

## Suppression of *Sitophilus zeamais* Motschulsky by the ectoparasitoid, *Anisopteromalus calandrae* (Howard)

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### Abstract

The use of natural enemies to control insect pests has been developed to reduce using insecticides for safety of human, animals and the environment. Most of the biological control practices are aimed for control of insect pests in the field and there is very little work done for post-harvest sector. *Anisopteromalus calandrae* (Howard) is an ectoparasitic wasp that is found throughout Thailand. The goal of this study is to describe the biology of *Anisopteromalus calandrae* and determine if it can control stored rice insect pests. An experiment on biology of this parasitoid wasp was tested for the wasp progenies in the *Sitophilus zeamais* when fed with milled rice at 32.5°C and 70% r.h. The egg laying stage observed is 1 d, 4.1±0.7 d for larvae, 6.3±0.9 d for pupae and 9.6±1.0 d. for adults. The life span from eggs to adults was 11.4 d. Female parasitoid wasps laid on average 37±14 females and 42±14 males for a total of 79±13 insects. The duration for egg laying is 11 d with the peak 12±5 insects on the 5<sup>th</sup> day. The parasitoid wasp sex ratio for female to male was 0.88: 1. Maize weevil larva at 21 d old reared on brown rice at 21d gave the highest yield of wasps (65±17). The number of the wasps produced was reduced to 57±17, 56±21 and 40±22 when raised with the weevil larva ages, 19, 23, and 25 d, respectively. The weevil larva age at 21 days was the most economic stage for the parasitoid wasp mass rearing. To obtain 1,124±236 wasps will use 220 g brown rice after 33 d rearing. The efficacies of the reared parasitoid wasps on controlling the *S. zeamais* was conducted by releasing 1,000 and 800 wasps into 25 kg rice at Rachaburi Rice Experiment Station yielded good control of the *S. zeamais* and with good rice quality. The rice without the parasitoid was heavily damaged and it had poor quality.

Keywords: *Sitophilus zeamais*, Ectoparasitoid, *Anisopteromalus calandrae*, Life cycle, Control efficacy

### 1. Introduction

Rice is an economic important cash crop for Thailand, both for exporting and for local consumption. Milled rice in storage is often faced with stored product insect infestation. The maize weevil (*Sitophilus zeamais* Motschulsky) is a common insect that infests rice in Thailand (Sukprakarn, 1985; Visarathanonth and Sukprakarn, 1988). Natural enemies have been extensively studied to control the stored product insect pests. The parasitoid wasp (*Anisopteromalus calandrae*) is a natural enemy of many stored product insects and it has been reared commercially in many countries (Perez-Mendoza et al., 1999; Haines, 1991; Hayashi et al., 2004; Subramanyam and Hagstrum, 1995). Amed (1996) reported the life cycle of this parasitoid wasp on lesser grain borer (*Rhyzopertha dominica* (F.)) fed with wheat kernels. This parasitoid wasp is widely distributed in stored products in Thailand (Konishi et al., 2004), so there is potential for its populations to be artificially reared and released to parasitize and control the stored rice insect pests in warehouses.

The goal of this study was to determine if the parasitoid wasp (*A. calandrae*) can be used to control *S. zeamais*, a major stored pest of the rice grains in Thailand.

### 2. Materials and methods

#### 2.1. Biology of parasitoid wasps

A pair of parasitoid wasps, *Anisopteromalus calandrae* (male and female) was reared with 21-d-old larvae of *S. zeamais* previously fed with 50 grains of milled rice in each plastic box at 32.5°C and 70% r.h. The wasps were released into new boxes of *S. zeamais* larvae after every other day until the wasps died. The new generations or progenies of the parasitoid wasps from each plastic box were checked for total ratios of the resulting male and female insects.

## 2.2. Effect host age on wasp production

One hundred *S. zeamais* were reared with 50 g of milled rice in each plastic box at 32°C and 70% r.h. for 1 d before removal all of the weevils. The infested rice in all plastic boxes was separately kept for 19, 21, 23, and 25 days before five pairs of wasps were released in each treatment. The wasp progeny were checked for male and female insect ratios.

## 2.3. Mass rearing of wasps

Three hundred *S. zeamais* were reared with 200 g of brown rice for 7 d, then all adults were removed and the rice stored for another 21 d at 31 - 35°C. Fifty pairs of the wasps were then released into the *S. zeamais* infested rice. After 14 -15 d, the number of emerged wasps was counted.

## 2.4. Large scale trial

Five hundred 2-3 wk old *S. zeamais* adults were released into 25 kg of rice in jute bags placed in plastic boxes with lids. The *S. zeamais* were left to lay eggs for 7 d, and then all adults were removed. Rice was left for another 21 d, allowing the *S. zeamais* to grow to the ideal larval stage for wasp reproduction. Five hundred *A. calandreae* were released into the *S. zeamais* infested rice and left for 14 days before removal all of the wasps. Three doses of the wasps were released; 1,000, 800, and 0 wasps/box. Each treatment had 4 replications. The wasps, at the various levels, were released into the rice every month for one year. At the same time at each month wasp released, a 250 g sample was taken from the 25-kg rice bulk and the number of *S. zeamais* adults counted.

Another set of experiments was conducted at the same time and inside the 25-kg rice bulks. Each month, twelve bottles with 200 g rice infested *S. zeamais* infested were prepared. One bottle was placed in each of the boxes within the 25 kg of rice. No additional parasites were added to these bottles. Bottles had lids that allowed the parasites to enter and leave at will but prevented the *S. zeamais* adults from entering or leaving the bottles. The bottles were replaced each month at the same time of 250-g rice samples were taken from the 25-kg rice bulk. The rice was shaken and the number of adult *S. zeamais* was counted.

The experiments were conducted at the Stored Product Insect Research Lab, Postharvest and Product Processing Research and Development Office, Department of Agriculture, Bangkok and the Rachaburi Rice Experiment Station in Rachaburi Province, Thailand, during October 2003 – September 2005.

## 3. Results and discussion

### 3.1. Biology of parasitoid wasps

The life cycle study of parasitoid wasp (*Anisopteromalus calandreae*) was studied by rearing this insect with 21-d-old- *S. zeamais* larvae previously fed with milled rice at 32.5°C and 70% r.h. The result indicated that *A. calandreae* egg, larva, pupa, and adult periods are 1, 4.1, 6.3, and 9.6 d, respectively (Table 1) for a total time from egg to adult of 11 d.

**Table 1** The duration of eggs, larvae, pupae, and adults of parasitoid wasps (*Anisopteromalus calandreae*) reared with 21-d-old *Sitophilus zeamais* fed with milled rice at 32.5°C and 70% r.h., n=20.

Insect stage	Duration Mean $\pm$ SD (d)	Range (d)
Egg	1.0 $\pm$ 0	1-1
Larva	4.1 $\pm$ 0.7	3-5
Pupa	6.3 $\pm$ 0.9	5-8
Adult	9.6 $\pm$ 1.0	7-11

The *A. calandreae* egg incubation periods on fully grown larvae of *R. dominica* are 36 h at 26°C and 27 h at 30°C (Amed, 1996). The larval stage, pre-pupal, and pupa lasted 6.9 and 5.4, 23.6 and 17.8, 5.4 and 4.6 d at 26 and 30°C (Amed, 1996), respectively. The total time from egg to adult lasted 18.9 days at 26°C and 14.6 days at 30°C. However, the life cycle from the eggs to adults of this parasitoid wasp on the *S. zeamais* in this study was approximately 11 d, this shorter time is likely due to the slightly higher temperature, 32.5°C, used in our experiments.

The duration for female parasitoid wasps (*A. calandreae*) to lay eggs was 11 d with 7.7 eggs at the first day and peaked at 12.4 eggs on the fifth day. The wasp progenies were then reduced afterward to the 11<sup>th</sup>

day on the last day of insect died. One female *A. calandreae* could lay 90.4 insects, 43.9 females and 46.5 males (Table 2). The sex ratio of female to male is 0.88:1. Amed (1996) has conducted a similar study to this study with *R. dominica* and could obtain 150 wasps at 26°C and 133 at 30°C with the insect ratio 2.1 and 2.3 at 26°C and 30°C, respectively. The wheat- *R. dominica* combination produced more wasps than our system using rice and *S. zeamais*. However, wheat costs more than rice in Thailand, therefore more work is needed to find the most cost effective production that still produces high numbers and high quality wasps.

**Table 2** Numbers of parasitoid wasp (*Anisopteromalus calandreae*) progeny per female, n=20.

Age (d)	Number of female progeny (Mean ± SD)	Number of male progeny (Mean ± SD)	Total wasps (Mean ± SD)
1	2.1 ± 2.2	5.6 ± 2.2	7.7 ± 3.1
2	2.4 ± 1.5	6.9 ± 2.2	9.2 ± 2.1
3	3.8 ± 3.3	7.1 ± 3.7	10.9 ± 4.4
4	5.1 ± 3.2	5.8 ± 3.5	10.8 ± 3.7
5	6.5 ± 4.3	5.9 ± 3.7	12.4 ± 5.0
6	5.6 ± 4.6	4.2 ± 4.6	9.8 ± 4.5
7	4.5 ± 2.6	2.5 ± 2.5	7.0 ± 2.4
8	3.4 ± 3.0	2.2 ± 2.6	5.5 ± 3.0
9	3.0 ± 2.2	1.6 ± 1.8	4.5 ± 2.3
10	2.8 ± 2.4	2.8 ± 2.6	5.5 ± 4.2
11	4.7 ± 3.3	1.9 ± 2.7	5.6 ± 2.5
Total	43.9 ± 32.6	46.5 ± 32.1	90.4 ± 64.7

### 3.2. Effect host age on wasp production

The progeny of wasps when raised on the *S. zeamais* previously fed with brown rice from all of the treatments were shown to have higher female ratios than male. This could benefit on using the female wasps for increasing new insect progenies. The 21-d-old larvae *S. zeamais* yielded 65.4 wasps, the highest number of wasp progenies for all ages of the host (Table 3).

**Table 3** Effects of *Sitophilus zeamais* age on the progeny production of the parasitoid wasps (*Anisopteromalus calandreae*), n=20.

<i>S. zeamais</i> age (day)	Number of parasitoid wasp progeny			Sex ratio (Female: Male)
	Female (mean ±SD)	Male (mean ±SD)	Total	
19	32.1 ± 12.4	24.7 ± 8.9	56.8 ± 21.3	1.3
21	43.1 ± 14.3	22.3 ± 7.4	65.4 ± 21.7	1.9
23	31.3 ± 13.3	24.3 ± 11.0	55.6 ± 24.3	1.3
25	26.2 ± 17.2	13.9 ± 6.8	40.1 ± 24.0	1.9

### 3.3. Mass rearing of wasps

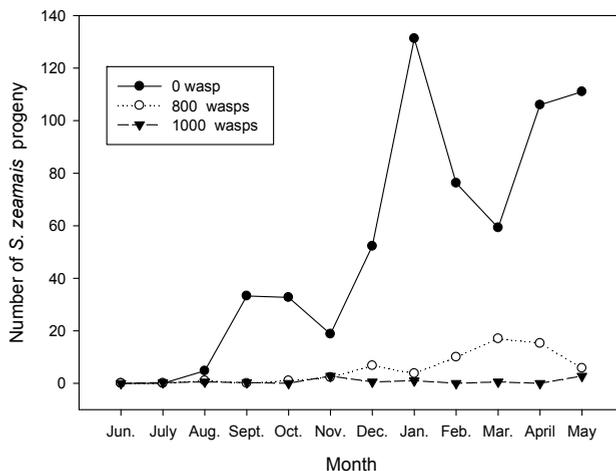
The 21-d-old *S. zeamais* larvae reared on brown rice yielded on average 1,124 wasps with a minimum of 708 wasps and a maximum of 1543 wasps per 200 g brown rice in the plastic boxes.

### 3.4. Large scale trial

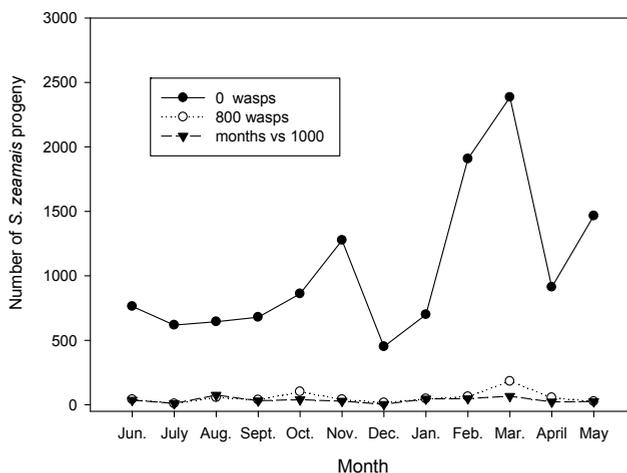
After 6 mo to one year 1000 wasps/25 kg rice reduced *S. zeamais* adults in large rice bulks more than releasing 800 wasps/25 kg, while the treatment without any wasps had the highest infestation of the *S. zeamais*. At the end of one year, the number of *S. zeamais* adults was 3, 6 and 111 weevils/250 g when treated with 1000, 800, and 0 parasitoid wasps, respectively (Fig.1). The weevil infested rice in the bottles at the same period of storage time yielded 24, 26, and 1465 insects when treated with 1000, 800, and 0 parasitoid wasps, respectively (Fig. 2).

Our results show that the parasitoid wasp, *A. calandreae* gave good control of *S. zeamais* in both large and small sizes of the rice samples in this study. The quality of the parasitoid wasps released rice remained

good with minor weevil infestations in a few replications. The results indicate that it may be possible to replace chemical insecticides for control of *S. zeamais* and to apply in the integrated control programs in the near future. The use of this biological control agent could reduce insecticide residues on rice, require fewer fumigants of rice in warehouses thereby improving worker safety. It could also reduce the development of insecticide resistance developing in insect populations and protect the other beneficial insects found in grain storages for both better stored product quality and quantity.



**Figure 1** Monthly numbers of *Sitophilus zeamais* adults in 250 g rice samples taken from 25-kg rice sacs at three densities of the parasitoid, *Anisopteromalus calandrae*.



**Figure 2** Monthly numbers of *Sitophilus zeamais* adults in 200 g rice samples taken bottles placed in a 25-kg rice sacs with three densities of the parasitoid, *Anisopteromalus calandrae*.

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