced in kernels enables other species to infest the grain and cause additional damage. In the northeast of Spain, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), has been reported as a serious pest of stored grain (Castañé and Riudavets, 2015; Trematerra et al., 2000). Since this species is more thermophilic than *Sitophilus* spp. (Hagstrum and Milliken, 1988), it is frequently found later in the season coexisting with *S. zeamais* but in lower abundance (personal observations). However, the presence of *R. dominica* in stored grain facilities is increasing due to the rising temperatures caused by climate change (Adler et al., 2022). *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) is a cosmopolitan and solitary ectoparasitoid that attacks late-instar larvae of coleopterans that develop concealed inside grain kernels, such as those of *S. zeamais* and *R. dominica* (Smith, 1993; Smith and Press, 1992). The female maize weevil feeds on the kernel, lays an egg inside it, and then seals the hole. The larva feeds inside the seed, pupates, and emerges as an adult (Longstaff, 1981). In contrast, *R. dominica* females lay their eggs loosely in the grain, and the larvae bore into a kernel near the germ to feed, pupate, and emerge as an adult (Edde, 2012). Parasitoid searching behavior depends on visual, tactile, and chemical stimuli received from certain factors, such as environmental conditions (Hong and Ryoo, 1991), host size, and host density (Choi et al., 2001; Steidle and Schöller, 2002).

Several studies have reported the significant potential of *A. calandrae* to reproduce and successfully suppress beetle populations in stored wheat (Mahal et al., 2005; Menon et al., 2002), maize (Wen and Brower, 1994), and rice (Belda and Riudavets, 2012; Chaisaeng et al., 2010; Nam et al., 2011). It was equally attracted to arenas of rice infested with *R. dominica* and *S. oryzae* larvae when presented in a Y-tube olfactometer test (Belda and Riudavets, 2010). In another study of infested rice samples, the parasitoid produced a higher progeny reduction of *S. oryzae* than of *R. dominica* (Belda and Riudavets, 2012). Although the parasitoid has been reported to have more progeny on *S. oryzae* than on *R. dominica*, parasitoid-induced mortality (PIM), which includes host feeding, unsuccessful parasitism and/or superparasitism, was higher in *R. dominica*, which means that its population may also be considerably reduced (Ghimire and Phillips, 2007; Solá et al., 2020). However, no information is available regarding parasitoid performance when both

Sitophilus spp. and *R. dominica* coexist.

The aim of this study was to assess whether the control efficacy of *A. calandrae* on *S. zeamais* is affected by the presence of the alternative host, *R. dominica*. We hypothesized that the parasitoid's effectiveness in suppressing *S. zeamais* would not be affected, and it would also be able to effectively control minor contaminations of *R. dominica*. To test this hypothesis, we first conducted a laboratory trial with different *S. zeamais* / *R. dominica* ratios and two parasitoid densities to determine parasitoid host selection on mixed populations of both pest species. Then, a subsequent experiment was done in large commercial bags, a common method of grain storage, filled with paddy rice and offering to the parasitoid only *S. zeamais*, or a combination of *S. zeamais* and a small proportion of *R. dominica* as hosts.

2. Materials and methods

2.1. Insect colonies

Insect colonies were maintained under controlled conditions (28 ± 2 °C, 70 ± 2 % relative humidity, 16:8 light:dark photoperiod). The laboratory experiment of host selection was performed under the same conditions. Stock colonies of the two coleopteran species and of the parasitoid were started with samples collected from commercial store-houses. *S. zeamais* was reared on brown rice, *R. dominica* on wheat, and *A. calandrae* on *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae) larvae that had developed on dried beans. The maize weevil identity was confirmed through genitalia dissection and molecular methods.

2.2. Host selection laboratory experiment

To determine the preference of the parasitoid for each pest species, we offered them a mix of second-third instar of *S. zeamais* larvae and fourth instar of *R. dominica* larvae, since it has been previously described as the preferred stage for the parasitoid (Wen et al., 1994; Solá et al., 2020). First, we planned to provide females with the following host ratios: 100/0, 75/25, 50/50, 25/75 and 0/100. However, as the larvae of both species develop inside the rice, and there is no external sign indicating that the grains contain a larva, it is not possible to know the exact number of hosts offered to the female parasitoids. Since this number can only be estimated from the number of adults that emerged in a control treatment, a preliminary test was done to determine the reproduction rates of each pest species when paddy rice was infested with 175 adults/kg of *S. zeamais* or *R. dominica* for one week. The emergence rate of *S. zeamais* was 0.69 adults/gr paddy rice, and that of *R. dominica* was 1.45 adults/gr paddy rice. According to these results, the amount of *S. zeamais* and *R. dominica* infested rice needed to offer a 100/0, 75/25, 50/50, 25/75 and 0/100 ratio of *S. zeamais*/*R. dominica* larvae was calculated (Table 1). To obtain the second and third-instar larvae of *S. zeamais*, the paddy rice was incubated for two weeks, and in the case of *R. dominica* for three weeks after being infested with adults as explained before for the preliminary test.

The amount of infested rice corresponding with each different ratio was placed in 710 mL plastic containers to which extra clean paddy rice was added up to 300 g. Containers had a meshed lid for ventilation, two small tubes containing sugary water, and a cotton plug for feeding the adult parasitoids. We released two different densities, one with 12 pairs (high) and one with three pairs (low) of *A. calandrae* (0–7 days old). After one week, the parasitoids were removed, and the containers were incubated until adult emergence. A control treatment without parasitoids was also included. Ten replicates per host ratio and parasitoid

density were performed.

2.3. Big bags experiment

This experiment was conducted in eight large woven polypropylene bags that were maintained under ambient conditions in a warehouse during spring and summer. The bags measured 90 cm × 90 cm × 110 cm (height) and were filled with 500 kg of paddy rice. Two stainless-steel screened cylindrical cages (7 cm long, 5 cm internal diameter, and 1 × 2.5 mm screen) containing either 54 g of rice infested with second- and third-instar *S. zeamais* larvae + 6 g of non-infested rice (*S. zeamais* treatment [Sz]), or 38 g of *S. zeamais* infested rice + 4.5 g of *R. dominica* infested rice + 17.5 g of non-infested rice (*S. zeamais*:*R. dominica* treatment [Sz/Rd]) were placed at the bottom of each bag. Six pairs of *A. calandreae* (0–7 days old) were released on the surface of the grain, together with a tube containing sugary water, to provide food for the females. Next, all the bags were closed with a rope and covered with polyester mesh. After two weeks, the screened cages were removed from the bags, and the rice inside the cages was incubated at 25 °C until pest emergence. Two control treatments were placed inside each bag. The first, with pests only, consisted of two stainless-steel cylindrical cages filled with the same amount of infested rice as in the treatment but with the perforated laterals covered with a sticky tape to prevent the entry of the parasitoids released in the big bag. The second, with parasitoid + pests, consisted of a plastic container with 120 g of rice (twice the same rice infestation than in the stainless-steel cylindrical cage) but included six pairs of *A. calandreae* (0–7 days old). Four rounds of experiments were carried out in mid-May, mid-June, mid-July, and mid-August. In all rounds, four replicates were conducted for each pest combination and control treatment.

2.4. Data analysis

In the host selection experiment, the percentage of emergence reduction vs. the control treatment of the total host offered, of *S. zeamais*, of *R. dominica*, the percentage of effective parasitism, and the percentage of PIM between the different *R. dominica*:*S. zeamais* ratios offered for each parasitoid density tested were analyzed using a generalized linear model (GLM) with a quasi-binomial error distribution, to account for overdispersion. Post hoc comparisons were conducted using the Tukey correction for multiple comparisons. PIM was computed as the difference between the total percentage of host mortality and the host mortality that produced adult parasitoids (effective parasitism). Differences in the percentage of emergence of each species in the parasitoid and in the control treatment for each host-to-host ratio and parasitoid density tested were compared with a GLM assuming a quasi-binomial error distribution. Differences in the percentage of reduction in emergence compared with the control treatment, percentage of effective parasitism, and percentage of PIM between both parasitoid densities when *S. zeamais* and *R. dominica* were offered alone were

compared using GLM (quasi-binomial error distribution). Significant deviation from 1:1 female-to-male in emerged *A. calandreae* was determined using the chi-squared test. In the big bags experiment, only the data from the cylindrical cages where parasitoids emerged were included in the analysis. The percentage of emergency reduction of *S. zeamais*, of parasitism, and of PIM were evaluated using a GLM (quasi-binomial error distribution), with % *S. zeamais* and treatment as factors. Post hoc comparisons were conducted using the Tukey correction for multiple comparisons. Also, the percentage of *R. dominica* reduction between the treatments was analyzed through GLM with quasibinomial error distribution. For all statistical analyses, a nominal significance of 5 % ($P < 0.05$) was applied. All statistical analyses were conducted with the R Studio software (RStudio Team, 2022).

3. Results

3.1. Host selection laboratory experiment

The emergence of each pest species in paddy rice differed from that estimated in the preliminary experiment, as expected (Table 1). Therefore, the parasitoid-to-host ratio tested ranged from the highest 1:53 in the 100 % *S. zeamais* to the lower 1:88 in the 100 % *R. dominica* when three *A. calandreae* females were released. When 12 *A. calandreae* females were released, the parasitoid-to-host ratios tested ranged from 1:09 in the 100 % *S. zeamais* to 1:19 in the 100 % *R. dominica* (Table 1).

Significant differences in total host emergence (*S. zeamais* + *R. dominica*), in comparison with the control treatment without parasitoids, were detected among each host ratio offered to the parasitoids and for both parasitoid densities tested: a higher host reduction was observed in ratios with a predominance of *S. zeamais* than in those with a predominance of *R. dominica* (Table 2). When examining each host separately, almost two times higher host reduction was observed for *S. zeamais* (ranging from 82 % to 95 %) than for *R. dominica* (ranging from 2 % to 45 %). When three pairs of parasitoids were released, there were significant differences in the emergence of *S. zeamais* among the ratios, with a higher reduction as the proportion of the weevil in the mixed treatments decreased. When 12 pairs of parasitoids were released, no significant differences in the emergence of *S. zeamais* among the ratios were detected, although a similar trend was observed. When three or 12 parasitoids were released, significant differences were found in the emergence of *R. dominica* among the ratios. In contrast to *S. zeamais*, the reduction was smaller, as the proportion of *R. dominica* in the mixed treatments was lower (Table 2). It is worth noting that the parasitoid densities tested did not differentially affect the emergence of each pest species when offered alone ($F_{1,18} = 1.75$, $P = 0.203$) for *S. zeamais* and ($F_{1,18} = 2.83$, $P = 0.110$) for *R. dominica*.

The preference of *A. calandreae* could also be observed by comparing the percentage of hosts that emerged from each host species with those that emerged in the control treatment for each host ratio offered (Fig. 1). In the case of no preference, the proportion of emergence of each host

Table 1

Amount (g) of infested rice with *S. zeamais* or *R. dominica* included in each ratio tested, number (mean ± SEM) of emerged pest adults (*S. zeamais* + *R. dominica*), of emerged *S. zeamais* adults, and of emerged *R. dominica* adults in the treatment without parasitoids (Control) and the resulting host-to-host and parasitoid-to-host ratios tested in the two parasitoid densities.

| A. Calandreae released (pairs) | S. zeamais/ R. dominica infested rice (g) | Total No. of adult hosts emerged | No. S. zeamais adult emerged | No. R. dominica adults emerged | S. zeamais: R. dominica ratio tested | Parasitoid/ host ratio tested |
|--------------------------------|---|----------------------------------|------------------------------|--------------------------------|--------------------------------------|-------------------------------|
| 3 | 261/0 | 158.4 ± 3.9 | 158.4 ± 3.9 | 0 | 100/0 | 1:53 |
| | 196/31 | 190.2 ± 10.9 | 128.5 ± 3.9 | 61.7 ± 12.7 | 70/30 | 1:63 |
| | 130/62 | 188.4 ± 8.5 | 82.2 ± 3.3 | 106.2 ± 6.9 | 44/56 | 1:63 |
| | 65/93 | 220.4 ± 5.7 | 42.9 ± 2.4 | 177.5 ± 5.0 | 19/81 | 1:73 |
| | 0/124 | 264.1 ± 5.5 | 0 | 264.1 ± 5.5 | 0/100 | 1:88 |
| 12 | 261/0 | 102.9 ± 3.8 | 102.9 ± 3.8 | 0 | 100/0 | 1:09 |
| | 196/31 | 130.5 ± 2.5 | 77.0 ± 3.3 | 53.5 ± 2.9 | 59/41 | 1:11 |
| | 130/62 | 160.7 ± 5.1 | 52.8 ± 3.0 | 107.9 ± 2.9 | 33/67 | 1:13 |
| | 65/93 | 190.2 ± 4.8 | 27.6 ± 1.9 | 164.6 ± 4.3 | 15/85 | 1:16 |
| | 0/124 | 225.6 ± 10.6 | 0 | 225.6 ± 10.6 | 0/100 | 1:19 |

Table 2

Number (mean \pm SEM) of emerged *S. zeamais* and *R. dominica* adults, and percentage (mean \pm SEM) of emergence reduction in comparison with the control treatment without parasitoids; of total hosts (*S. zeamais* + *R. dominica*); of *S. zeamais* or of *R. dominica*, at each host-to-host ratio offered and for the two parasitoid densities tested.

| <i>A. calandreae</i> released (pairs) | <i>S. zeamais</i> : <i>R. dominica</i> ratio | No. <i>S. zeamais</i> adult emerged | No. <i>R. dominica</i> adult emerged | % Total host reduction | % <i>S. zeamais</i> reduction | % <i>R. dominica</i> reduction |
|---------------------------------------|--|-------------------------------------|--------------------------------------|-----------------------------------|----------------------------------|------------------------------------|
| 3 | 100/0 | 19.8 | 0.0 | 87.5 \pm 1.7 a | 87.5 \pm 1.7b | 0 |
| | 70/30 | 11.3 | 54.5 | 65.4 \pm 4.4b | 91.2 \pm 1.8 ab | 19.1 \pm 4.7 bc |
| | 44/56 | 4.3 | 90.9 | 49.5 \pm 2.0c | 94.8 \pm 0.6 a | 15.4 \pm 2.8c |
| | 19/81 | 2.8 | 127.7 | 40.8 \pm 2.3c | 93.5 \pm 1.6 ab | 28.1 \pm 2.8b |
| | 0/100 | 0.0 | 145.6 | 44.9 \pm 2.6c | 0 | 44.9 \pm 2.6 a |
| | | | | $F_{4,45} = 41.51$ $P < 0.001$ | $F_{3,36} = 4.71$ $P < 0.01$ | $F_{3,36} = 18.59$ $P < 0.001$ |
| 12 | 100/0 | 17.6 | 0.0 | 82.9 \pm 3.2 a | 82.9 \pm 3.2 | 0 |
| | 59/41 | 8.5 | 60.3 | 47.3 \pm 3.1b | 89.0 \pm 1.9 | 2.0 \pm 1.4c |
| | 33/67 | 5.6 | 76.1 | 49.2 \pm 2.6b | 89.4 \pm 1.5 | 29.5 \pm 3.4b |
| | 15/85 | 4.1 | 97.2 | 46.7 \pm 2.2b | 85.1 \pm 2.3 | 41.0 \pm 2.3 a |
| | 0/100 | 0.0 | 130.0 | 42.4 \pm 2.8b | 0 | 42.4 \pm 2.8 a |
| | | | | $F_{4,45} = 22.15$ $P < 0.001$ | $F_{3,36} = 1.84$ $P = 0.157$ | $F_{3,36} = 154.40$ $P < 0.001$ |

Values in the same column for each parasitoid density followed by the same letter are not significantly different (Tukey test, $P < 0.05$).

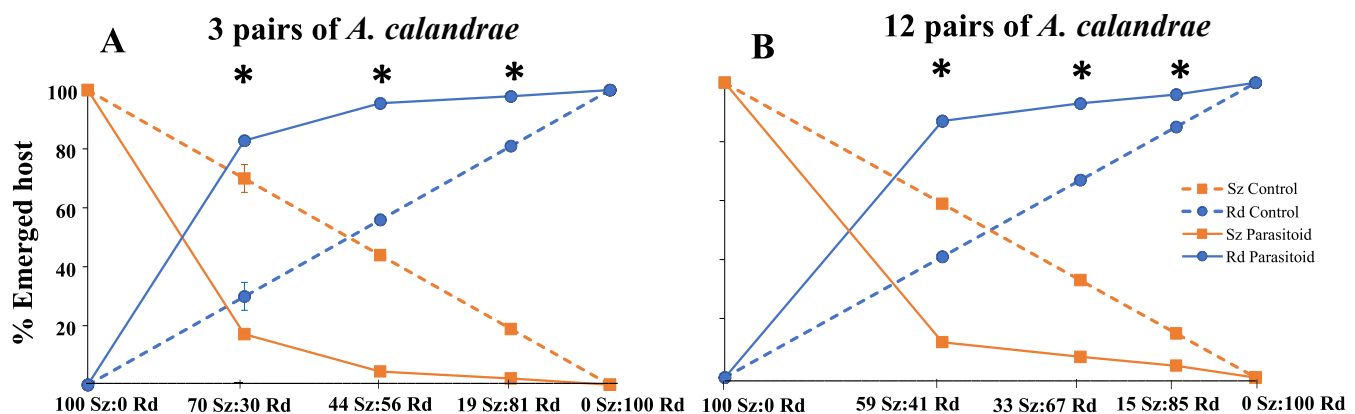


Fig. 1. Percentage (mean \pm SEM) of *S. zeamais* and *R. dominica* adults emerged in the parasitoid (solid line) versus control treatment (dotted lines) at the different host-to-host ratios offered when: A) three female's and B) 12 female's parasitoids were released. * Denotes significant differences ($P < 0.001$) in pest emergence rates between parasitoid and control treatments.

species at each tested ratio would be similar in both the parasitoid and the control treatments. However, the emergence rates of *S. zeamais* and *R. dominica* were significantly different from those of the control treatment in both parasitoid density releases. In all cases, the proportion of *S. zeamais* that emerged was lower than expected. For three pairs of *A. calandreae* released, $F_{1,18} = 184.55$, $P < 0.001$ (70Sz/30Rd), $F_{1,18} = 404.98$, $P < 0.001$ (44Sz/56Rd) and $F_{1,18} = 51.22$, $P < 0.001$ (19Sz/81Rd), and for 12 pairs of *A. calandreae*, $F_{1,18} = 83.64$, $P < 0.001$ (59Sz/41Rd), $F_{1,18} = 320.70$, $P < 0.001$ (33Sz/67Rd) and $F_{1,18} = 304.46$, $P < 0.001$ (15Sz/85Rd). Conversely, the proportion of *R. dominica* that emerged was higher than expected.

When *S. zeamais* was offered alone, almost three times more parasitoids emerged from three pairs than from 12 pairs of parasitoids released (Table 3) ($F_{1,18} = 72.46$, $P < 0.001$) and, consequently, a significantly lower percentage of parasitism was obtained in the high parasitoid density. On the contrary, when *R. dominica* was offered alone, no differences in parasitism were observed between parasitoid-released densities ($F_{1,18} = 1.06$, $P = 0.316$). Also, when three female parasitoids were released, a bias toward female production in most of the ratios tested was observed, while no bias was obtained in any ratio when 12 female parasitoids were released (Table 3). When parasitoid preferences were evaluated as the percentage of effective parasitism (adult parasitoids emerged) at the different pest species ratios, it significantly increased with the proportion of *S. zeamais* at both parasitoid-released densities ($F_{4,45} = 181.61$, $P < 0.001$ and $F_{4,45} = 63.60$, $P < 0.001$ for three and 12 pairs of *A. calandreae*, respectively) (Fig. 2, darker bars).

Table 3

Number (mean \pm SEM) and sex ratio of the *A. calandreae* emerged at each host-to-host (*S. zeamais*: *R. dominica*) ratio offered and for both parasitoid densities (three or 12 pairs) released.

| <i>A. calandreae</i> released (pairs) | <i>S. zeamais</i> : <i>R. dominica</i> ratio | No. <i>A. calandreae</i> adults emerged | Sex ratio ¹ |
|---------------------------------------|--|---|------------------------|
| 3 | 100/0 | 149.3 \pm 3.9 | 0.64 \pm 0.07* |
| | 70/30 | 98.1 \pm 3.8 | 0.76 \pm 0.03* |
| | 44/56 | 77.5 \pm 3.0 | 0.77 \pm 0.02* |
| | 19/81 | 45.0 \pm 3.9 | 0.74 \pm 0.02* |
| | 0/100 | 24.2 \pm 3.6 | 0.61 \pm 0.05 |
| 12 | 100/0 | 52.2 \pm 4.5 | 0.43 \pm 0.60 |
| | 59/41 | 41.7 \pm 2.9 | 0.51 \pm 0.06 |
| | 33/67 | 34.4 \pm 2.7 | 0.53 \pm 0.04 |
| | 15/85 | 24.9 \pm 1.9 | 0.40 \pm 0.04 |
| | 0/100 | 16.9 \pm 2.1 | 0.42 \pm 0.04 |

¹ Ratio of females to the total number of emerged parasitoids.

* Denotes a significant deviation from 1:1 female-to-male (chi-squared test).

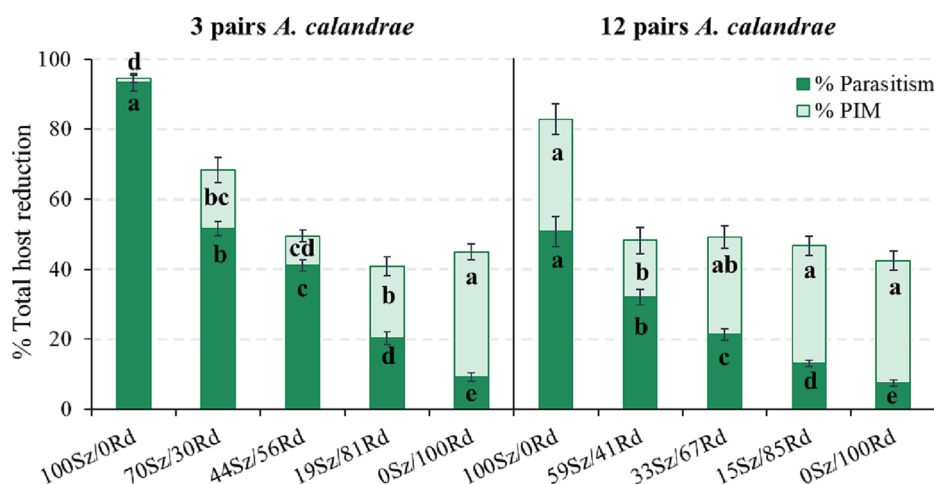


Fig. 2. Percentage (mean \pm SEM) of host reduction in comparison with the control treatment and its components: effective parasitism and PIM at the different host-to-host ratios offered when three females and 12 females were released. *S. zeamais*: *R. dominica* ratios with the same lowercase letter for parasitism (darker bars) or for PIM (lighter bars) in each parasitoid density are not significantly different (Tukey test, $P < 0.05$).

PIM significantly varied among the different host-to-host ratios in both parasitoid release rates ($F_{4,45} = 25.32$, $P < 0.001$ and $F_{4,45} = 4.84$, $P < 0.001$ for three and 12 parasitoid females, respectively), increasing as the proportion of *R. dominica* increased (Fig. 2, lighter bars). When *S. zeamais* was offered alone, a significant increase in PIM was observed when 12 pairs of parasitoids were released ($F_{1,18} = 49.13$, $P < 0.001$). However, when *R. dominica* was offered alone, no significant differences were detected between parasitoid-released rates ($F_{1,18} = 0.04$, $P = 0.851$).

3.2. Big bags experiment

Temperature in the warehouse during the trial ranged from 17.8°C to 30.36°C, with a daily mean of $25.0 \pm 0.5^\circ\text{C}$; and humidity ranged from 77.8 % to 52.3 %, with a daily mean of 68.6 ± 0.5 %. Temperatures inside the bags were very similar to those of the warehouse, ranging from 19.1°C to 27.9 °C, with a mean of 24.5 ± 0.5 °C, and the relative humidity ranged from 74.6 % to 72.0 %, with a mean of 73.7 ± 0.1 %.

The mean infestation of rice offered to the parasitoid (data in the control treatment estimated as adult emergence) when only *S. zeamais* was offered was 68.3 ± 3.4 larvae. In contrast, when *S. zeamais* + *R. dominica* were offered, the mean infestation obtained was 83.4 ± 5.2 of *S. zeamais* larvae and 2.8 ± 2.2 of *R. dominica* larvae, corresponding to a host-to-host ratio of 97 *S. zeamais*: 3 *R. dominica* (Fig. 3). In all

replicates of the parasitoid + pests control treatment, we recovered adults of *S. zeamais* and of *R. dominica* when offered both species and of the parasitoid. However, in the parasitoid + pests treatment, pest individuals were recovered in all replicates, but parasitoids were not present in six of the thirty-two replicates conducted in the four rounds, three in the *S. zeamais* treatment and three in the *S. zeamais* + *R. dominica* treatment. The resulting parasitoid-to-host ratio was 1:14 and 1:12 for the *S. zeamais* and *S. zeamais* + *R. dominica*, respectively, considering the four rounds together.

In each treatment, *S. zeamais* reduction by the parasitoid was similar when offering only *S. zeamais* than when offering *S. zeamais* + *R. dominica*. This reduction was more than 90 % in the parasitoid + pests control treatment, being higher than in the parasitoid + pests treatment (43–53 % reduction) (treatment $F_{1,62} = 33.81$, $P < 0.001$; % *S. zeamais* $F_{1,61} = 0.07$, $P = 0.790$; treatment \times % *S. zeamais* $F_{1,60} = 3.67$, $P = 0.060$) (Fig. 4). The reduction of *R. dominica* emergence was similar in the parasitoid + pests control than in the parasitoid + pests treatment (25.0 % and 25.9 %, respectively) ($F_{1,22} = 0.02$, $P = 0.885$), but lower than the reduction of *S. zeamais* emergence (Fig. 4).

When examining host reduction in relation to the number of parasitoids emerged and the hosts killed by other causes (PIM), parasitism was similar in the control (parasitoid + pests) than in the treatment, and it was not affected by the presence of *R. dominica* (treatment $F_{1,62} = 1.91$, $P = 0.167$; % *S. zeamais* $F_{1,62} = 0.07$, $P = 0.798$; treatment \times %

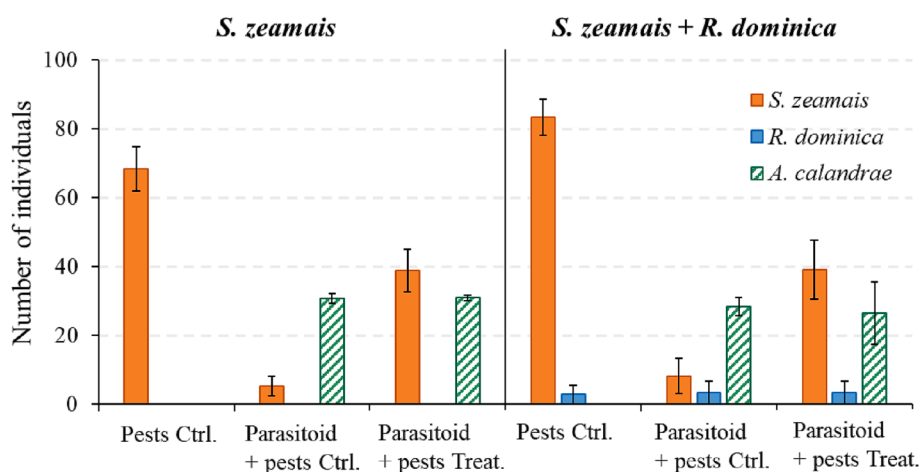


Fig. 3. Number (mean \pm SEM) of *S. zeamais*, of *R. dominica*, and of *A. calandreae* adults emerged in the pests control, in the parasitoid + pests control, and in the parasitoid + pests treatment, when rice was infested only with *S. zeamais* or with both pest species.

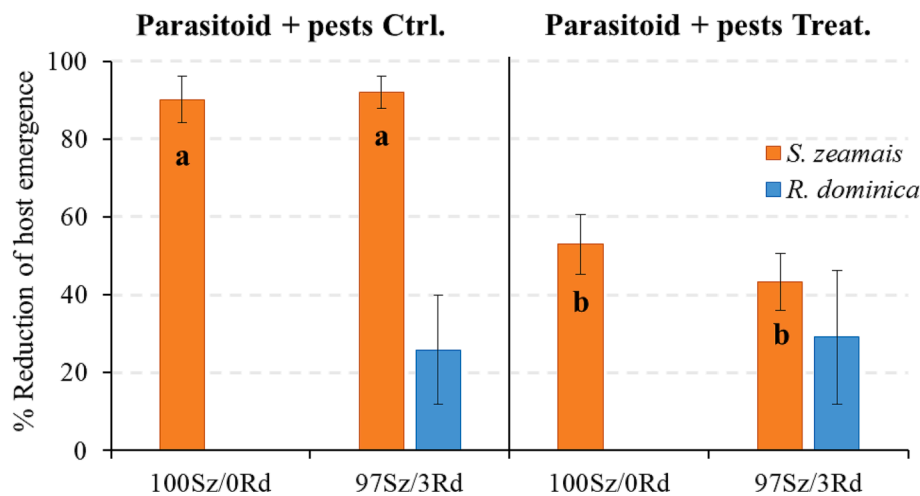


Fig. 4. Percentage (mean \pm SEM) of *S. zeamais* and *R. dominica* reduction in emergence in the control with parasitoid + pests and in the parasitoid + pests treatment, when rice was infested only with *S. zeamais* or with *S. zeamais* + *R. dominica*. Comparison of host ratios between parasitoid + pests control and parasitoid + pests treatment with different letters are significantly different (Tukey's test, $P < 0.05$).

S. zeamais $F_{1,62} = 0.06$, $P = 0.801$) (Fig. 5). However, PIM was significantly higher in the control group than in the treatment group (treatment $F_{1,62} = 42.27$, $P < 0.001$; % *S. zeamais* $F_{1,62} = 1.32$, $P = 0.250$; treatment \times % *S. zeamais* $F_{1,62} = 0.43$, $P = 0.513$) (Fig. 5). Therefore, the fact that there was more host reduction in the parasitoid + pests control than in the parasitoids + pests treatment was due to the difference in PIM values.

4. Discussion

Results obtained in this study showed that the presence of *R. dominica* at low levels did not diminish the efficacy of the parasitoid *A. calandreae* in reducing *S. zeamais* emergence at either a small (laboratory) or large (big bags) scale. In the host selection laboratory experiment, we offered different proportions of rice infested by each pest species. As mentioned, we conducted a previous test to obtain the approximate emergence ratios of both pest species in paddy rice and used these results for calculating the proportions of infested rice to use in the experiment. It turns out that in the laboratory experiment, the emergence of *R. dominica* was higher than the one observed in the

previous test, with a higher reproduction of *R. dominica* compared to *S. zeamais*, as can be observed by the total number of emerged adults from each species when they were not mixed (100 % *R. dominica* or 100 % *S. zeamais*). This large variability in hosts' emergence in both tests is inherent to the methodology, since neither the sex nor the age of the individuals used in the rice infestation were determined. Sex determination of adults of both pest species is not an easy task, and if done, individuals are injured. Although the siliceous hull of paddy rice protects it from attack by many storage pests (Nadeem et al., 2011), the mouthparts of *R. dominica* compared to those of *S. zeamais* may facilitate access to the rice grain. It is also worth noting that the parasitoids were able to disperse and parasitize the infested grains despite the presence of the hull, as already reported in Riudavets et al. (2021).

To a large extent, the effectiveness of parasitoids in controlling a specific insect pest infestation depends on its host preference. Differences in parasitism rates among host species are used as an estimate of host preference and host suitability for the parasitoid (Timokhov and Gokhman, 2003). The host selection laboratory experiment showed that *A. calandreae* preferred to parasitize *S. zeamais* larvae in comparison with *R. dominica* larvae. This result was supported by: a) a higher reduction in host emergence, almost double, occurred when *S. zeamais* larvae were offered alone to the parasitoid than when *R. dominica* larvae were offered alone; b) a reduction in *S. zeamais* emergence in the mixed host ratios was always above 80 %, while that of *R. dominica* was at the most around 41 %; c) when host emergence in the parasitoid treatment was compared to the control treatment, significantly fewer *S. zeamais* adults and more *R. dominica* adults emerged than expected; and d) *A. calandreae* preferentially reproduced on *S. zeamais* larvae than on *R. dominica* larvae. These results are in line with earlier studies of host suitability, which found higher parasitism rates of *A. calandreae* in *S. zeamais* and *S. oryzae* than in *R. dominica* (Solá et al., 2020; Belda and Riudavets, 2012; Ghimire and Phillips, 2007). Ghimire and Phillips (2008) also indicated that *A. calandreae* has an innate preference for *S. zeamais* over *R. dominica*. Nevertheless, it is worth noting that Timokhov and Gokhman (2003) showed intraspecific variation of different strains of *A. calandreae* in host preference between Curculionidae or Anobiidae families.

The higher effective parasitism observed in maize weevil larvae at the lowest parasitoid density suggests that *A. calandreae* females tend to use hosts of larger sizes for oviposition and offspring development, whereas the smallest ones are used for their nutrition (Choi et al., 2001). This is because the host size on which the parasitoids develop affects the size of the emergent parasitoid and thereby, also its reproductive

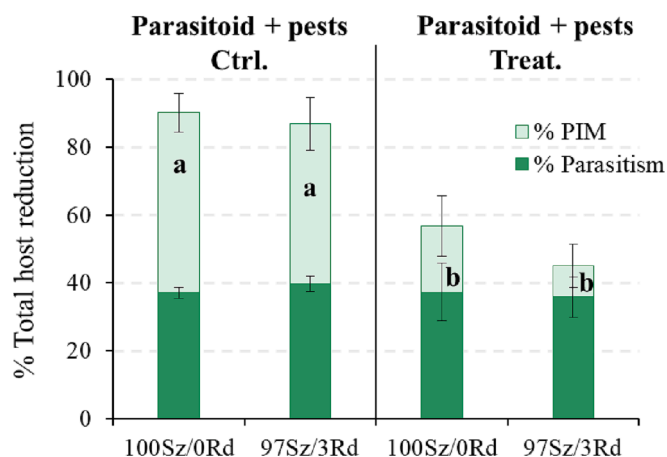


Fig. 5. Percentage (mean \pm SEM) of host reduction in emergence and its components (effective parasitism in dark green bars and PIM in light green bars) in the parasitoid + pests control and in the parasitoid + pests treatment, when rice was infested only with *S. zeamais* or with *S. zeamais* + *R. dominica*. Comparison of host ratios between control and treatment with different letters are significantly different (Tukey's test, $P < 0.05$).

potential (Kim et al., 1995). Bruins et al. (1994) proposed that the relative host size may influence host selection because larger hosts are perceived earlier than smaller ones. We deliberately offered *S. zeamais* larvae of a younger instar than that preferred by *A. calandreae* to avoid size-host selection between the two pest species. However, because the rice infestation period was one week and our pests are internally developing species, the assessment of the larval size offered was just an estimate, and we may have offered smaller *R. dominica* and larger *S. zeamais* than expected.

In contrast with what could be expected, parasitoid offspring were almost double in the low parasitoid density than in the high density when *S. zeamais* was offered alone. This was probably caused by superparasitism due to intraspecific competition between female parasitoids, as documented in other studies (Choi et al., 2001; Lebreton et al., 2009; Solá et al., 2020). *Anisopteromalus calandreae* females are unable to identify hosts parasitized by sibling females (Benkhellat et al., 2015). Therefore, increasing the parasitoid density increases the probability of females laying eggs on already parasitized larvae, which could end up killing the host since only one egg could develop in one host larva (Lebreton et al., 2009). Therefore, quadrupling the number of parasitoids introduced did not result in further host reduction in either of the two pest species. In the case of *S. zeamais* alone, it increased the PIM. In the case of *R. dominica*, as supported by other authors who studied host suitability (Ghimire and Phillips, 2007; Solá et al., 2020), higher PIM values were observed in both parasitoid densities because of host feeding or high mortality during parasitoid development.

The female-biased sex ratio observed when three female parasitoids were released could be explained by the regulation of the sex ratio of pteromalid offspring, which preferentially deposit female eggs on large hosts and male eggs on small hosts due to its lower nutritional value (Choi et al., 2001; Lebreton et al., 2009). Therefore, the fact that the sex ratio was balanced with *R. dominica* suggests that it is a less suitable host for parasitoid development, as previously reported by Solá et al. (2020), as well as when compared with *S. oryzae* (Belda and Riudavets, 2012; Ghimire and Phillips, 2008, 2007). No bias resulted when offered 12 female parasitoids; in the case of superparasitism, Choi et al. (2001) estimated a balanced progeny sex ratio.

In the big bags, the parasitoid was able to successfully disperse through the paddy rice kernels and locate host larvae at 1 m depth in the bottom of the bags, as already shown in Riudavets et al. (2021). Preference of *A. calandreae* for *S. zeamais* larvae was also confirmed in this experiment because a similar reduction of *S. zeamais* adult emergence was observed when it was offered alone than when it was offered together with a small proportion of *R. dominica*. Likewise, the reduction in *R. dominica* adult emergence was lower, as occurred in those ratios with less *R. dominica* in the host selection trial. The differences in *S. zeamais* reduction between the parasitoid + pests control and the parasitoid + pests treatment were probably due to the larger dimensions of the container and the amount of rice to be explored. In the big bags, the odors used by the parasitoids to find the host larvae (Belda and Riudavets, 2010; Ghimire and Phillips, 2008) were more diluted than in the parasitoid + pest control plastic container. Similar results in the decrease of parasitoid efficacy against *S. zeamais* were obtained in Riudavets et al. (2021) when comparing results obtained in PVC pipes (filled with 30 kg of paddy rice) and in big bags (500 kg of paddy rice). In this experiment, effective parasitism was similar in the parasitoid + pest control than in the treatment, indicating that the priority of parasitoid females would be first to lay eggs and second to prey on the hosts. However, we would expect parasitism to be higher in the parasitoid + pest control, as the larvae were more accessible. However, perhaps due to the high values of PIM obtained, superparasitism could also have occurred.

In conclusion, the effectiveness of *A. calandreae* in controlling *S. zeamais* in big bags would not be hampered by the presence of *R. dominica* at any studied host proportion. However, the effectiveness of the parasitoid in reducing *R. dominica* populations in the presence of

S. zeamais would be reduced, and this reduction would increase as the proportion of *S. zeamais* increased. Nevertheless, *S. zeamais* is the key pest in paddy rice, and the use of *A. calandreae* seems to be a feasible alternative to the use of fumigants or contact insecticides when this commodity is stored in big bags.

CRediT authorship contribution statement

Lidia del Arco: Methodology, Investigation, Data curation, Writing – review & editing. **Jordi Riudavets:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Funding acquisition. **José Miguel Campos-Rivela:** Methodology, Investigation, Data curation, Writing – review & editing. **María Teresa Martínez-Ferrer:** Methodology, Investigation, Data curation, Writing – review & editing. **Nuria Agustí:** Conceptualization, Writing – review & editing. **Cristina Castañé:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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