

Laboratory studies on control of the maize weevil *Sitophilus zeamais* by the parasitoid *Anisopteromalus calandrae*

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ABSTRACT: *Anisopteromalus calandrae* (Howard), a cosmopolitan parasitoid, attacks several beetle pests of stored products, including the maize weevil, *Sitophilus zeamais* (Motschulsky). Two laboratory experiments were conducted at ambient conditions (25–29 °C, 60–70% RH, natural photoperiod). A short-term experiment evaluated the density of *A. calandrae* (0, 4, 8, 12, 16, or 20 per box) for the optimal production of parasitoids in 3825-cm³ boxes containing milled rice. The percentage of parasitoid emergence was highest and the percentages of weevil emergence and parasitoid-induced mortality i.e., parasitoid mortality resulting from superparasitism were relatively low with 16 females per box rather than with smaller (0 to 12) or larger (20) numbers of females added per box. The decline in parasitoid emergence at the highest parasitoid density tested (20 females per box) was probably due to superparasitism. A long-term experiment was conducted to determine the parasitoid densities (0, 2, 4, 6, 8, or 10 per bottle) that most effectively suppress weevils in milled rice during 6 months of storage in 1-l bottles. To simulate repetitive release of parasitoid, the same number of parasitoids was introduced after each monthly sampling. The number of maize weevils decreased as parasitoid density increased. The best control was obtained with 10 female parasitoids per bottle. At this density, the number of emerged weevil remained stable from the third to the sixth month. Based on the short-term experiment, a parasitoid-host ratio of 1:47 will produce the largest number of parasitoids. Based on the long-term experiment, a parasitoid-host ratio of 1:30 will provide the best control of maize weevils in a monthly release program. The current results indicate that large-scale experiments on biological control of the maize weevil with *A. calandrae* are now needed.

KEYWORDS: biological control, ectoparasitoid, induced mortality, repetitive release

INTRODUCTION

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is one of the most destructive pests of stored cereals. It is the dominant *Sitophilus* species found in rice granaries in all but the southern part of Thailand¹. Its biology was previously reported by Longstaff². Weevil adults attack whole grains, and larvae cryptically feed and develop within grains³. Infestation by this weevil begins in the field⁴ but most damage occurs during storage. Damage to grain caused by this weevil includes reductions in nutritional value, germination, weight, and commercial

value⁵. The maize weevil can be conventionally controlled by residual pesticides and fumigation³. Methyl bromide fumigation, however, has been banned in developed countries since 2005 and will be banned in developing countries in 2015 because it destroys ozone and endangers human health⁶. One of the most promising alternatives to pesticides and fumigants for postharvest pest management is biological control⁷, which should be particularly effective in the closed environment where grain is stored⁸.

Among natural enemies that could act as biological control agents of the maize weevil, the wasp *Anisopteromalus calandrae* (Howard) (Hymenoptera:

Pteromalidae) is a dominant parasitoid naturally found in granaries^{9,10}. It is a solitary ectoparasitoid that parasitizes numerous stored-product beetles including *S. zeamais*^{9,10}, *S. oryzae*^{7,11,12}, *S. granarius*⁸, *Callosobruchus maculatus* (Coleoptera: Bruchidae)¹³, and *Rhyzopertha dominica* (Coleoptera: Bostrichidae)^{14,15}. The female wasp parasitizes coleopteran larvae that are feeding inside the grain kernels. The potential for control of *S. zeamais* by *A. calandreae* in maize has been demonstrated^{9,10,16,17}. In the laboratory, Helbig¹⁸ found that *A. calandreae* suppressed *S. zeamais* on maize by 23% relative to the untreated control. Wen and Brower¹⁹ found that both single and multiple releases of *A. calandreae* substantially reduced the population increase of maize weevils in drums of maize; suppression was > 90% with all rates of both single and multiple releases.

The research reported here evaluated *A. calandreae* introduction rates on the control of *S. zeamais* in milled rice. A short-term experiment determined the optimum host-parasitoid ratio for mass rearing the parasitoid. A long-term experiment was carried out to estimate the suitable release rate for repeated, monthly parasitoid releases. The baseline data generated from this study will be used to develop an efficient method for control of *S. zeamais* in rice granaries.

MATERIALS AND METHODS

Insect rearing

The maize weevil, *S. zeamais*, and its parasitoid, *A. calandreae*, were obtained from the Postharvest Technology Research and Development Laboratory, Postharvest and Product Processing Research and Development Office, Department of Agriculture, Bangkok. For stock cultures of *S. zeamais*, 200 maize weevils were reared on 250 g of milled rice in a glass jar (12 cm height × 5 cm diameter). For stock cultures of *A. calandreae*, 100 adult *A. calandreae* were introduced into a clear plastic box (15 cm width × 30 cm length × 6 cm height) containing milled rice infested with 4th instar maize weevils. All insects were reared at ambient conditions (25–29 °C and 60–70% RH) and under natural photoperiod. Both experiments were conducted under these conditions. Under these conditions, the generation times for the weevil and parasitoid are 48 and 13 days, respectively (unpublished data). The parasitoid produces one progeny per parasitized weevil larva (unpublished data).

Short-term experiment

This experiment examined the optimum host-parasitoid ratio for mass rearing the parasitoid. In

preparation for this experiment, 20 unsexed adult maize weevils, less than 2 weeks old, were introduced into each of five 140-ml glass bottles (12 cm height and 5 cm diameter) containing 50 g of milled rice. The bottles were then sealed with blotting paper. The adult weevils were allowed to oviposit for 7 days and then were removed by passing the milled rice through a coarse sieve. The weevil-infested rice was then returned to the bottles, which were maintained at rearing conditions. After 25 days, the infested rice was transferred to small (12 cm width × 10 cm length) cheesecloth bags (50 g of infested rice per bag), which were sown shut with thread. Adult *A. calandreae* that had emerged within 1 day from the rearing colony were used in the experiment.

The short-term experiment used 35 clear plastic boxes (17 cm width × 25 cm length × 9 cm height, 3835 cm³). Each box contained a pile of four cheesecloth bags filled with weevil-infested milled rice. The boxes had tight fitting lids. Each lid had two 3-cm diameter holes which were covered with a polyester screen to allow gas exchange but prevent insect escape. Different numbers of mated female *A. calandreae* were introduced into each box (0, 2, 4, 8, 12, 16, or 20 per box). There were five replicate boxes per parasitoid level. After 1 month, the boxes were frozen at 0 °C for 2 days. The numbers of maize weevils and parasitoids in each box were determined. Note that the holes in the cheesecloth were large enough to permit passage of adult parasitoids.

Long-term experiment

This experiment examined the long-term interaction of weevil and parasitoid as affected by numbers of parasitoids released at monthly intervals. In preparation for this experiment, 10 unsexed adult maize weevils were introduced into each of 6 1-l bottles, each containing 500 g of freshly milled rice. After 25 days, different numbers of presumably mated female *A. calandreae* were added to the bottles (0, 2, 4, 6, 8, or 10 per bottle). There were 30 bottles per parasitoid level. One month after addition of *A. calandreae* to the bottles, 5 bottles of each parasitoid density were randomly selected and destructively sampled; the numbers of emerged maize weevils and parasitoids were counted, and the sex of the parasitoids was determined. After the 1-month bottles were removed, each of the remaining bottles received the same number of parasitoids as initially added to simulate repetitive release of parasitoids in the field. The monthly removal of 5 replicate bottles and addition of parasitoids to the remaining bottle was continued for 5 more months.

Data analysis

The parasitoid-induced mortality (PIM, referring to parasitoid mortality caused by superparasitism) was calculated from $PIM = [(the\ average\ number\ of\ rice\ weevils\ that\ emerged\ when\ no\ parasitoids\ were\ added) - (the\ number\ of\ all\ insects\ including\ parasitoids\ that\ emerged\ in\ each\ replicate)] / [(the\ average\ number\ of\ rice\ weevils\ that\ emerged\ when\ no\ parasitoids\ were\ added)]$. The parasitoid emergence (PE) was given by $PE = [(the\ number\ of\ parasitoids\ that\ emerged\ in\ each\ replicate)] / [(the\ average\ number\ of\ rice\ weevils\ that\ emerged\ when\ no\ parasitoids\ were\ added)]$. The weevil emergence (WE) was given by $WE = [(the\ number\ of\ rice\ weevils\ that\ emerged\ in\ each\ replicate)] / [(the\ average\ number\ of\ rice\ weevils\ that\ emerged\ when\ no\ parasitoids\ were\ added)]$ ^{16,17}.

In the short-term experiment, one-way ANOVAs were used to determine the effect of parasitoid density on PIM, PE, WE, and progeny sex ratio (female:male). In the long-term experiment, two-way ANOVAs were used to determine the effect of parasitoid density and time after parasitoid introduction on WE, PE, and the progeny sex ratio. Before the ANOVAs were conducted, the homogeneity of variance was checked²⁰. The PE and sex ratios were logarithmically transformed to meet the assumption of homogeneity of variance. The differences among treatments were assessed by Fisher's least significant difference (LSD) test.

RESULTS AND DISCUSSION

Short-term experiment

The number of rice weevils (mean \pm SE) that emerged from the treatment without parasitoids was 748 ± 55 per box. Parasitoid density significantly affected the WE ($F = 175$, $df = 5, 24$; $P \leq 0.001$), the PE ($F = 19$, $df = 5, 24$; $P \leq 0.001$), and the PIM ($F = 9.9$, $df = 5, 22$; $P \leq 0.001$). The WE decreased as parasitoid density increased while the PIM values tended to increase with parasitoid density (Table 1). The PIM was significantly higher with 20 female parasitoids per box than with smaller numbers per box. These results are consistent with those of Wen and Brower²¹ and Ryoo et al¹⁷. Similarly, Mahal et al²², who studied the effect of *A. calandreae* on the control of *S. oryzae* in wheat grain, found that the PIM was positively correlated with the parasitoid density.

PE also increased with parasitoid density in the short-term experiment (Table 1). PE tended to be lower, however, with 20 female parasitoids added per box than with 16 female parasitoids added per box. This apparent decline in PE at 20 females per box was

probably due to superparasitism, as suggested by Wen and Brower²¹. At this density, the PIM was nearly twice as high as the PIM with other densities while the number of hosts encountered by the parasitoid did not decrease. This was probably due to intraspecific competition among female parasitoids when the available hosts were relatively limiting¹⁷. For example, although the *A. calandreae* female usually avoids ovipositing on previously parasitized hosts, it is likely to lay more than one egg per host when the number of available hosts becomes insufficient. The supernumerary larvae may be eliminated, leading to a lower percentage of emerged parasitoids.

Given the conditions of this experiment (infested milled rice kept in a pile of 4 cheesecloth bags and producing 748 maize weevils per box), our results suggest that the appropriate parasitoid density for parasitoid production was 16 female parasitoids per box. At this density, the PE was highest and the WE and the PIM were relatively low. The low PIM is advantageous for the mass rearing and releasing of parasitoids in the field.

The sex ratio (female:male) of parasitoid progeny was significantly affected by parasitoid density ($F = 15$, $df = 5, 24$; $P \leq 0.001$). The progeny sex ratio tended to decrease as parasitoid density increased (Table 1). This reduction in the number of female versus male offspring was probably due to the deficiency of larval hosts rather than to the high parasitoid density. However, the effect of parasitoid density on progeny sex ratio was inconsistent. For example, the sex ratio was higher at 12 females than at 8 females per box (Table 1). Our results agree with those of Wen et al¹⁶, who reported that the sex ratio (female:male) of *A. calandreae* progeny decreased as the number of parasitoids added per box increased. In contrast, Ryoo et al¹⁷ found that the sex ratios of *A. calandreae* progeny were not significantly affected by the numbers of parasitoids added.

Long-term experiment

The numbers of maize weevils that emerged in the bottles without parasitoid ($n = 5$) at the end of 1–6 months after the release of parasitoid averaged 305 ± 15 , 574 ± 23 , 695 ± 97 , 765 ± 18 , 929 ± 57 , and 1344 ± 107 , respectively.

Percentage of maize weevil emergence

Both parasitoid density and time after parasitoid introduction significantly influenced the WE (Table 2). The statistically significant interaction between density and time indicated that the difference among the 5 densities was not constant at all times after the release

Table 1 Effect of parasitoid density on PE, WE, PIM, and sex ratio of parasitoid progeny in the short-term experiment.

| Parasitoid density (no. of mated females/box) | PE (%) | WE (%) | PIM (%) | Sex ratio (female:male) of parasitoid progeny |
|--|--------------------------|------------|-------------------------|--|
| 2 | 24.4 ± 2.0 | 49.1 ± 1.2 | 24.4 ± 2.3 ^a | 1.07 ± 0.17 |
| 4 | 36.1 ± 1.0 ^a | 38.2 ± 1.9 | 25.7 ± 2.1 ^a | 1.40 ± 0.11 |
| 8 | 42.1 ± 4.3 ^{ab} | 31.0 ± 0.9 | 26.8 ± 4.4 ^a | 0.43 ± 0.65 ^a |
| 12 | 50.4 ± 3.1 ^c | 26.9 ± 0.7 | 26.9 ± 4.7 ^a | 0.71 ± 0.08 ^b |
| 16 | 58.0 ± 1.7 | 14.3 ± 1.1 | 27.7 ± 2.4 ^a | 0.62 ± 0.11 ^{ab} |
| 20 | 45.0 ± 1.2 ^{bc} | 7.6 ± 0.6 | 47.7 ± 1.7 | 0.57 ± 0.03 ^{ab} |

Values are means (± SE) of 5 replicate boxes. Means in a column followed by the same superscript are not significantly different ($P \geq 0.05$; Fisher's LSD).

Table 2 WE, PE, and sex ratio of parasitoid progeny (SR) as affected by parasitoid density (D) and time (t) after parasitoid introduction in the long-term experiment: analysis of variance table. $P \leq 0.001$ in all cases.

| Source | df | MS | | | F | | |
|--------|-----|--------|--------|------|--------|--------|-----|
| | | WE (%) | PE (%) | SR | WE (%) | PE (%) | SR |
| Model | 29 | 1111 | 4.2 | 0.28 | 25 | 29 | 9.3 |
| D | 4 | 5023 | 1.3 | 0.13 | 112 | 9.1 | 4.5 |
| t | 5 | 1460 | 22 | 1.4 | 33 | 147 | 46 |
| Dt | 20 | 242 | 0.44 | 0.09 | 5.4 | 3 | 3.1 |
| Error | 120 | 45 | 0.15 | 0.03 | | | |

of the parasitoid^{20,21}. Because of this interaction, it was not meaningful to compare the effect of density and time on the WE²⁰.

For each parasitoid density, the WE was lower at 6 months after parasitoid introduction than 1 month after (Fig. 1). At 2 female parasitoids per bottle, host mortality was nearly 55%. At 4, 6, and 8 female parasitoids per bottle, the immature stages of *S. zeamais* were suppressed by nearly 70%. At 10 female parasitoids per bottle, *A. calandreae* was suppressed by about 80%. Thus, the best control of weevils in the long-term experiment was obtained with 10 females per bottle. The results suggest that a high level of weevil control will require the release of large numbers of parasitoids.

Percentage of parasitoid emergence

The PE was significantly affected by parasitoid density and time after parasitoid introduction (Table 2). The statistically significant interaction between density and time indicated that the difference among the 5 densities was inconsistent among the times after parasitoid introduction^{20,21}. Because this interaction was significant, the effects of levels of the main factors on the PE were not compared²⁰.

At each parasitoid density, the PE decreased as the time after the release of parasitoid increased (Fig. 2). The decline in PE at each parasitoid density was

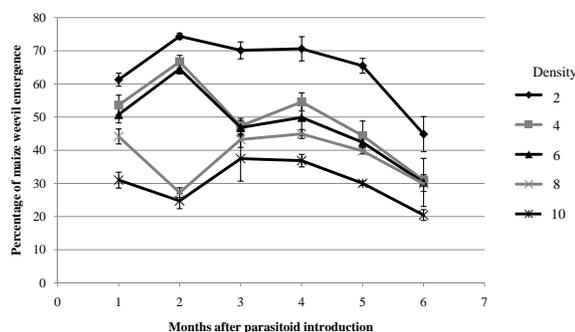


Fig. 1 The WE (mean ± SE of 5 replicates) as affected by parasitoid density and time after parasitoid introduction in the long-term experiment.

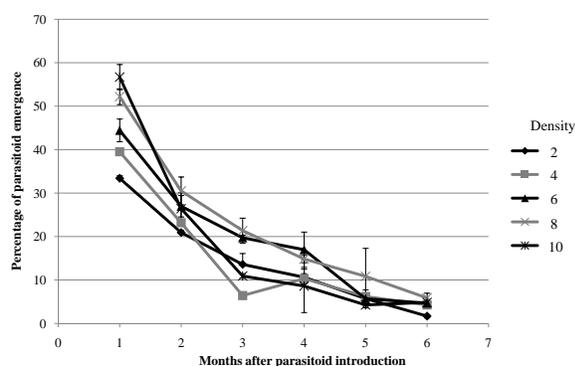


Fig. 2 The PE (mean ± SE of 5 replicates) as affected by parasitoid density and time after parasitoid introduction in the long-term experiment.

probably due to superparasitism, which led to high mortality during larval development, rather than to reduced rates of oviposition. Similar effects of parasitoid density were reported for *Muscidifurax zarapter* Kogan and Legener (Hymenoptera: Pteromalidae), which is a solitary pupal parasitoid of the house fly *Musca domestica*; the decline in *M. zarapter*

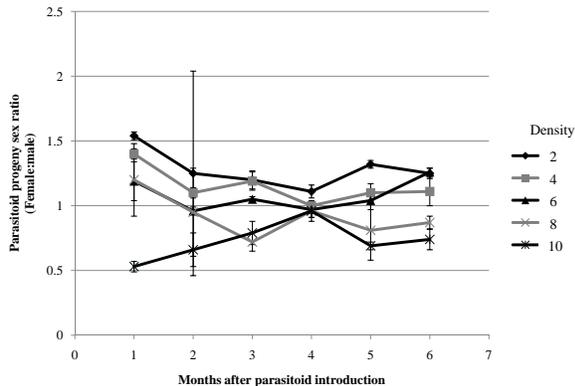


Fig. 3 Parasitoid progeny sex ratio (mean \pm SE of 5 replicates) as affected by parasitoid density and time after parasitoid introduction in the long-term experiment.

emergence at higher parasitoid-host ratios was due to superparasitism²³.

Sex ratio of the parasitoid progeny

Parasitoid density as well as time after parasitoid introduction significantly affected the sex ratio (female:male) of the parasitoid progeny (Table 2). The statistically significant interaction between density and time indicated that the difference among 5 densities was inconsistent among the times after the release of parasitoid^{20,21}. Because this interaction was significant, the effects of levels of the main factors on the sex ratio of parasitoid offspring were not compared²⁰.

The sex ratio of parasitoid progeny decreased with the increase of parasitoid density (Fig. 3). Hassell²⁴ previously presented data from a number of laboratory studies demonstrating that the sex ratio (% female) of parasitoid progeny decreased as a function of female parasitoid density. Our results differ from those reported by Choi and Ryoo²⁵, who found that the sex ratios of *A. calandraye* progeny parasitizing a different host (*C. chinensis*) were unrelated to parasitoid density. They suggested that mutual interference among individual *A. calandraye* reduces host searching efficiency but does not affect progeny sex ratio.

As demonstrated in the current laboratory experiments, the number of parasitoids released can greatly affect weevil control and parasitoid establishment. Similarly, Ngamo et al¹³ reported that control of cowpea weevils depended on the number of *A. calandraye* females introduced into the infested cowpea. In granaries, host-parasitoid ratio is also likely to affect the control of maize weevil. If a large number of parasitoids are released, superparasitism may cause the decline in *A. calandraye* emergence. As a result,

the parasitoid progeny could disappear before maize weevils are adequately controlled. In addition to the number of parasitoids released, the frequency of release could also greatly affect weevil control. In our long-term experiment, control could have been greater with weekly rather than monthly releases of *A. calandraye*. The long-term experiment, however, focused on the effects of parasitoid density rather than on frequency of parasitoid release.

In commercial rice storage, the use of biological control may be limited by many factors. First, the use of *A. calandraye* for suppressing maize weevil may increase costs. Second, releasing large numbers of parasitoids in vertical silos or rice stacks may be difficult, and the efficiency of the parasitoids in such situations may be poor. Press¹¹ compared the penetration by two weevil parasitoids, *A. calandraye* and *C. elegans*, in stored wheat infested with the weevil *S. oryzae*; although both parasitoids were effective against rice weevils near the surface of the grain mass, *A. calandraye* could not move downwards to suppress the weevil host in the bottom of the grain mass. Third, there are many pest species associated with stored rice and not all are attacked by parasitoids. Fourth, the presence of the parasitoids in the mill may not be acceptable in the retail trade.

CONCLUSIONS

Based on our studies, the pteromalid parasitoid, *A. calandraye*, may be an effective biological control agent if it is introduced in sufficient numbers at the beginning of the storage period so as to suppress the initial increase of maize weevil populations. Given that the host-parasitoid ratio greatly affects biological control in this and other systems, the weevil population size in the field should be estimated and the optimum number of parasitoids should be calculated before release. For long storage periods, the parasitoid may need to be added repeatedly to prevent weevil numbers from increasing when the parasitoid numbers decrease.

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