EFFECT OF PARASITOID *Anisopteromalus calandrae* (HOWARD)(HYMENOPTERA: PTEROMALIDAE) DENSITY ON THE POPULATION OF MAIZE WEEVIL, *Sitophilus zeamais* (MOTSCULSKY)(COLEOPTERA: CURCULIONIDAE) IN MILLED RICE

INTRODUCTION

In Southeast Asian countries, approximately 10-30% of postharvest losses of grains have been estimated. The damages caused mainly by improper drying, insect infestation during crop storage and distribution, under hot and humid climatic condition. Rice is the most important staple crop in Thailand. Paddy and milled rice was infested by insects resulting in great losses and poor yield. Thus this problem could affect the exporting rice (Hayashi *et al*., 2004). Methyl bromide (MeBr) is one of the most effective fumigant for controlling stored product pests. However, this fumigant has been banned in developed countries since 2005. It also will be forbidden in developing countries in 2015 because it has harmful side effects by destroying the ozone and has negative effects on human health (Nishi *et al*., 2004).

Despite the progressive reduction of methyl bromide for fumigating stored product insect pests, many control options remain but few have been evaluated thoroughly (White and Lessch, 1996; Lucas and Riudavets, 2002 a). The options include physical (Lucas and Riudavets, 2002 c), chemical (Harrein, 1991) and biological control methods (Lucas and Riudavets, 2002 b). Parasitoids are one of important factors regulating insect pest population dynamics. Important natural enemies are parasitoid wasps in several families such as Braconidae, Ichneumonidae, Pteromalidae and Bethylidae. In addition predators could be found in some situations, and these include pirate bugs, beetles, pseudoscorpions, and predatory mites. Pathogenic bacteria and viruses occur naturally within storage insect communities. *Bacillus thuringiensis,* the bacterium commonly known as Bt, affects stored product moth larvae and is available commercially for use on grains. However, many reports are coming in of insect resistance to this pathogenic pesticide. Scientists in USDA's Agricultural Research Service, America, conducted operational field trials of parasitoids and predators in large metal bins of grain identical to those used on farms and at county elevators. Results of these studies confirmed that natural enemies could be used in bulk grains to reduce pest populations, and some application methods were recommended. The use of natural enemies against stored-product pests is legally permissible in the U.S., but the regulatory process applied to these organisms resulted from interesting series of events. As research results on the efficacy of storage natural enemies became generally known, a commercial insectary began producing some of these species for distribution (Phillips, *et al*., 2000).

Thus biological control should be one of insect pest management alternatives for postharvest agriculture to reduce the use of pesticides on food and to produce high quality food products in Thailand.

OBJECTIVES

To find the optimum host parasitoid ratio for controlling *Sitophilus zeamais*, the important stored product insect pests in milled rice.

LITERATURE REVEIW

Natural Enemies

Natural enemies such as parasitoids and predators are well known as control agents for controlling the field insect pests, since they are safe without any harmful results to produces. Another pest management strategy is the use of natural enemies for storage pests. Several parasitic wasps and predators are common in tropical stores and their potential to control storage pests has been investigated in some cases. Parasitic wasps are natural components of stored ecosystems, some of which belong to order Hymenoptera. Twenty one species of parasitoid belonging to seven families, Chalcididae, Eurytomidae, Pteromalidae, Eulophidae, Evaniidae, Braconidae and Bethylidae were found in rice stores in Thailand. Among them, nine parasitoids (with asterisk) were abundant or frequently found (Hayashi *et al*., 2004).

Chalcididae

This family is cosmopolitan in distribution. They can be easily distinguished from other families of hymenopterous parasitoids collected in rice stores by conspicuously enlarged hind femur, with a comb of teeth on the ventral margin. The Chalcididae is important as a natural enemy of stored product moths. *Proconura minusa* Narendran*, *Proconura caryobori* (Hanna)*, *Antrocepphalus mitys* (Walker), *Notaspidiella clavata* Narendran and Konishi, *Notaspidium thailandicum* Narendran and Konishi and *Hockeria* spp. were found in rice stores in Thailand.

Eurytomidae

The only one species, *Eurytoma* sp. was found in rice stores in Thailand. There have no report on its insect host, but this species was collected often with *P. minusa*, thus it is thought to be a secondary parasitoid of *P. minusa.*

Eulophidae

Aprostocetus hagenowii was only one species found in Thailand.

Evaniidae

Species of this family are known to parasitize the egg case of cockroaches. A female lays a single egg in each egg case and a hatched larva feeds all the eggs in the egg case.

Braconidae

*Bracon hebetor** is cosmopolitan in distribution. In Thailand this parasitoid was abundant and found on polished rice. It is a gregarious ectoparasitoid of caterpillars feeding on storage grains. Females paralyze and lay eggs on late instar moth larvae. Each female produce about 100 eggs. This species was sold as biopesticide of stored product moths in USA, and is important as a parasitoid of *Corcyra cephalonica.*

Bethylidae

This family is distinctively and easily recognized by their antlike habitat. They are small, usually less than 5 mm and nearly always less than 10 mm long. The Bethylidae is an important natural enemy of colepterans feeding externally. Five species found in Thailand were *Cephalonomia tarsalis* (Ashmead)*, *Cephalonomia* sp., *Plastanous* sp., *Holepyis sylvandis** (Brethes) and *Holepyis* sp.

Pteromalidae

Pteromalidae is a large cosmopolitan family. This family is one of the most difficult to identify because it is structurally diverse and the identification is based primarily on negative morphological characteristic (Gordh and Hartman, 1987). In Thailand, the Pteromalidae is important as a natural enemy of colepterans in grains such as *Theocolax elegans* (Westwood)*, *Cerocephala dinoderi* (Gahan), *Anisopteromalus calandrae* (Howard)* and *Lariophagus distinguendus* (Forter).

Anisopteromalus calandrae (Howard) is a dominant parasitoid attacking coleopteran larvae in stored grains (Konishi *et al*., 2004).

A*nisopteromalus calandrae* (Howard)

Original name : *Pteromalus calandrae*

Synonym : *Aplastomopha pratti*, *Bruchobius mediums*, *Meraporusvandinei*, *Neocatolaccus australiensis*, *N. indicus*, *N. mamezophagus*, *Pteromalus oryzae*.

Host: *Acanthoscelides obtectus* (Coleoptera: Bruchidae), *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae), *Apomyelois ceratoniae* (Lepidoptera: Pyralidae), *Araecerus fasciculatus* (Col. Anthribidae), *Athesapeuta cyper* (Col. Curculionidae), *Bruchus pisorum* (Col. Bruchidae), *B. rufimanus* (Col. Bruchidae), *Callosobruchus analis* (Col. Bruchidae), *C. chinensis* (Col. Bruchidae), *C. maculatus* (Col. Bruchidae), *C. phaseoli* (Col. Bruchidae), *Carpophilus obsoletus*

(Col. Nitidulidae), *Caulophilus oryzae* (Col. Curculionidae), *Ceutorhynchus assimilis* (Col. Curculioidae), *Ephestia cautella* (Lep. Pyralidae), *E. elutella* (Lep. Pyralidae*), E. kuehniella* (Lep. Pyralidae), *Lasioderma serricorne* (Col. Anobiidae), *Oryzaephilus surinamensis* (Col. Silvanidae), *Pempheres affinis* (Col. Curculionidae), *Piezotrachelus variius* (Col. Curculionidae), *Prostephanus truncates* (Col. Bostrichidae), *Rhyzopertha dominica* (Col. Bostrichidae), *Sitophilus granarius* (Col. Curculionidae), *S. oryzae* (Col. Curculionidae), *S. zeamais* (Col. Curculionidae), *Sitotroga cerealella* (Lep. Gelechiidae*), Stegobium paniceum* (Col. Anobiidae), *Tribolium castaneum* (Col. Tenebrionidae), *Tricorynus herbarium* (Col. Anobiidae), *Trogoderma granarium* (Col. Dermestidae), *Zabrotes subfasciatus* (Col. Bruchidae)

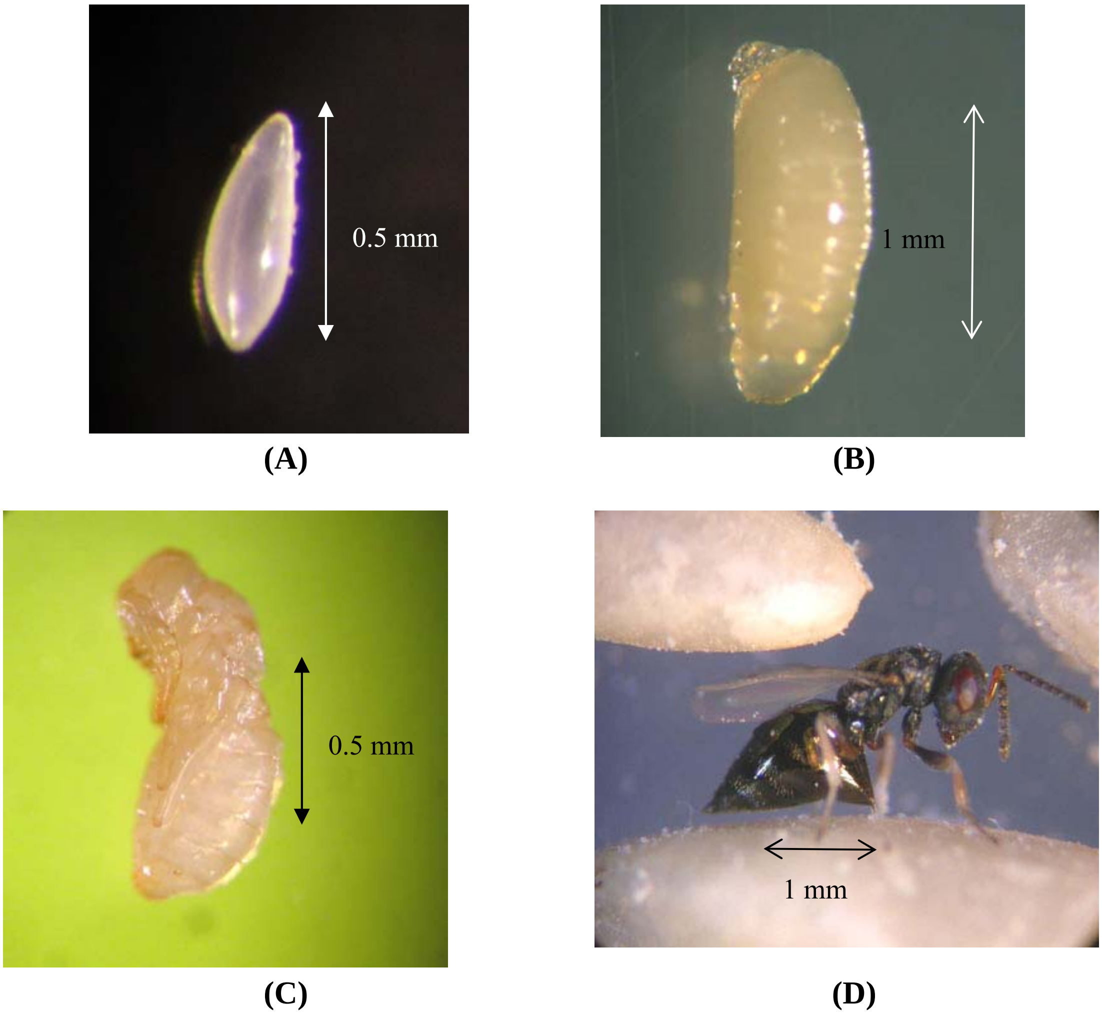


Figure 1 Life stage of *Anisopteromalus calandrae*.
(A) Egg
(B) Larva
(C) Pupa
(D) Adult

Description

Egg: The egg is oblong in shape, small size and cannot be seen by naked eyes. It is whitish or colorless and nearly transparent. The outer shell is smooth and is covered with sticky substance that aids in attaching the egg to surfaces and causes small particles to adhere (Fig. 1A).

Larva: Mature larva is 2 mm long and 0.5-1 mm wide, white and legless (Fig. 1B).

Pupa: The pupa is uniformly white in color when it's first formed; then gradually darkens and assumes adult structure before fully mature. It is 2.5-4.00 mm in length and 3.5 mm in width (Fig. 1C).

Adult: An adult of *A. calandrae* is sexually dimorphic; a female is entirely black and 2.5 mm in length while a male has partly milky-white abdomens and 2.0 mm in length (Onodera *et al*., 2002) (Fig. 1D and 2). Forewings of both sexes do not have a tuft of hairs and cloud, always with wings. Femora legs are brown or black.

Life history

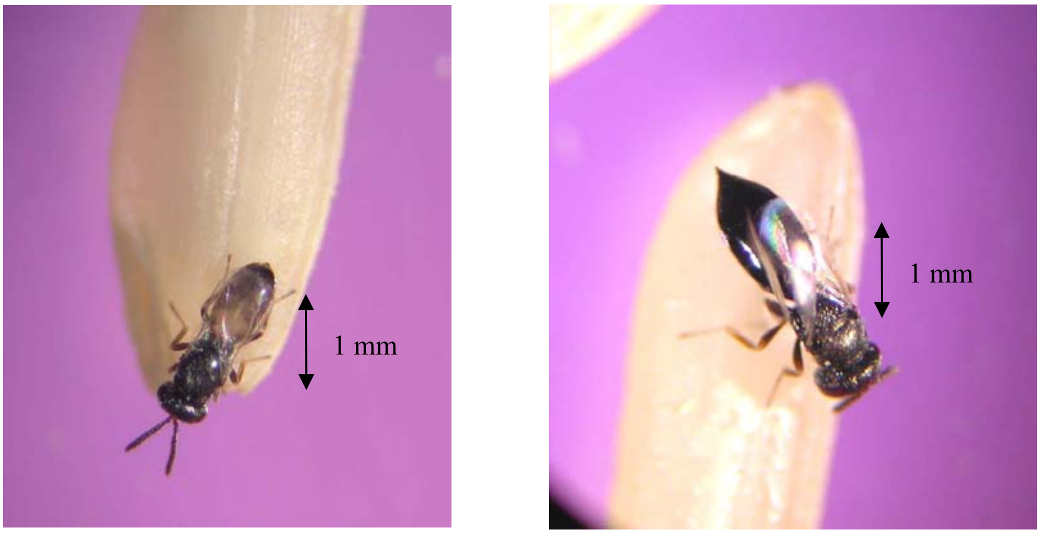
Anisopteromalus calandrae is a solitary and larval ectoparasitic wasp that parasitizes numerous coleopterans (Fig. 3). This wasp does not feed on the grain. It normally dies within five to ten days if no weevils are available in the stored grain. This parasitoid is found naturally in stored grain suggesting that once released it tends to continue suppressing pests for many years. When a grain containing host larva is given to a female, the following sequential behaviors are observed; the female walks and examines surface of the grain with antennal tapping, bends her abdomen vertically. After detecting an internally feeding host, the female parasitoid uses its ovipositor to drill through the grain surface, paralyzes the larva and lays a single egg on it. Sting behavior is only observed when the female locates grains that contain host larvae (Onodera *et al*., 2002). The hatched larva may use the hole made by its mother or makes its own hole to extract the host body fluid. The larval host dies when the parasitoid larva is fully grown. Its fecundity is approximately 280 eggs. Unlike other adult parasitoid females, *A. calandrae* female does not feed on the body fluid of the host, but it may feed on flower nectar (Ahmed, 1996 a). The adult longevity depends on food supplementary.

Ahmed (1996 b) studied *A. calandrae*'s biology. *Anisopteromalus calandrae* was reared in laboratory on full grown larvae of lesser grain borer, *Rhyzopertha dominica* (Fab.). The incubation period of the parasitoid's egg was 36 h at 26°C and 27 h at 30°C. The larval stage lasted 6.9 and 5.4 days, the pre-pupal stage lasted 23.6 and 17.8 h, and the pupa lasted 5.4 and 4.6 days at 26 and 30°C, respectively. The total developmental time, from egg to adult, averaged 18.9 and 14.6 days at 26 and 30°C, respectively. Adult parasitoid required 14 days to emerge from parasitized *S. granarium*. An increase in temperature reduced the duration of immature stages by nearly 20%. The sex of field collected material was close to that from material that bred in the laboratory, when available hosts were not limited. Sex ratio, female: male, was 2.1: 1 in the field and 2.3: 1 in the laboratory. The daily and total numbers of egg laid per female were 6.7, 150.4 and 8.3, 132.6 at 26 and 30°C, respectively. The adult parasitoid female and male, fed on honey, lived for 32.6 and 25.5 days at 26°C, respectively. The egg production significantly increased with the increase of temperature. On the other hand, the total eggs laid per female and their life span decreased as the temperature rising. The short life span during hot conditions may result from the higher metabolic rate and energy utilization causing death sooner. The increase of temperature may activate hormonal activity, expediting the utilization of more vitelline precursors producing more eggs in shorter time and making up for reduction of life span (Ahmed, 1996 b).

Distribution

Anisopteromalus calandrae has been sold as a natural enemy of stored product beetles. Its distribution is world wide. The records ranged from Argentina, Australia, Austria, Bangladesh, Bermuda, Bolivia, Brazil, Columbia, Cuba, Czech Republic, Egypt, El Salvador, France, Greece mainland, Guyana, Hungary, India, Iraq, Israel, Italy, Jamaica, Japan, Kenya, Korea, Madeira Islands, Malawi, Malaysia, Mauritius, Mexico, Morocco, Myanmar, New Zealand, Nigeria, Pakistan, Papua New Guinea, Peru, Puerto Rico, Romania, Seychelles, Slovakia, Solomon Islands, Taiwan, Tanzania, Thailand, USA, United Kingdom and Venezuela. This parasitoid is one of the dominant species in Thailand (Hayashi *et al*., 2004**).**

Williams and Floyd (1971) and Arbogast and Mullen (1990) studied the potential for suppressing population of maize weevil in corn by *A. calandrae* and *T. elegans* (Westwood). They similarly reported that in stored corn, *A. calandrae* was more efficient at parasitizing immature maize weevil than *T. elegans*. The sex ratio (percentage female) of *T. elegans* was significantly reduced by the presence of *A. calandrae* but not vice versa.



(A) **(B)**

Figure 2 *Anisopteromalus calandrae* (A) male and (B) female.

Wen and Brower (1994) studied competition between *A. calandrae* and *T. elegans* at different parasitoid densities on immature rice weevil in wheat. They found that under competitive conditions, the sex ratio of *T. elegans* was decreased by the presence of *A. calandrae* but the sex ratio of *A. calandrae* was not affected by *T. elegans*. Competition reduced emergence of both species. However, *A. calandrae* was clearly the dominant species when rice weevils were exposed to equal numbers of both parasitoid species.

Several studies have focused on *A. calandrae* life history (Smith, 1992), resistance to pesticides (Baker and Weaver, 1993 and Baker and Thorne, 1995), competition with other parasitoids and regulation of progeny sex in relation to host preference, host vulnerability and host size (Wen and Brower 1995; Ryoo *et. al*., 1996 a). No research has been done so far to evaluate the optimum density of *A. calandrae*, parasitoid-host ratio, for controlling *S. zeamais*, the important stored pest in milled rice.

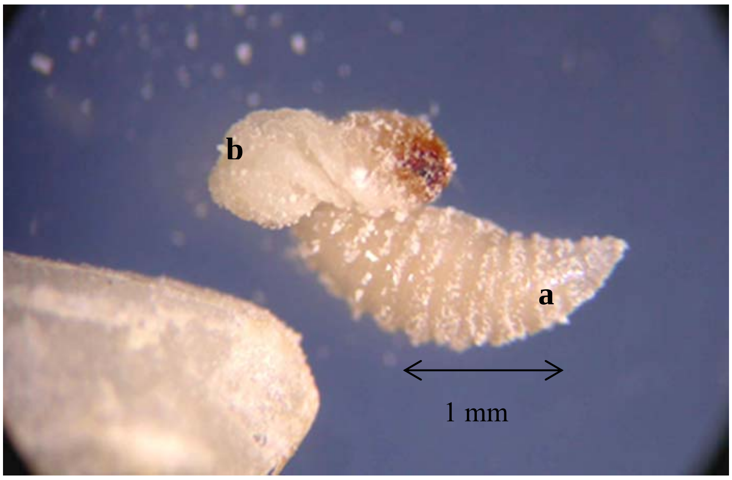


Figure 3 *Anisopteromalus calandrae* feeding on its host *Sitophilus zeamais*.
a: *Anisopteromal calandrae*
b: *Sitophilus zeamais*

Stored Product Insect Pest

The custom of storing plant materials, mainly grain and its products, provides a habitat with shelter, feeding grounds and breeding sites to which a group of insects, known as stored product insect pest, has successfully adapted. About 100 insect species, mainly from the order Coleptera, are able to complete their development and spend entire life cycle in stored products. Worldwide losses of stored products due to postharvest insect pest attack are estimated to be 15 % annually and tremendous costs are involved to protect product commodities against insect infestation (Plarre and Vanderwel, 1999).

According to their small body size, these insects smartly conceal themselves particular in grains. Many of them have achieved their extensive distribution through transportation to all parts of the world commercially, henceforth they become truly cosmopolitan in distribution. However, the conditions in all parts of the world are not equally favorable for the development of all of these insects. For example, in some regions where some species flourish whilst others are barely able to exist (Kengkarnpanich, 2003). The amount of damage inflicted by stored product insect pests in a particular storage situation is largely a function of their feeding habits and the population levels that they are able to attain. The population level depends upon the number initially present, a time available for population growth, and its biotic potential modified by the combined action of predators, parasites and other adverse factors.

The earliest identifiable references to storage species in Britain date back to 1825, but in USA, there is some archeological information for the previous century. There are also some archeological record from Egypt and Roman Britain, but the history of human beings, their agricultural pursuits and the insects associated therewith, extend much further into the past (Howe,1987).

Sitophilus zeamais (Motschulsky)

Common name: maize weevil
Family: Curculionidae
Order: Coleoptera

Sitophilus spp. are the most serious insects in rice storage in Thailand, especially rice weevil, *S. oryzae* and maize weevil, *S. zeamais*. These two species are morphologically similar leading to difficulty in identifying (Fig. 4). They were first described by Linnaeus in 1763 as *Curculio oryzae*, this first name was later revised by Clairwille and Schltenberg in 1798 as *Calandrae oryzae*. Many worker realized that two distinct forms of species existed which were described as the small and large forms. Motschulsky recognized a large form as a distinct species, which he named *Sitophilus zeamais* in 1855. Takahashi complicated matters by raising the small form to specific status as *Calandrae sasakii* in 1928 (Boudreaux, 1968 a; Kiritani, 1965). This confused situation still continued. Howe (1952) described his work on the biology of *S. zeamais* under name *S. oryzae*. In fact, Linnaeus has originally described the smaller species, and Motschulsky's description of larger species was valid (Boudreaux, 1968 b). Since the size difference between *S. oryzae* and *S. zeamais* was not constant, so it is not possible to be sure that the small or the large form refer to *S. oryzae* or *S. zeamais* (Jumroenma, 1992 a). However, *S. zeamais* is more dominant than *S. oryzae* in laboratory.

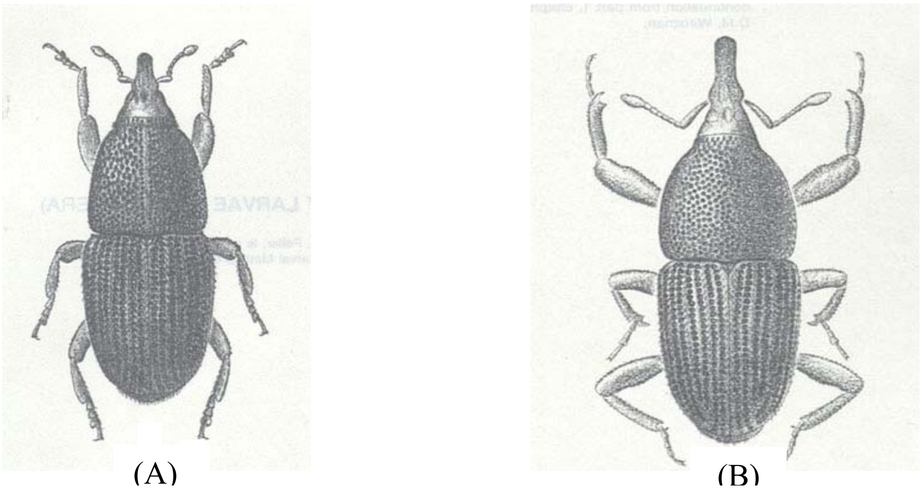


Figure 4 *Sitophilus* spp. (A) Rice weevil, *Sitophilus oryzae* and (B) Maize weevil, *Sitophilus zeamais*.

Source: Whitehead (1987)

Separation of the species can be achieved with certain only by examining the genitalia: *S. oryzae*, upper surface of median lobe of aedeagus evenly convex; *S. zeamais*, upper surface of median lobe of aedeagus flattened and with a distinct longitudinal impression on each side of midline (Halstead, 1963). Whitehead (1987) identified *Sitophilus* species as an illustrate key below (Fig. 5).

> Upper surface of median lobe of aedeagus evenly convex (Fig. 5A); free sclerite at base of median lobe of aedeagus small, with apex rounded (Fig. 5B, 5C); Y shaped sternite VIII (speculum ventrale) of female lateral lobe parallel sided and rounded apically (Fig. 5D); midline of pronotum usually puncture-free (Fig. 5E) -- rice weevil, *S. oryzae*
>
> Upper surface of median lobe of aedeagus flattened, with a distinct longitudinal impression on each side of midline (Fig. 5F); free sclerite of aedeagus large, with apex cute (Fig. 5G, 5H)(Fig. 6a); lobes of sternite VIII of female gradually and evenly tapered and vaguely acute apically (Fig. 5I)(Fig. 6b); punctures usually present along midline of pronotum (Fig. 5J).
> -- maize weevil, *S. zeamais*

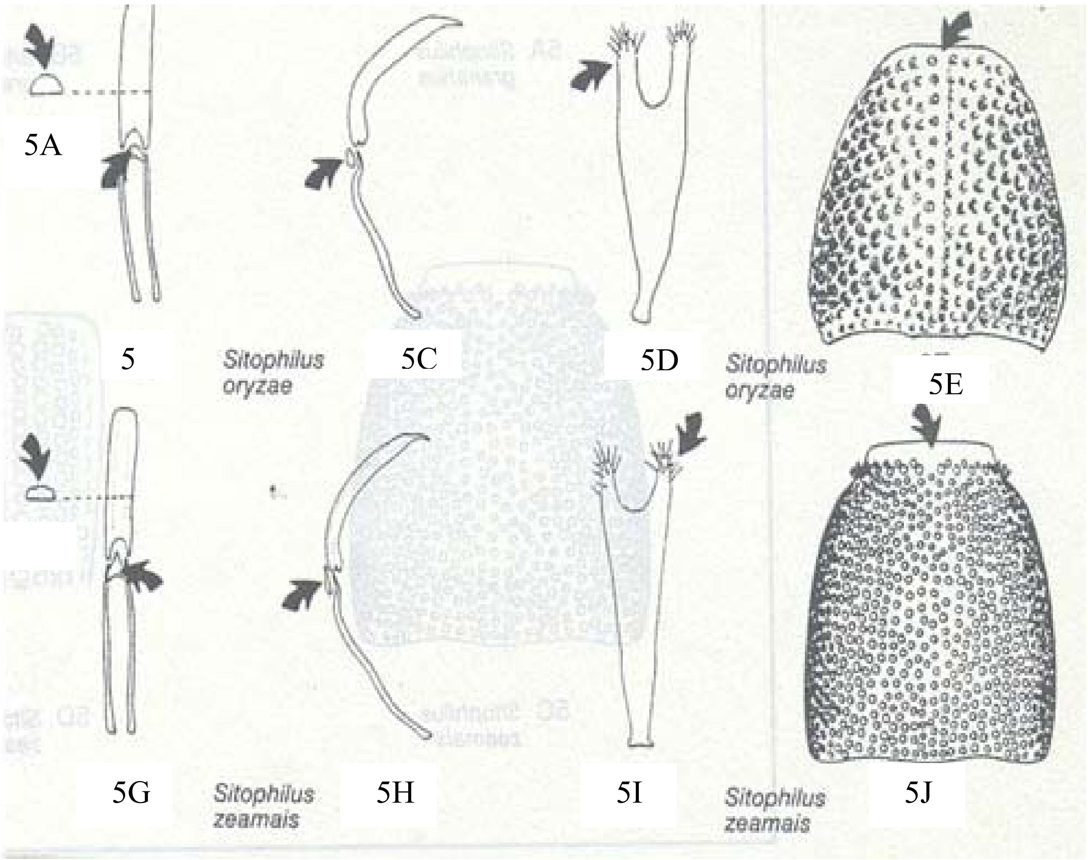


Figure 5 Identification of *Sitophilus* spp.

Source: Whitehead (1987)

The lateral and dorsal surface of the male snout is covered by irregular pits producing a rough appearance (Fig. 7a), the pits on the female snout are ovoid, regularly spaced, and surface between the pit is smooth with shiny appearance (Fig. 7b) (Dobie *et al*., 1984).

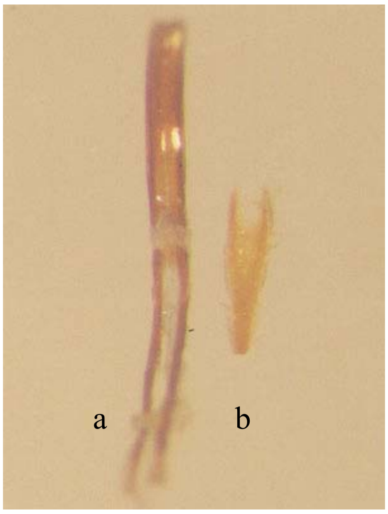


Figure 6 Genitalia of maize weevils.
a: male
b: female

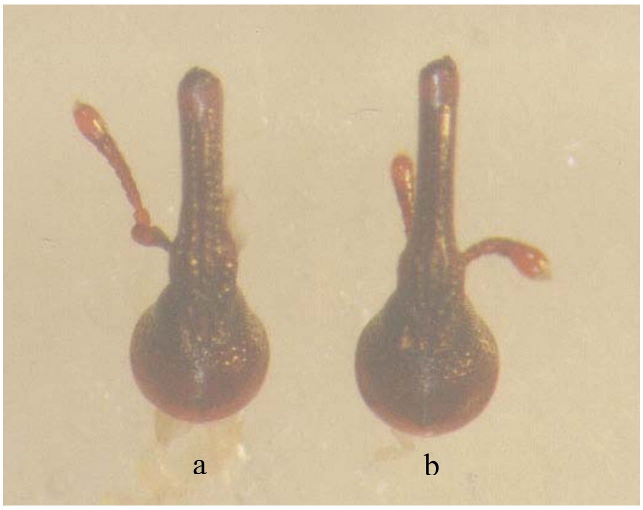


Figure 7 Snout of maize weevils.
a: male
b: female

Description

Egg: The egg is opaque, shining white; ovoid to pear shaped, widest below middle; bottom broadly rounded, neck narrowing gradually toward top, which is somewhat flattened and has a small rounded protuberance that fits into a cap or plug cementing the egg in place. It's small size and cannot be seen by naked eyes. Length 0.65±0.04 mm, width about 0.27± 0.02 (Nualvatna *et al.*, 2005 a) (Fig. 8A).

Larva: There are four instars in the larval stage. A mature larva is 2.50 ±2.75 long, pearly white, legless, fleshy and very thick body. The head and mouth parts are usually intact structures as food fragments. The larval instar could be identified by measuring the width of the head capsules of larvae dissected from kernels. In corn, the average widths of tunnels are 0.33 mm, 0.55 mm, 0.80 mm and 1.49 mm for first, second, third and fourth instars, respectively (Jumroenma, 1992 c). The mean developmental periods for stadia 1-4 at 27°C and 69±3 % RH are 3.6, 4.7, 4.8 and 5 days, respectively (Fig. 8B).

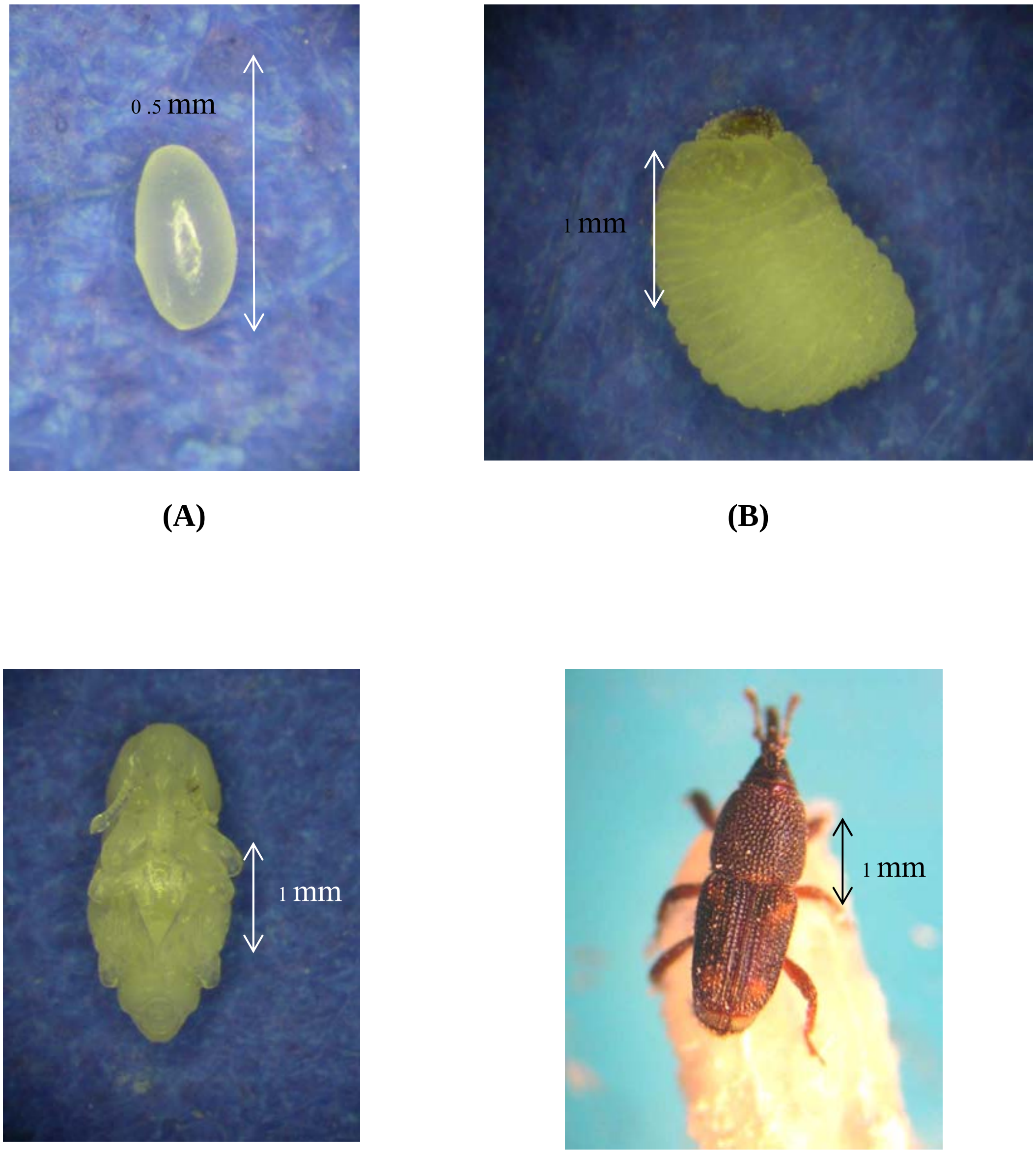
Pupa: The pupa uniformly white in color when it's first formed, then gradually darken and assumes adult structure before fully mature. Length 3.75-4.25 mm, width 1.75 mm, which is not constant, because their body size depends on the kind of food rearing (Fig. 8C).

Adult: The color of adult is brown to black. Some adults have four reddish orange circular marking on elytra. Snout cyrindrical with dorsal margin straight; antenna inserted near base of snout, just in front of eyes, funicle with 6 segments and club with basal segment shiny, pygidium largely exposed. Thorax is longer than wide constricted near apex, sides feebly curved, gradually divergent to base; disk densely and coarsely punctured, body length depends upon food rearing (Fig. 8D) (Table 1).

Table 1 The effect of food type on the body length of *Sitophilus zeamais* adult.

Type of food	Adult body length (mm) Mean (range)
Corn	4.38 (3.9-4.9)
Wheat	3.70 (3.0-4.6)
Milled rice	3.60 (2.9-4.3)
Paddy	3.40 (2.7-3.2)

Source: Nualvatna *et al.* (2005 b)



(A) (B)

(C) (D)

Figure 8 Life stage of maize weevil, *Sitophilus zeamais*.
(A) Egg
(B) Larva
(C) Pupa
(D) Adult

Life history

The adult has a much prolonged life span, several months to one year. Each female laid 150- 300 eggs within the seed by excavating a hole within the grain equal to the length of its slender proboscis. The proboscis is then withdrawn, whereby the cavity is located by the abdomen from which the ovipositor is thrust into the cavity and an egg is deposited. Before the ovipositor is removed, a translucent mass of gelatinous material is discharged on the top of its eggs and it tamped down level with the surface harden, holding the eggs in place and forming a protective covering on it. The young larva starts feeding by burrowing through the tissues of seed. When its development is complete, the newly developed adult chews its way out leaving a large characteristic emergence hole. The total developmental period ranges from 35 to over 110 days under optimal condition (Kengkarnpanich, 2003). Jumroenma (1992 a) and Nawanich (1996 a) showed that the maize weevil did not mate before the age of 3 days. In corn, the female weevil preferably made holes in the endosperm (42%) rather than in germ perimeter (37%) and germ center (21%). In milled rice, all part of grain was equally selected for making hole.

The actual length of developmental life cycle, from the oviposition to the emergence of the adult, depends upon the quality of grains being infested, temperature and humidity. Dobie *et al.* (1984) and Jumroenma (1992 a) reported that, in different varieties of corn, developmental period of maize weevil varied from 31 to 37 days at 27°C and 70% RH. Nawanich (1996 b) showed the activity of maize weevil increased weekly for 4 weeks and virgin males were more active than virgin females of the same age. Tipping *et al.* (1986) and Jumroenma (1992 a) studied the feeding activity of *S. zeamais* and reported that corn weevil exhibited two types of feeding behavior on corn seeds. Adults sometimes penetrated the pericarp over an irregularly area and fed lightly on the surface of the endoderm (shallow feeding). On the other occasions they chewed through the pericarp in a localized area and fed to a much greater extent on the endosperm (deep feeding). The corn weevil can damage stored grain and seed by lowering germination and also creating conditions favorable to the growth of *Aspergillus flavus* (Jumroenma (1992 b). Dix and All (1986) studied the interaction between weevil infestations and infection by *A. flavus* in stored corn and reported that weevil infested corn began heating significantly (by 2°C) within 9 weeks and began losing weight within 12 weeks. The increasing temperature and moisture in the infested grain appeared to create a favorable environment for mold growth.

Distribution

The maize weevil also infests corn, rice and cereal ears in the field and may oviposit in them while they are still green. This insect disperses to corn or rice fields from storage entirely by flight and the adult weevils do not crawl up the stalk but fly directly to the ears of corn or rice. Jumroenma (1992 d) studied the flight activity of corn weevil in the field. He found that the flight periodicity curve was normal and very little flight occurred at night. In the store, the flight periodicity curve was flat topped and flight activity was prolonged far into the night. Temperature appears to be the most important factor on flight activity. The flight periodicity curve of corn weevil had a peak between 15 and 17 hours and corn weevil can fly over long distance, at least a quarter of mile. The time of greatest dispersal flight occurred between 16 and 18 hours. Almost 100 % of the females leaving the bin have been mated. Maize weevil was found in all warm and tropical parts of the world in the maize planting areas. It is strong and can fly ever far, thus it can distribute rapidly. The maize weevil and other stored insect pests were moved and distributed around the world because of the global transportation of stored products. Moreover, the differences in stored condition and regional climate are supportive to maize weevil adaptation and survival.

Food preferences

The maize weevils breed in all common grains such as corn, rice, oats, barley, rye and wheat. They cannot breed in finely farinaceous material such as flour, but breed readily in manufactured cereal products, such as macaroni and noodles. After breeding, the milled cereals have become badly caked from excess moisture. The adult feeds on a variety of seeds and cereal products including flour. It can develop on newly developed cereal crop triticale (a wheat x rye cross) in the laboratory and *Sitophilus* sp. has been reported attacking triticale in the field.

Nualvatana *et al*. (2005 c) reported that a food preference of maize weevil was variable, but the most favorable food was maize grain. Nawanich (1996 a) also reported that the most preference food of maize weevil was corn grain. In the case of rice, detailed surveys in Indonesia showed that maize weevil was predominant on milled rice more than on paddy (Dobie *et al*., 1984; Nawanich, 1996 a). Sukprakarn *et al*. (1984) surveyed maize weevil in Thailand. The survey was conducted in seed stores of farmer, a cooperative, middleman, export warehouse and silo during December 1983 to January 1984. They found that on paddy, milled rice, maize, sorghum, wheat and barley were only infested by maize weevil. Nakakita and Visarathanonth (1991) randomly took *Sitophilus* spp. samples from different location in Chiang Rai provinces, Thailand. The samples were then identified. They observed that the dominant *Sitophilus* spp. in rice storage place was *S. zeamais* rather than *S. oryzae.*

Natural enemies

Sitophilus zeamais is a host of following hymenopterous wasps: *A. calandrae* (Howard), *Theocolax elegans* Westwood, *Holepyris sylvanhnidis* (Bretes), *Cerocephala donodiri* Graham, *Meraparus requistitus* (Tuker), *Dibrachys cavus* (Walker) and *Lariophagus distinguendus* (Forster). These parasites, however, have limited potential as an agent for biological control (Kengkarnpanich, 2003).

MATERIALS AND METHODS

Rearing and preparation of insects for experiments

1. Mass rearing of insects

Maize weevils, *S. zeamais* and its parasitoid, *A. calandrae* used in this study were obtained from the Postharvest Technology Research and Development Laboratory, Postharvest and Product Processing Research and Development Office. Department of Agriculture, Bangkok. Two hundred maize weevils were reared on 250 g milled rice in glass jar (12 cm height x 5 cm diameter). One hundred of adult *A. calandrae*, collected from stock cultures, were introduced into each clear plastic box (15 cm width x 30 cm length x 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.

2. Preparation of insects

Host, immature maize weevil

Twenty unsexed adults maize weevils less than two weeks old, were introduced into each of 140 glass bottles (12 cm height x 5 cm diameter) containing 50 g milled rice, sealed with filter paper. The adult weevils were allowed to oviposit for 7 days and then removed by sifting out from the culture media. The bottles of infested rice were maintained at the rearing condition for 25 days. Afterwards infested rice was transferred to small cheesecloth bags and then sewed with thread. Each bag contained 50 g of infested milled rice.

2.2 Parasitoid, *A. calandrae*

Anisopteromalus calandrae adults that emerged from the clear plastic box within 1 day were used in the experiment (Fig. 9).

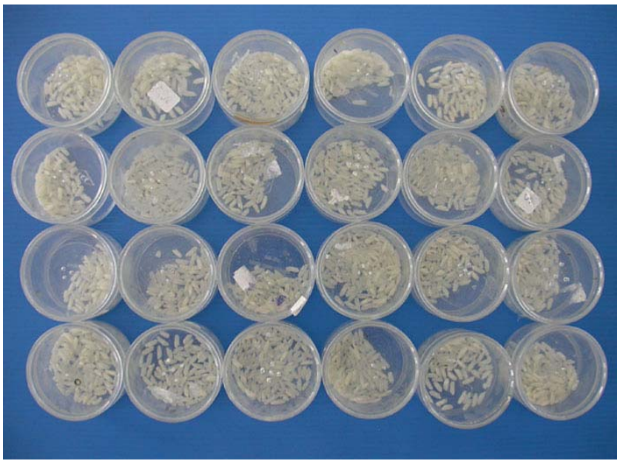
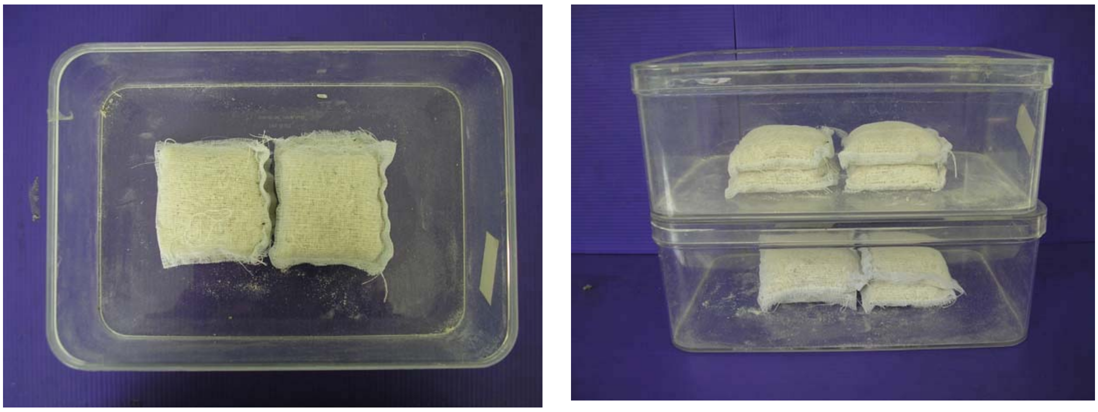


Figure 9 Preparation of newly emerged parasitoid.

3. Short term experiment

A short term experiment was conducted in 35 clear plastic boxes (17cm width x 25 cm length x 9 cm height). Each box contained a pile of four cheesecloth bags (12 cm width x 10 cm length) filled with infested milled rice (Fig. 10). Each box has tight fitting lid with a hole covered with mesh polyester screen for preventing insect escape. Six densities of mated female *A. calandrae*, 0, 2, 4, 8, 12, 16 and 20, were introduced into each treatment box and held for one month. Afterwards, all boxes were frozen for two days. All *S. zeamais* adults were counted to determine percentage of damaged grain.

Each parasitoid density was replicated 5 times. The numbers of maize weevils emerged from the treatment without parasitoids were used as controls.



(A) (B)

Figure 10 Four cheesecloth bags filled with infested milled rice in clear plastic boxes**.**
(A) Top view
(B) Side view

4. Long term experiment

To examine long term host-parasitoid interaction, 10 unsexed adults maize weevil were introduced into each of 6 bottles, 1,000 ml, containing 500 g fresh milled rice. After 25 days, 6 densities of mated female *A. calandrae*, 0, 2, 4, 6, 8 and 10, were added into each bottles (Fig. 11).

One hundred and eighty bottles, 30 bottles per parasitoid density, were prepared. Five bottles of each parasitoid density, thus a total of 30 bottles, were randomly sampled monthly. All emerged maize weevils were counted. To simulate repetitive release of parasitoids in the field, the equal number of parasitoids was added to the remaining bottles after each sampling. The schedule for addition of parasitoids and for maize weevil sampling is presented in Table 2.

The numbers of maize weevils emerged from the treatment without parasitoids were served as controls.

Figure 11 Bottles containing rice grain infested with weevils and their parasitoids in the long term experiment.

Table 2 The schedule for addition of equal number of *Anisopteromalus calandrae* and for *Sitophilus zeamais* sampling.

Experimental set	*A. calandrae* density	Month / Activity*					
		1	2	3	4	5	6
1	0, 2, 4, 6, 8, 10	S	-	-	-	-	-
2	0, 2, 4, 6, 8, 10	A	S	-	-	-	-
3	0, 2, 4, 6, 8, 10	A	A	S	-	-	-
4	0, 2, 4, 6, 8, 10	A	A	A	S	-	-
5	0, 2, 4, 6, 8, 10	A	A	A	A	S	-
6	0, 2, 4, 6, 8, 10	A	A	A	A	A	S

* A= Addition of equal number of *A. calandrae*.
S= *Sitophilus zeamais* sampling.

Data Analysis

The percentage of parasitoid induced mortality (PIM), the percentage of parasitoid emergence and the percentage of weevil emergence were calculated as described by Wen and Brower (1994) and Ryoo *et al*., (1996 b).

PIM = (WC-WPT) X 100/ WC
The percentage of parasitoid emergence = (PE/ WC) X 100
The percentage of weevil emergence = (WT/ WC) X 100

Where WC is the number of rice weevils that emerged in the control
PE is the number of parasitoids that emerged in the treatment
WT is the number of rice weevils that emerged in the treatment
WPT is the number of all insects including parasitoids that emerged in the treatment

Statistical analysis

The effects of parasitoid density on percentage of *S. zeamais* population, the percentage of parasitism and the percentage of weevil emergence and PIM were analyzed by one way ANOVA (Vanichbancha, 2003). The homogeneity of variance was checked by Levene's test. The data were logarithmic transformed to linearlise the data and to meet the assumption of homogeneity of variance. The differences between treatments were assessed by Fisher's least significant difference, LSD. The transformed data were converted back to the original unit prior to presenting in the table.

Places and Duration

Places

The experiment was carried out at the Postharvest Technology Research and Development Group, Postharvest and Product Processing Research and Development Office, Department of Agriculture, Bangkok.

Duration

January 2005 to December 2005.

RESULTS AND DISCUSSION

Short term experiment

The mean number of rice weevils that emerged from the treatment without parasitoids was 748±55.41 per box. The percentages of maize weevil emergence, of parasitoid emergence and of PIM were significantly different among 6 parasitoid densities (Table 3). The percentage of maize weevil emergence significantly decreased from 49.14±1.19 at 2 female parasitoids per box to 7.59±0.62 at 20 female parasitoids per box (Table 4). The mean percentage of *A. calandrae* emergence significantly increased from 24.41±2.02 at 2 female parasitoids per box to 57.96±1.70 at 16 female parasitoids per box, but thereafter decreased to 45.00±1.18 at 20 female parasitoids per box. The mean percentage of PIM significantly increased with parasitoid density. The PIM increased (24.43±2.38 to 27.72±2.37) gradually with the density of parasitoids up to 16 female parasitoids per box**.** The PIM increased to 47.72±1.69 at parasitoid density of 20, which was significantly higher than that of other densities.

In the short term experiment, the highest percentage of parasitoid emergence was 57.96±1.70 at 16 female parasitoids per box, although it did not significantly differ from that of 20 female parasitoids per box. The trend of parasitoid emergence increased with the increasing parasitoid density, but at 20 female parasitoids per box, the parasitoid emergence was only 45.00±1.18, which was lower than that of 16 female parasitoids per box. The decline in parasitoid emergence at high parasitoid density was likely due to superparasitism. At 20 female parasitoids per box, the PIM was nearly two times higher than that of other densities and the number of hosts encountered by the parasitoid did not decrease. This was probably due to intraspecific competition because the number of available host was scarce. Although *A. calandrae* is a solitary parasitic wasp, females avoid ovipositing on previously parasitized hosts, but they are forced to lay more than one egg per host when the number of available hosts becomes scarce. The supernumerary larvae may be eliminated and resulted in a lower percentage of emerged parasitoid.

This result suggested that the appropriate parasitoid for controlling 748±55.41 maize weevils was 16 female parasitoids on condition that the infested milled rice was kept in a pile of four cheesecloth bags (12 cm width x 10 cm length). At this parasitoid density, the percentages of parasitoid emergence was highest, the percentage of weevil emergence and of the PIM were relatively low. The low PIM would be a great advantage for mass rearing and mass releasing of parasitoids in the field.

The results of this experiment suggested that *A. calandrae* could move downward to parasitize infested rice grains which were kept in cheesecloth bags and put into a stack simulating the gunny bags containing rice grain piled up in warehouse or godown. Mahal *et al.* (2005) studied efficiency of *A. calandrae* suppression rice grain infested by *S. oryzae* in five different bag types; jute, calico or American cloth, polypropylene, polythene and nylon. They found that the parasitoids could suppress host populations in all types of bag, except in those made from polythene. The highest percentage suppression, 81%, occurred in calico bags and the lower suppression, 51%, was observed for polypropylene bags. They suggested that the PIM was positively correlated with the parasitoid density.

The sex ratio (female: male) significantly decreased with the increasing parasitoid density (Table 5). The sex ratio decreased from 1.07±0.17 at 2 female parasitoids per box to 0.57±0.03 at 20 female parasitoids per box.

The increase of female parasitoid per box did not affect their progeny sex ratio (female: male). The sex ratio of *A. calandrae* was not related to the density of parasitoid because the sex ratio of *A. calandrae* was higher at 12 females per box than that of 8 females per box. This result was similar to that reported by Ryoo *et al.*, (1996 a). They found that the sex ratio of *A. calandrae* at 6 densities (1, 2, 4, 8, 16, and 20 pairs) per 500 ml beaker containing 210 g fresh brown rice was not significantly different among the 6 density combinations. By contrast, Wen and Brower (1994) reported that the sex ratio of *A. calandrae* at 2 pairs (female: male) per 0.95 liter jar containing 450 g infested corn was significantly higher than those of other densities (4, 8 and 16 pairs per liter jar).

Table 3 Analysis of variance of effect of parasitoid density on the percentage of maize weevil emergence, the percentage of parasitoid emergence and the percentage of PIM in the short term experiment.

Source		Sum of Squares	df	Mean Square	F	P
% Weevil emergence						
	Between Groups	5825.453	5	1165.091	174.710	.000
	Within Groups	160.049	24	6.669		
	Total	5985.501	29			
% Parasitoid emergence						
	Between Groups	2.442	5	.488	18.918	.000
	Within Groups	.620	24	.026		
	Total	3.061	29			
% PIM	Between Groups	1924.937	5	384.987	9.943	.000
	Within Groups	851.821	22	38.719		
	Total	2776.758	27			

Table 4 Effect of parasitoid density on the percentage of parasitoid emergence, the percentage of maize weevil emergence and the percentage of PIM in the short term experiment.

Parasitoid density	Mean ±SE*		
	The percentage of parasitoid emergence	The percentage of maize weevil emergence	The percentage of PIM
2	24.41±2.02a	49.14± 1.19a	24.43± 2.28a
4	36.12±0.97b	38.18± 1.92b	25.69± 2.07a
8	42.14±4.25c	31.04± 0.89c	26.81± 4.41a
12	50.40±3.12d	26.89± 0.67d	26.87± 4.68a
16	57.96±1.70e	14.30± 1.11e	27.72± 2.37a
20	45.00±1.18e	7.59± 0.62f	47.72± 1.69b

*Means followed by the same letter in a column are not significantly different ($P \geq 0.05$; Fisher’s least significant difference, LSD).

Table 5 Analysis of variance of effect of parasitoid density on the progeny sex ratio in the short term experiment.

Source		Sum of Squares	df	Mean Square	F	P
Sex ratio	Between Groups	3.201	5	.640	14.620	.000
	Within Groups	1.051	24	.044		
	Total	4.252	29			

Table 6 Effect of parasitoid density on the sex ratio (female: male) in the short term experiment.

Parasitoid density	Sex ratio (female: male) Mean ±SE*
2	1.70± 0.17b
4	1.40± 0.11a
8	0.43± 0.65d
12	0.71± 0.08c
16	0.62± 0.11cd
20	0.57± 0.03cd

*Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD).

Long term experiment

The mean numbers of maize weevil emerged in bottles without parasitoid (n=5) at the end of one to six months after releasing parasitoid were 305.20±15.49, 574.40±23.80, 695.75±97.59, 765.80±18.49, 929.00±57.03 and 1344.40±107.52, respectively. The percentage of maize weevil emergence significantly increased with the decrease of parasitoid density (Table 7). The effect of various parasitoid densities on percentage of maize weevil emergence, and on percentage of parasitoid emergence at the end of each month are shown in Table 8-13.

One month after parasitoid introduction, the emergence of maize weevil decreased as parasitoid density increased (Table 8). The percentages of emerged maize weevil at 8 and 10 female parasitoids per bottle were significantly lower than those of 2, 4 and 6 female parasitoids per bottle (Table 8). On the other hand, the percentage of parasitoid emergence increased as parasitoid density increased. The similar trend was observed at the end of the 2nd month after releasing of parasitoids (Table 9).

At the end of the third month, the increasing of parasitoid density led to the reduction of percentage of maize weevil emergence. On the contrary, the parasitoid progeny emergence increased as parasitoid density increased, except at 4 and 10 female parasitoids per bottle. The percentage of parasitoid emergence at 4 female parasitoids per bottle was lower than that of 2 female parasitoids per bottle. Moreover, at 10 female parasitoids per bottle, the percentage of parasitoid emergence was significantly lower than these of 6 and 8 female parasitoids per bottle (Table10).

At the end of the fourth month after parasitoid introduction, the emerged maize weevil still decreased with the increase of parasitoid density. On the contrary, the percentage of parasitoid emergence increased with the parasitoid density up to 6 parasitoids per bottle, but thereafter decreased significantly at 10 female parasitoids per bottle. The similar trend was observed at the end of the 5th and 6th months after parasitoid introduction (Table 11-13).

At the end of the fifth month after parasitoid release, the percentage of emerged maize weevil still decreased with the increase of parasitoid density. The percentage of parasitoid emergence increased with the parasitoid density, but there was no significant difference in the parasitoid progeny emergence among five parasitoid densities (Table 12). This trend was also noticed at the end of the sixth month after parasitoid releasing. The maize weevil emergence reduced with increasing parasitoid density especially at 10 female parasitoids per bottle. The percentage of maize weevil emergence was significantly lower than those of other densities. The percentage of the emerged weevil did not relate to the percentage of parasitoid emergence, at the same density. The percentage of parasitoid emergence did not significantly differ among 4, 6 and 8 and 10 female parasitoids per bottle (Table 13).

Table 7 Analysis of variance of effect of parasitoid density and time after parasitoid introduction on the number and percentage of maize weevil emergence.

Source	df	MS	F	P
Model	29	111.24	24.75	0.000
Density	4	5022.90	111.90	0.000
Month	5	1459.71	32.52	0.000
Density*Month	20	241.80	5.38	0.000
Error	120	44.88		

Table 8 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the first month after releasing parasitoid.

Parasitoid density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	33.41± 0.57a	61.27± 1.97a
4	39.52± 0.26b	53.62± 2.99b
6	44.44± 2.61c	50.77± 2.54b
8	52.19± 1.82d	44.14± 2.27c
10	56.71± 2.88d	31.01± 2.40d

*Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD).

Table 9 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the second month after releasing parasitoid.

Parasitoid density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	20.92± 0.51a	74.37± 0.96a
4	23.18± 0.19ab	66.67± 1.95a
6	26.98± 2.46ab	64.53± 1.55a
8	30.50± 3.21b	27.22± 1.46b
10	26.42± 3.68ab	24.72± 2.36b

* Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher’s least significant difference, LSD).

Table 10 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the third month after releasing parasitiod.

Parasitoid density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	13.59±2.49b	70.14±2.54a
4	6.41±0.24a	47.34±2.30b
6	19.72±0.59c	46.85±2.14b
8	21.36±2.87c	43.23±2.37b
10	10.92±0.53b	37.48±6.78b

* Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher’s least significant difference, LSD).

Table 11 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the fourth month after releasing parasitoid.

Density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	10.63± 1.91ab	70.61±3.64a
4	10.31± 1.16ab	54.58±2.76b
6	16.97± 4.07b	49.90±5.53b
8	14.83± 0.84b	44.92±1.36b
10	8.61± 6.09a	36.87±1.91c

* Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD).

Table 12 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the fifth month after releasing parasitoid.

Density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	5.73±0.60a	65.48±2.22a
4	6.19±0.49a	44.39±4.41b
6	5.68±2.07a	42.36±1.41b
8	10.84± 6.44a	39.80±0.92b
10	4.26±0.25a	30.03±0.69c

*Means followed by the same letter in a column are not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD).

Table 13 Effect of parasitoid density on the percentage of parasitoid emergence and the percentage of maize weevil emergence at the end of the sixth month after releasing parasitiod.

Density	Mean ±SE*	
	The percentage of parasitoid emergence	The percentage of maize weevil emergence
2	1.68± 0.36a	44.86± 5.31a
4	4.25± 0.41b	30.97± 1.66b
6	4.68± 0.33b	30.30± 7.27b
8	5.87± 1.09b	29.87± 2.33b
10	4.84± 0.65b	20.44± 1.63c

*Means followed by the same letter in a column are not significantly different ($P \geq 0.05$; Fisher's least significant difference, LSD).

Effect of parasitoid density on the number and percentage of maize weevil emergence

The number of remaining maize weevil in the bottle significantly increased with the decrease of parasitoid density (Table 7).

The number of maize weevil in the control was higher than those of the treatments at all densities. The result suggested that *A. calandrae* could be an important factor in regulating maize weevil population (Fig. 12). At the highest parasitoid density, 10 females per bottle, the number of maize weevil at the end of the 3rd month after parasitoid introduction did not significantly differ from those of the 4th to 6th months (Appendix Table 1). This result suggested that the number of maize weevil remained stable. Thus, the best parasitoid density for controlling of weevil population in the long term experiment was 10 females per bottle. To maximize suppression of weevil population, a large number of parasitoid are required.

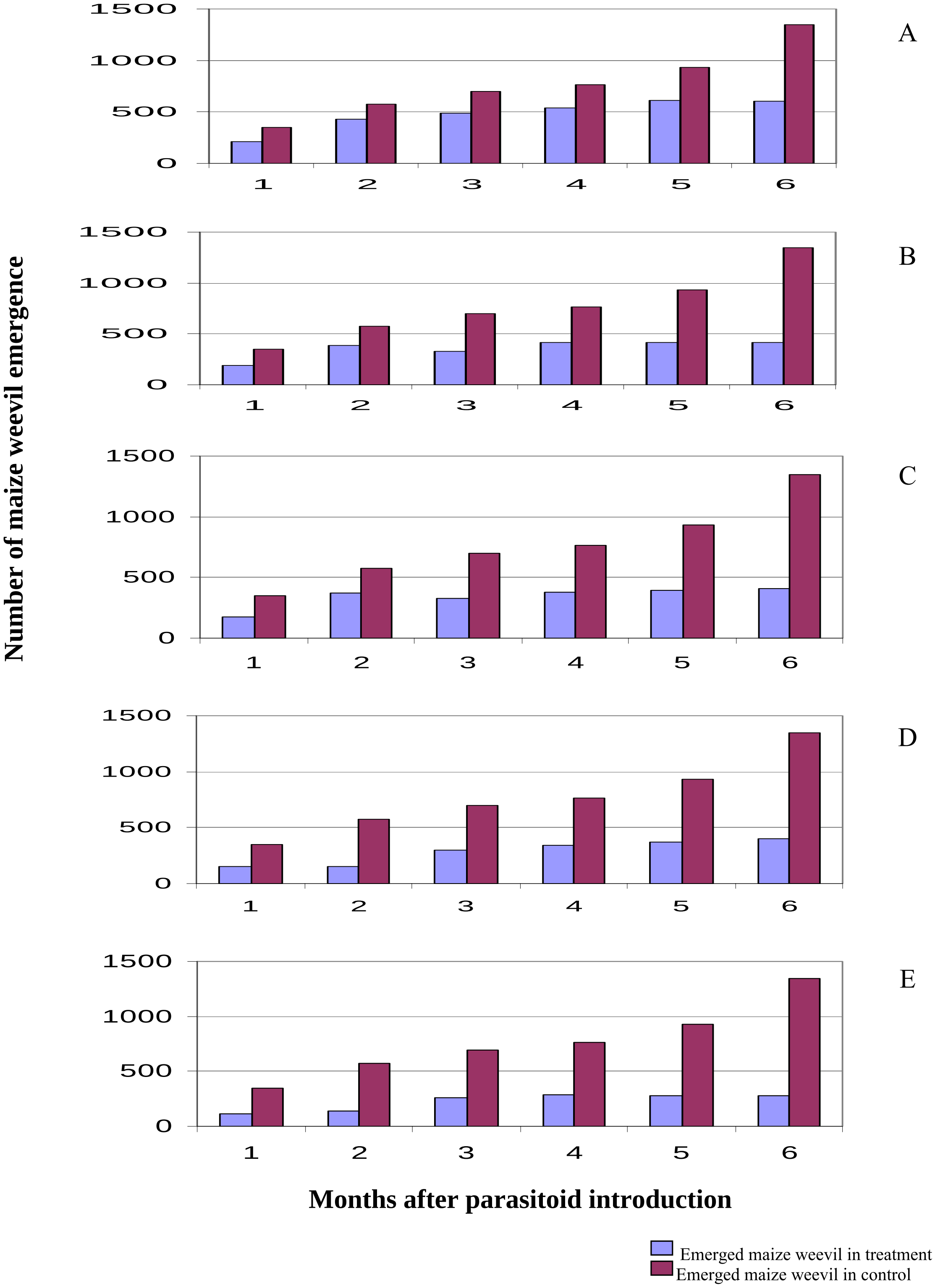


Figure 12 The average number of maize weevil emergence as a function of time after parasitoid release (n= 5). A =2 parasitoids/ bottle, B= 4 parasitoids/ bottle, C=6 parasitoids/ bottle, D=8 parasitoids/ bottle and E=10 parasitoids/ bottle.

Relationship between time after parasitoid release and the number and percentage of maize weevil emergence

At 2 female parasitoids per bottle, the percentage of emerged maize weevil increased at the end of 2nd month after parasitoid introduction as showed in Figure13. At the end of the 3rd month, the percentage of maize weevil emergence gradually decreased. At 4 female parasitoids per bottle, the percentage of emerged maize weevil increased at the end of 2nd month after releasing parasitoid, but it declined at the end of the 3rd month. Then, it increased at the end of the 4th month, but it turned to decrease at the end of the 5th and the 6th months after releasing parasitoid. The similar fluctuation trend was observed in the density of 6 female parasitoids per bottle. At 8 and 10 females per bottle, the percentage of maize weevil emergence decreased at the end of 2nd month after the release of parasitoid. Then it increased at the end of the 3rd month. The emerged weevil declined at the end of the 3rd to 6th months after parasitoid introduction.

One month after parasitoid introduction, the number and the percentage of maize weevils decreased as the parasitoid density increased. In the last experimental month, the sixth month after releasing parasitoid, the percentage of maize weevil emergence was 20.44±1.63. This result suggested that *A. calandrae* suppressed nearly 80% of maize weevil at 10 female parasitoids per bottle. The percentages of emerged maize weevil at 8, 6 and 4 female parasitoids per bottle were 29.87±2.33, 30.30±7.27 and 30.97±1.66, respectively. Thus the immature of *S. zeamais* population was suppressed nearly 70%. At 2 female parasitoids per bottle, host mortality was nearly 55% and the percentage of emerged maize weevil was 44.86±5.31.

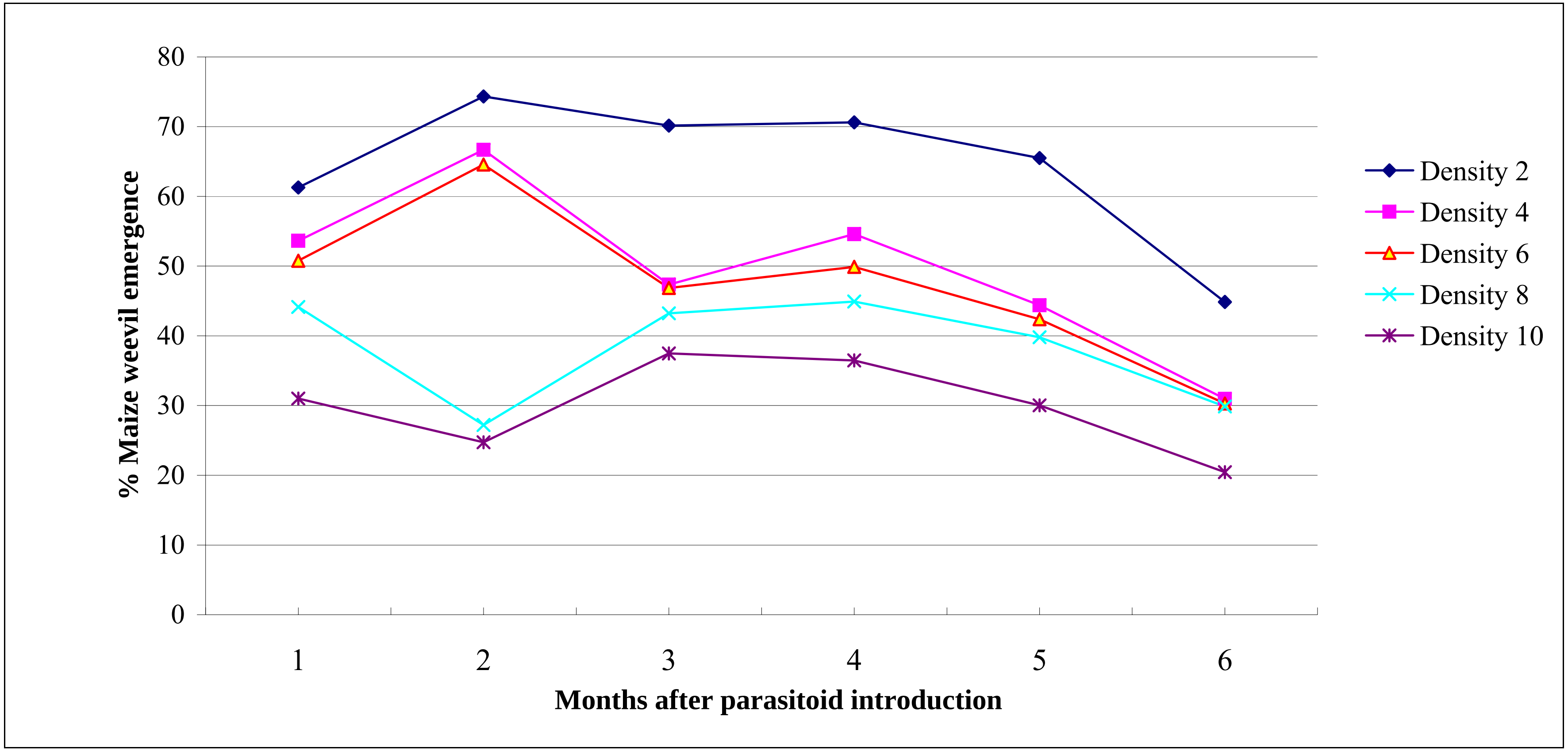


Figure 13 The percentage of maize weevi emergence among five parasitoid densities as a function of time after parasitoid introduction.

Effect of parasitoid density on the number and percentage of parasitoid emergence

The mean percentage of parasitoid emergence was significantly different among 6 parasitoid densities (Table 14). One month after parasitoid introduction, percentage of parasitoid emergence increased with increasing parasitoid density. Two months after releasing parasitoid, the percentage of parasitoid tended to increase at 2 to 8 female parasitoids per bottle and then declined. The similar trend was observed after three to six months (Fig. 14).

Table 14 Analysis of variance of effect of parasitoid density and time after parasitoid introduction on the percentage of parasitoid emergence.

Source	df	MS	F	P
Model	29	4.193	28.579	0.000
Density	5	1.329	9.059	0.001
Month	4	21.509	146.595	0.000
Density*Month	20	0.437	2.980	0.000
Error	120			

The density of 10 female parasitoids per bottle seems to be the best parasitoid density for controlling weevil population growth because the number of emerged weevils was lowest (Table 10-13). However, this density was not the best density for parasitoid emergence because the percentage of parasitoid emergence at this density was lower than that of 8 female parasitoids density, two to six months after releasing parasitoid (Table 9-13).

This result suggested that the decline in parasitoid emergence at higher parasitoid densities was probably due to superparasitism because the number of hosts suppressed by *A. calandrae* at 10 female parasitoids per bottle did not decrease. Parasitoid progeny decreased because of high mortality during larval development rather than low oviposition by *A. calandrae.* Corresponding with the number of parasitiod emergence (Appendix Fig. 1) at the end of the 2nd, 3rd and 4th months after parasitoid introduction, parasitoid number was significantly lower at 10 female parasitoids per bottle than that of 8 female parasitoids per bottle. Similar patterns, in relation to density of parasitoids, were reported for *Musicidofurax zarapter* Kogan and Legener, which is a solitary pupal parasitoid of the house fly *Musca domestica* L. The decline in *M. zarapter* emergence at higher parasitoid-host ratios was caused by superparasitism (Ryoo *et al*., 1996 b).

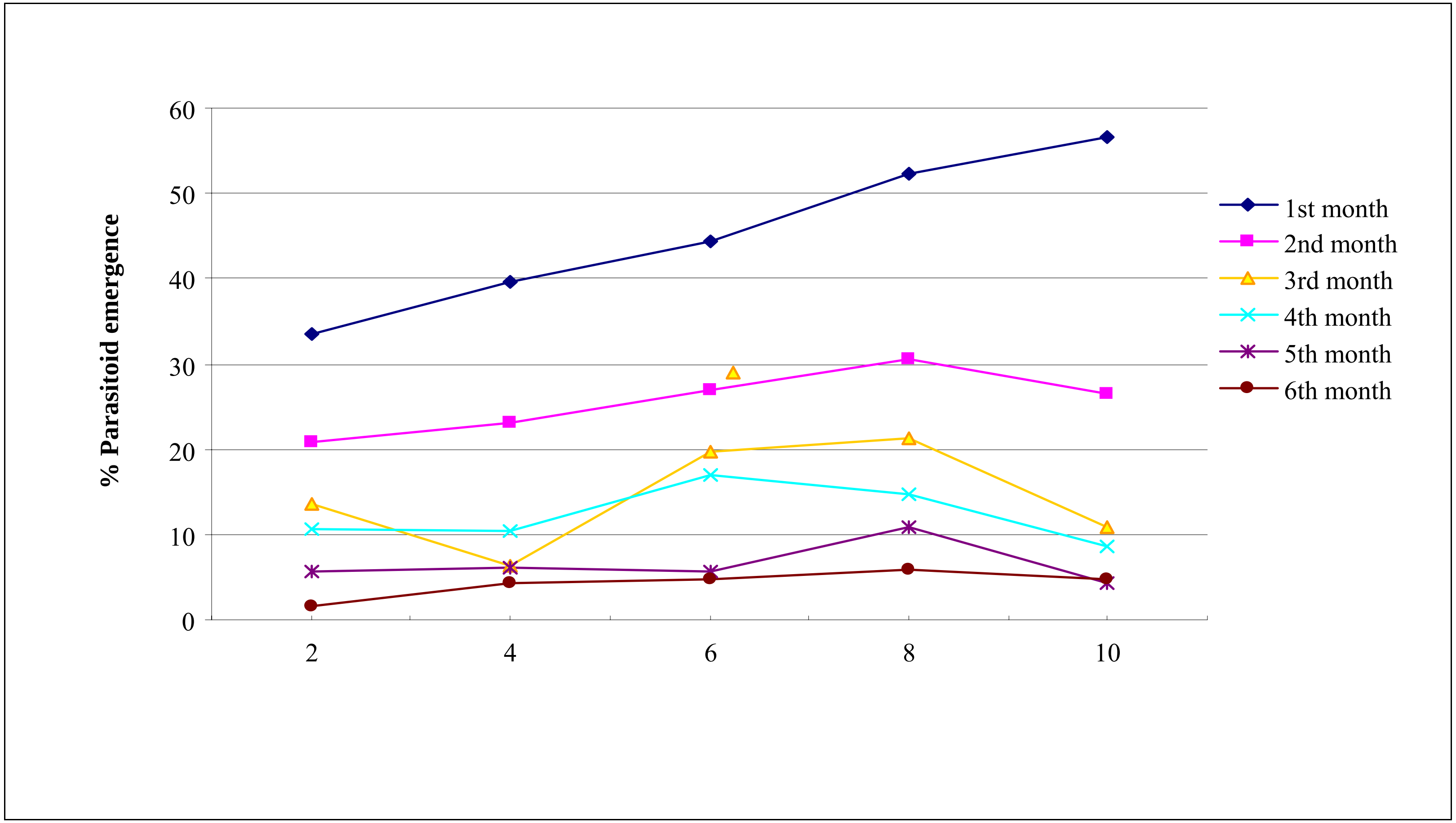


Figure 14 The percentage of parasitoid emergence among six months after releasing parasitoid as a function of parasitoid densities.

Relationship between time and the number and percentage of parasitoid emergence after releasing parasitoid

The emerged parasitoid number decreased with time after releasing parasitoid, at the end of the 1st month to 6th month. The similar trend was observed for the percentage of parasitoid emergence (Fig. 15).

At all densities, the number of *A. calandrae* reached greater densities at the end of the 1st month after parasitoid introduction than at other months. At 10 female parasitoids per bottle, the number of parasitoid progeny was highest, 198.6 ±10.11 (Appendix Fig. 1) because there was a large number of larval host produced by original weevil parent and there was the highest released parasitoid initially.

The parasitic wasp had effect on available larval host. At 10 female parasitoids per bottle, the high superparasitism probably caused parasitoid become extinct before the end of experiment. If there were no parasitoids in the bottles, the weevil population would increase again because some adult weevil still lived in the bottle. Moreover, small larvae and mature pupae which escaped parasitism even at high density of parasitoid would develop into adults. The small larvae might release weak attack stimuli which were not strong enough for the parasitoid to locate them. This wasp is larval parasitoid, so the old pupae were not parasitized by *A. calandrae* (Assem *et al*., 1984). In this laboratory experiment, *A. calandrae* were monthly released, thus the extinction of parasitoid population was unlikely to occur.

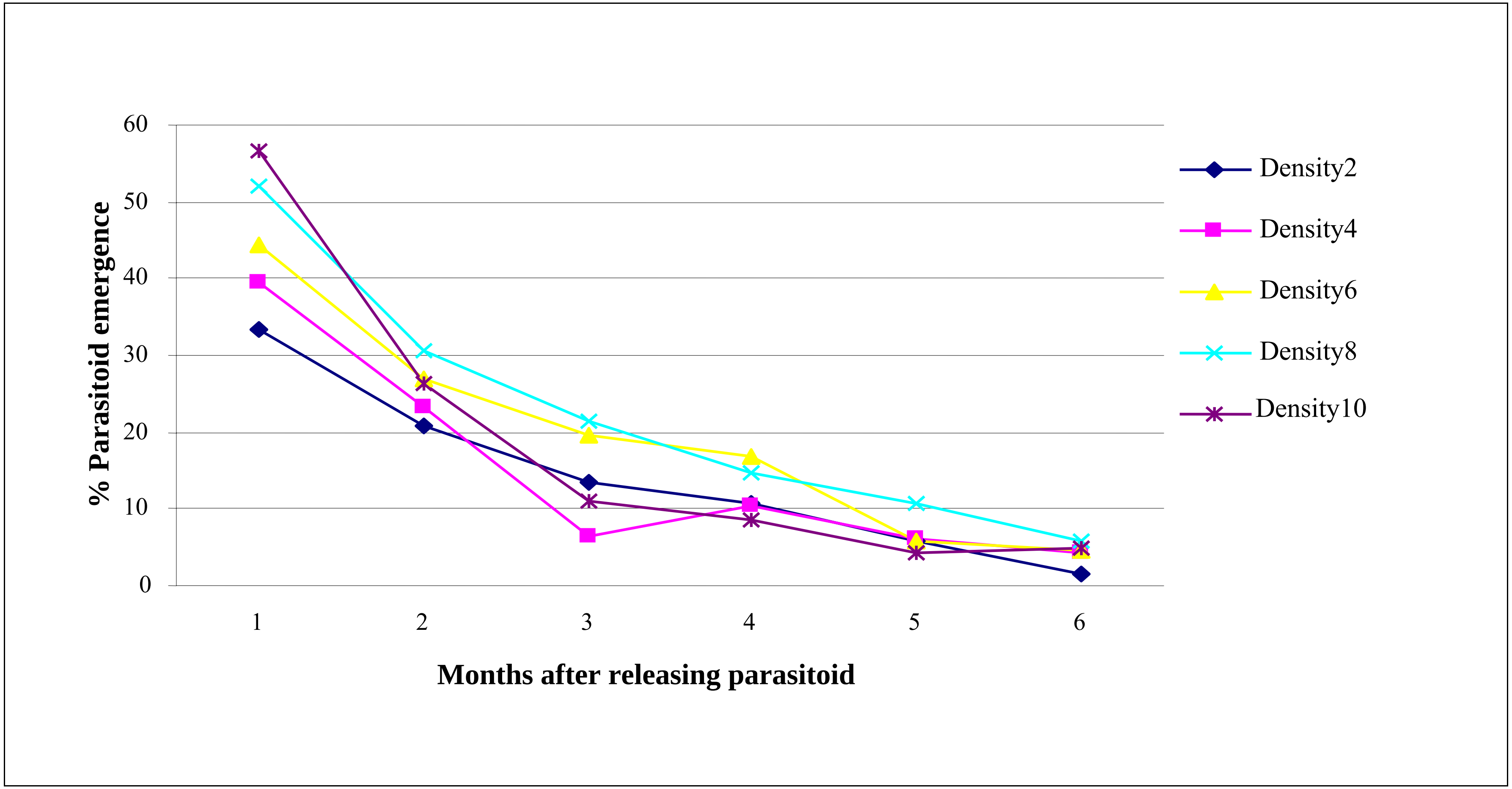


Figure 15 The percentage of parasitoid emergence among five parasitoid densities as a function of time after parasitoid introduction.

Effect of parasitoid density on parasitoid sex ratio

That the density of female parasitoid per bottle significantly affected the sex ratio (female: male) of parasitoid progeny (Table 15). The sex ratio of parasitoid decreased with the increase of parasitoid density (Table 16). The reduction of female proportion may result from both natural mortality and superparasitism. The explanation based on two reasons. Firstly female avoided ovipositing on the formerly parasitized hosts because the chemical marks left by the preceding females on the previously oviposited hosts. Secondly, the female parasitoids encountered other females more often while laying eggs on hosts at high parasitoid densities and they may adjust progeny sex ratio according to the number of other females colonizing a patch. Hassell *et al*. (1978) also presented data from a number of laboratory studies which demonstrated that sex ratio (% female) was a decreasing function of the female parasitoid density.

This result was contrast to that reported by Ryoo and Choi (2002 a). They found that although parasitism of *A. calandrae* was related to density of *A. calandrae*, the host preference and the larval size, the produced progeny sex ratios of *A. calandrae* on *C. chinensis* were not related to those factors. Mutual interference among individual *A. calandrae*, which reduced host searching efficiency, did not induce alternation of progeny sex ratio. Assem *et al*. (1984) reported that the parasitoid of *S. granarius* allocated progeny sex based on the host size with respect to three basic processes; a fixed number of males produced per female; resetting male producing factor completely after oviposition of male egg; and rejection of low hosts quality. The influence of female parasitoid on progeny sex ratio remained unclear. However, Choi *et al*. (2001) found that parasitoid progeny sex ratio was dependent on host size. This parasitoid preferentially fed on small hosts and oviposited on large hosts.

Ryoo and Choi (2002 b) considered that *A. calandrae* had a preference for a large host (third larval instar of *C. chinensis*) over a small one. Their study showed that the mortality of small larvae (<12 days old) was lower than that of the large ones. This could be explained by the low vulnerability of small larvae to *A. calandrae* than that of the large ones (>12 days old). The small larvae of *C. chinensis* settle deep in the adzuki beans, so that *A. calandrae* could hardly locate them, whereas the large larvae settle near the surface of the adzuki bean. They explained that if parasitism rate of *A. calandrae* were related to host size preference, the lower parasitism rate on small larvae could be compensated with higher PIM because of the selective feeding of *A. calandrae* on small larvae.

It is well known that female parasitoid of *A. calandrae* regulated her progeny sex ratio by host size dependent determination; depositing female eggs on large hosts (old larvae) and male eggs on small hosts (young larvae). Under condition which available hosts were scarce, *A. calandrae* had no choice for parasitism. They oviposit on nonpreferred small larvae hosts. Therefore, the decrease in parasitoid sex ratio as parasitoid density increased was probably due to host shortage rather than high parasitoid density.

Table 15 Analysis of variance for effect of parasitoid density and time after parasitoid introduction on the parasitoid sex ratio (female: male) in the long term experiment.

Source	df	MS	F	P
Model	29	0.276	9.254	0.000
Density	4	0.134	4.484	0.001
Month	5	1.366	45.795	0.000
Density*Month	20	9.333	3.129	0.000
Error	119	2.982		

Table 16 Effect of parasitoid density on the parasitoid progeny sex ratio (female: male) in the long term experiment.

Parasitoid Density	Sex ratio of parasitoid at the end of each month after releasing parasitoid Mean ±SE*					
	1	2	3	4	5	6
2	1.54±0.03a	1.25±0.79a	1.20±0.07a	1.11±0.05a	1.32±0.03a	1.25±0.04a
4	1.40±0.04b	1.10±0.04ab	1.19±0.07a	1.00±0.03a	1.10±0.07ab	1.11±0.11a
6	1.19±0.15c	0.96±0.03b	1.05±0.02a	0.97±0.06a	1.04±0.07ab	1.26±0.03a
8	1.20±0.28c	0.95±0.34b	0.72±0.07b	0.96±0.05a	0.81±0.23bc	0.87±0.05b
10	0.53±0.04d	0.66±0.13c	0.79±0.09b	0.96±0.08a	0.69±0.03c	0.74±0.08b

*Means followed by the same letter within a column is not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD)

This long term experiment indicated that *A. calandrae* could be used as a control agent against maize weevil. The number of parasitoid release at the start of experiment was an important factor for keeping the weevil population under control and for the success of parasitoid establishment.

In the field, the appropriate host-parasitoid ratio is also important for the control of maize weevil. The effectiveness of parasitoid will be low if the number of released parasitoid is too high (in the short time). The reason is that superparasitism may occur leads to the decline in *A. calandrae* emergence. As a result, the parasitoid progeny will be disappeared before maize weevils are exterminated. In addition to the parasitoid density, the extrinsic factor such as the temperature dynamic was important for population growth of maize weevil. Flinn (1998) studied the effectiveness of the parasitoid wasp *Chetospila elegans* (Westwood) for controlling *Rhyzopertha dominica* (F), lesser grain borer in wheat at 25 and 32°C. He found that the percentage of reduction in *R. dominica* density by *C. elegans* was greater at 25°C than at 32°C. At the warmer temperature, the weevil laid more eggs and developed faster than at the lower temperature. Thus more *C. elegans* must be introduced at the start of experiment in order to effectively suppress the grain borer population.

However, the potential influence of a parasitoid on the population of its host relied, among other factors, on the parasitoid's life span, its fecundity and host searching capacity. The vast majority of adult parasitoids needed food supplement, such as nectar or honeydew, as their main source of energy (Wacker, 2001 a). Sugar feeding could increase parasitoid longevity (Wacker, 2001 b) as well as fecundity (Olson and Andrew, 1998; Schmale *et al*. 2001; Wacker, 2001a). In addition, well fed parasitoids were also more actively concentrated in searching out their herbivorous hosts. Each of these three fitness parameters was directly related to the number of hosts which a parasitoid can attack. Therefore, the accessibility of sugar sources can be a key factor in the population dynamics of a parasitoid-host system. Wacker (2001 a) reported that *A. calandrae* failed to establish in the absence of food supplements. The provision of honey as food supplement magnified parasitoid efficacy dramatically. The prolong lifespan possibly increased the time window during which bruchid bean, *C. chinensis* larvae were exposed to parasitoid attack in cowpeas. Through this prolongment, *A. calandrae* lived long enough to encounter larger larvae that were preferably oviposited for producing female progeny.

In field system, the frequency of parasitoid releasing is one of important factors for the success of weevil control. This experiment, if *A. calandrae* were released weekly they might suppress more maize weevils at all densities. However, the objective of this experiment was to compare the range of parasitoid releasing densities rather than the parasitoid released frequency.

Lucas and Riudavest (2002 a) evaluated parasitoid impact on the rice weevil *S. oryzae* in rice by focusing on the combination of mechanical and biological methods. The mechanical method included the conventional polishing process, exploited either before or after infestation. This process resulted in reduced nutritional quality plus the added effect of mechanical impact in the presence of the pest. The biological method included parasitism by the pteromalids, *A. calandrae* and *L. distinguendus*. They found that the joint use of parasitoids and the mechanical process was effective in reducing the pest population. Therefore, as polishing is required in the commercial production of white rice, the use of pteromalid parasitoids before the polishing could have positive impact on weevil control.

The long term study confirms that *A. calandrae* has potential in suppressing *S. zeamais* population in small scale farms where storages are not fully disinfested and rice grain is not stored for a long period.

In the commercial rice storage, the use of biological control method may be limited by many factors. Firstly, the use of *A. calandrae* for suppressing maize weevil may increase cost. Secondly, releasing high population of parasitoids in vertical silos or rice stacks may be very difficult, and the efficiency of the parasitoids in such a different situation may be reduced. Press (1992) studied the comparative penetration efficacy in wheat between two weevil parasitoids, *A. calandrae* and *C. elegans*. He found that both parasitoids were equally efficient against rice weevil population occurring near the surface of grain mass. However, *A. calandrae* could not move downward to suppress *S. oryzae* host which settled in the bottom of 2.2 meters columns of wheat. Thirdly, there are many pest species associated with rice and not all are susceptible to attack by parasitoids. Furthermore, the presence of the parasitoids in parts of the mill may not be acceptable in the retail trade.

CONCLUSION

The results of the short term and the long term experiments indicated that the parasitoid, *A. calandrae* could be used as a biological control agent for *S. zeamais*. The optimum parasitoid density for the control of weevil population was 16 females per box in the short term experiment and 10 females per bottle in the long term experiment. At 16 female parasitoids per box, there was highest percentage of parasitoid emergence, relatively low percentages of emerged weevil and PIM. When the number of available host became scarce, *A. calandra*e had to oviposit on previously parasitized hosts leading to the decrease of parasitoid offspring and subsequently the failure in parasitoid establishment. For this reason, the exceeding number of parasitoids was not recommended for parasitoid mass rearing in the laboratory.

In the long term experiment, the highest density, 10 females per box, the superparasitism was highest. However, at the density of 10 female parastoid per bottle was more efficient for control maize weevil than other parasitoid densities. The number of emerged weevil remained stable since at the end of 3rd month after parasitoid introduction. The objective of the long term experiment was to find the optimum host parasitoid ration for the control of maize weevil, so the best parasitoid density was 10 female per bottle.

In the short term experiment, the increase of parasitoid density did not affect progeny sex ratio. On the contrary, in the long term experiment, the sex ratio of parasitoid emerged decreased with the increase of parasitoid density. Thus, the effect of parasitoid density on sex ratio was ambiguous. The reduction in parasitoid sex ratio as parasitoid density increased was probably due to the deficiency of larval hosts rather than the high parasitoid density. The total progeny declined with the increase of parasitoid density in both experiments.

After the parasitoid release, the infestation rate of grains was slow comparing to the control. Six months afterward, *A. calandrae* suppressed nearly 80% of maize weevil at the density of 10 female parasitoids. At lower parasitoid densities, 8, 6, and 4 female parasitoids per bottle, they also parasitized almost 70 % of host weevil. Thus, the parasitoid, *A. calandrae* could be used as a biological agent to control pest in storage. This parasitoid should help to reduce the frequency of pesticide application on food thus providing high quality food product.

According to the importance of host parasitoid ratio, in the field, the weevil population size should be estimated and the optimum number of parasitoid should be calculated prior to the release.

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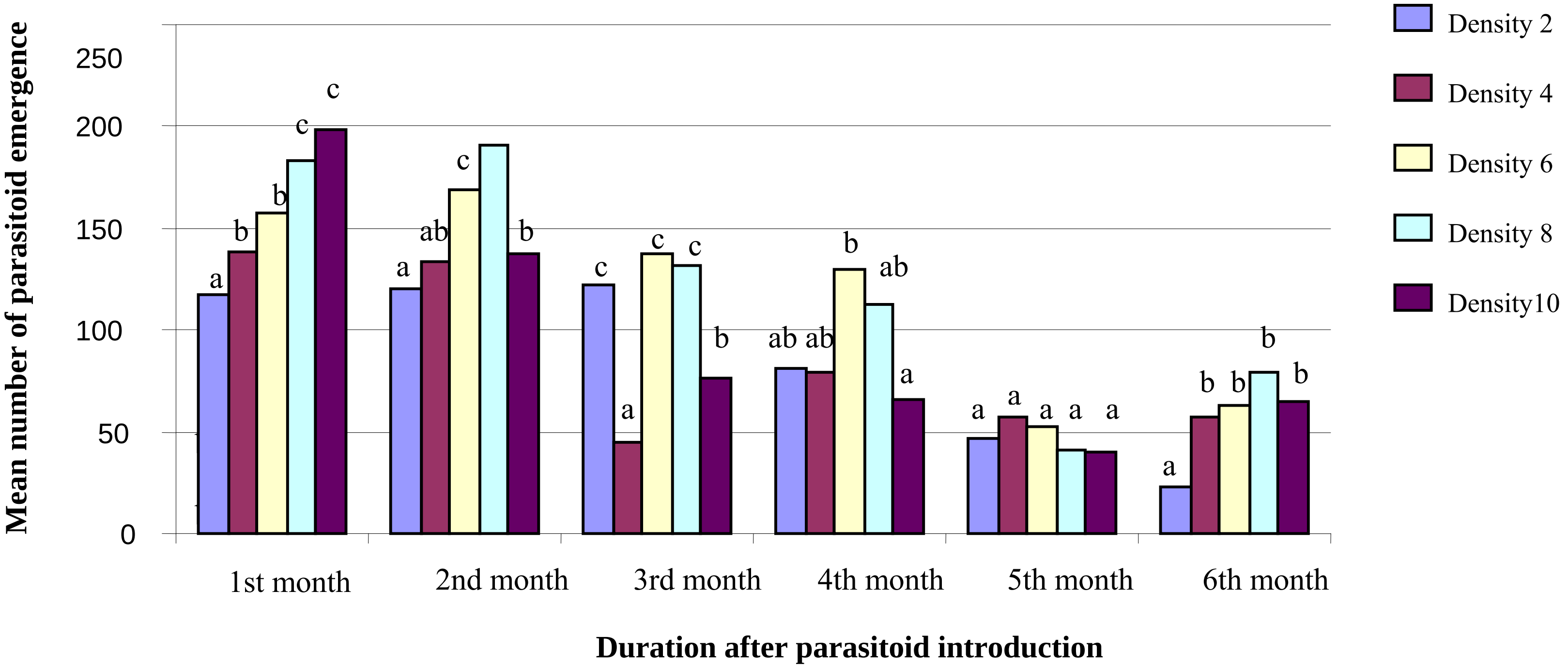
APPENDIX

Appendix Table 1 Effect of parasitoid density on the number of maize weevil emergence.

Month after parasitoid introduction	Maize weevil emergence at five densities Mean± SE *				
	2	4	6	8	10
1	214.6± 6.93a	187.0± 4.68a	177.8± 3.99a	154.6± 3.55a	108.6± 3.76a
2	427.2± 5.55b	383.0±11.23bc	370.8± 8.92b	156.4± 8.41a	142.0±13.59a
3	488.0±17.72bc	329.0±16.06b	326.0±14.50b	300.8± 16.52b	260.8±47.17b
4	540.0±27.92cd	418.0±21.18c	382.2±42.39b	344.0± 10.44bc	282.4±14.90b
5	608.0±20.66d	421.0±41.03c	393.6±13.13b	369.8± 8.62cd	279.0± 6.47b
6	604.0±71.38d	416.0±17.37c	407.4±97.80b	401.6± 31.40d	274.8±21.93b

* Means followed by the same letter within a column are not significantly different (*P*≥ 0.05; Fisher's least significant difference, LSD).



Appendix Figure 1 The mean number of *Anisopteromalus calandrae* emergence in each treatment as a function of duration after parasitoid introduction*.

*Means followed by the same letter within the same duration are not significantly different ($P \geq 0.05$; Fisher's least significant difference, LSD).