



Original Article

Host-substrate preference of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)Saruta Sitthichaiyakul,^{a, b, 1} Weerawan Amornsak^{a, *}^a Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand^b Post-harvest and Processing Research and Development Division, Department of Agriculture, Bangkok 10900, Thailand

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ABSTRACT

The solitary parasitoid *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) was investigated attacking larvae of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions. *T. elegans* parasitoids were mass reared on 21-day-old *S. zeamais* fed with different host substrates consisting of brown rice, maize, sorghum and wheat. The developmental time of *S. zeamais* was observed. The widest head capsule was recorded from *S. zeamais* developing in brown rice grain kernels. The head capsule width was used to determine the age of the larval instars. The sex ratio of *T. elegans* progeny emerging from brown rice was the same in the choice and no-choice tests (1.8:1.0 and 1.8:1.0, respectively). Female parasitoids preferred to oviposit on *S. zeamais* developed in brown rice grain kernels in both tests. The number of parasitoid progeny emerging from different host substrates was different in the choice and no-choice tests. The progeny of *T. elegans* females and males were fully winged, short winged and wingless.

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Introduction

Stored-product insect pests infest grain stores around the world, with serious damage to stored grains being caused by stored-product insect pests such as the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Toews et al., 2007), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Campbell and Hagstrum, 2002), cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Timokhov and Gokhman, 2003), and cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) (Ghimire and Phillips, 2007). These pests cause economic loss in terms of the quality and quantity of the products. Biological control is a method used to control stored-product insect pests and the potential benefit of natural enemies such as parasitoids has been acknowledged for many years (Bellows, 1985; Shin et al., 1994; Adarkwah et al., 2014). However, insecticides remain the primary tool for controlling stored product insect pests, and

consequently, resistance to fumigation and contact insecticides has been reported with some stored product insects (Boyer et al., 2012; Kang et al., 2013).

Pteromalid parasitoids are important biological control agents (Howard and Liang, 1993) and occur naturally in stored grain (Williams and Floyd, 1971). Species include *Anisopteromalus calandrae* (Howard), *Lariophagus distinguendus* (Förster) (Shin et al., 1994) and *Pteromalus cerealellae* (Ashmead) (Wen et al., 1995). *Theocolax elegans* (Westwood) is a pteromalid ectoparasitoid used to suppress the larval stage of several stored-product insect pests (Wen and Brower, 1995). These beneficial parasitoids have been shown to attack coleopteran and lepidopteran insect pests (Flinn et al., 1994). They have been used as a biological control agent in stored grain (Gordh, 1979; Germinara et al., 2009). Flinn and Hagstrum (2001) reported that augmentative releases of *T. elegans* reduced damage from the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Investigations of *T. elegans* larvae revealed survival and the preference of the parasite for the fourth and pupal stages of the host (Sharifi, 1972; Flinn and Hagstrum, 2001). The effect of temperature was reported to be a functional response in the reproduction of the progeny of the parasitoid *T. elegans* (Flinn, 1998; Toews et al., 1998, 2001; Flinn and Hagstrum, 2002). The successful parasitism of the female parasitoid

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depends on many factors including finding a target host (Steidle and Schöller, 2002), acceptance of the target host and the development of target host size (Bergeijk et al., 1989; Bouchier et al., 1993). Parasitoids acquire nutrients during their larval development (Strand and Casas, 2008). Host preference and host suitability have been studied in pteromalid parasitoids using different host species in stored grain (Timokhov and Gokhman, 2003; Grimire and Phillips, 2007). Host quality was related to host size and affected parasitoid progeny allocation (Cónsoli and Vinson, 2012). However, little information on *T. elegans* is available compared with other pteromalid parasitoids. The current study provided basic information on host substrate preferences by *T. elegans* on larval *Sitophilus zeamais*.

Materials and methods

Insects host and parasitoid

S. zeamais was taken from silos in Amnatcharoen province, Thailand during January 2013. *T. elegans* was taken during 2011 from silos in Petchaburi province, Thailand. These hosts and parasitoids were maintained at the Post-harvest and Processing Research and Development Division, Department of Agriculture, Chatuchak, Bangkok, Thailand. Both insects were mass reared at the National Biological Control Research Center, Kasetsart University, Bangkok Campus, Bangkok, Thailand under laboratory conditions (24–26 °C, 50–60% relative humidity, 12 h light:12 h dark as the natural photoperiod).

Mass rearing

The host species (*S. zeamais*) was mass reared on brown rice (*Oryza sativa* L. (Poaceae)), maize (*Zea mays* L. (Poaceae)), sorghum (*Sorghum bicolor* (L.) Moench (Poaceae)) and wheat (*Triticum aestivum* L. (Poaceae)) using glass containers holding 50 g each of brown rice, maize, sorghum and wheat. One hundred unsexed adults of *S. zeamais* were placed in each container. Each glass container (5.5 cm diameter, 15 cm tall) was covered with a filter paper. The adults of host species oviposited for 5 d and were then removed. The bottle was then maintained under laboratory conditions until the larvae were used for other trials.

T. elegans was mass reared on *S. zeamais* feeding on brown rice. Ten pairs (females and males) of *T. elegans* were released when the larvae of *S. zeamais* were aged 21 d in a glass bottle container (5.5 cm diameter, 15 tall cm) with a filter paper cover. *T. elegans* were allowed to parasitize the hosts.

Head capsule size of *S. zeamais*

S. zeamais was mass reared on brown rice, maize, sorghum and wheat and then placed individually with 5 g of plant substrate in test tubes. Five sexed pairs were determined based on the surface, size and shape of snout characteristics (Tolpo and Morrison, 1965; Dobie et al., 1984; Throne and Eubanks, 2002). The individual females were released on substrate to lay eggs for 24 h and covered with filter paper. After an additional 21 d, *S. zeamais* larvae were removed from the grain kernel and the width of larval head capsules was measured under a compound microscope (Olympus BH-2 BHS Research Microscope; Olympus Corp.; Tokyo, Japan). A visual record was made using the software program Ulead VideoStudio SE DVD (©2007, InterVideo Digital Technology Corp; Fremont, CA, USA). The measurement of head capsule size was under taken using the software program Image-Pro PLUS (version 6.0.0.260, Media Cybernetics Inc.; Rockville, MD, USA).

Host substrates

A glass cylinder (12.5 cm diameter, 12.5 cm height) was divided into quarters using four acrylic partitions (Fig. 1). For the choice experiments, 50 g of infested brown rice, maize, sorghum and wheat with 21-day-old *S. zeamais* were placed in each quadrant. An acrylic circle covered with a hole enabled parasitoids to contact the hosts. *T. elegans* female parasitoids mated and were fed with honey for 24 h before being released in the center of the quadrants. The experiment was replicated 20 times.

In the no-choice experiments, glass bottles (5.5 cm diameter, 15 cm height) containing 50 g of infested host substrates (brown rice, maize, sorghum or wheat), separately were used as treatments. Twenty pairs of *T. elegans* fed with honey were allowed to mate for 24 h. Mated females were released into glass bottles for parasitism. Each trial involved 20 replications. After the offspring of *T. elegans* emerged, they were frozen for determination of the progeny number and sex ratio.

Statistical analysis

Data were analyzed using the SPSS Statistics for Windows software (version 20.0; IBM Corp.; Armonk, NY, USA). The developmental time of *S. zeamais* larvae was estimated using the mean \pm SD. The numbers of *T. elegans* progeny that emerged from different host substrates in the choice and no-choice tests were determined using one-way analysis of variance to compare means via an F test in a completely randomized design.

Results

Head capsule width of *S. zeamais*

The head capsule width of *S. zeamais* was measured at age 21 d following development on the different substrate grains—brown rice, maize, sorghum and wheat. The greatest mean width of the head capsule \pm SD was with brown rice (0.6 ± 0.0 mm), which was not significantly different from maize (0.6 ± 0.1 mm) but these two were significantly different from sorghum (0.5 ± 0.0 mm) and also wheat (0.4 ± 0.0 mm) which were both significantly different also, as shown in Fig. 2.

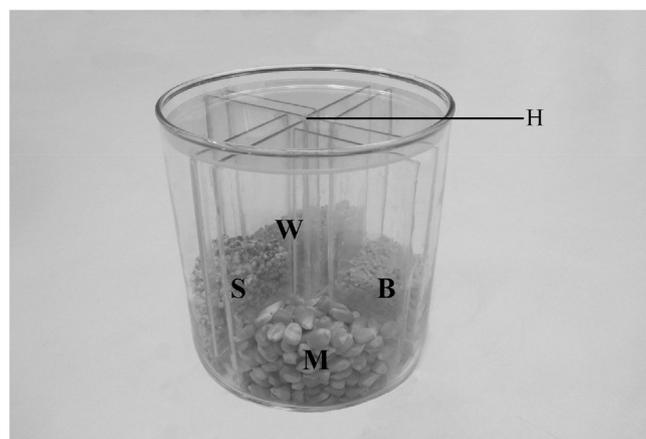


Fig. 1. Glass cylinder with *Theocolax elegans* used to parasitize *Sitophilus zeamais* larvae in different host substrates. B = brown rice, H = hole for entry of a female parasitoid, M = maize, S = sorghum, W = wheat.

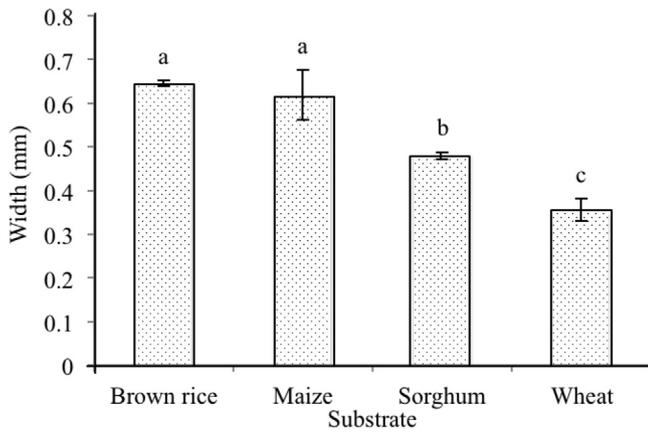


Fig. 2. Head capsule width of *Sitophilus zeamais* larvae developing in different substrates. Lowercase letters above each column indicate a significant different ($P < 0.05$) and errors bars indicate mean \pm SD.

Table 1
Sex ratio of *Theocolax elegans* emerging from *Sitophilus zeamais* larvae in different host substrates in choice and no-choice tests.

<i>S. zeamais</i> larvae in host substrates	<i>T. elegans</i>			
	Choice tests (female:male)		No-choice tests (female:male)	
	Sex ratio	n	Sex ratio	n
Brown rice	1.8:1.0	111:61	1.8:1.0	141:78
Maize	0.3:1.0	2: 6	1.8:1.0	29:16
Sorghum	1.5:1.0	6:4	1.5:1.0	52:35
Wheat	5.0:1.0	5:1	1.7:1.0	45:27

Sex ratio progeny's host substrate

The results showed that the proportion of females and males of *T. elegans* progeny differed among host substrates. More total female progeny emerged from *S. zeamais* developing in brown rice grain kernels than in the other grains. (See Table 1).

T. elegans progeny's host substrate

The results showed that the *T. elegans* adults were fully winged (macropterous), short winged (brachypterous) or wingless

(apterous). The highest mean number of *T. elegans* fully-winged progeny was produced with *S. zeamais* larvae on brown rice used as the host substrate. The highest numbers of progeny in the choice tests (5.0 ± 5.3 female and 2.6 ± 2.3 male) and no-choice tests (6.7 ± 3.2 female and 3.0 ± 1.7 male) were produced with *S. zeamais* in brown rice (Tables 2 and 3).

Discussion

The study found that the developmental time of *S. zeamais* was different in various host substrates. The head capsule width of *S. zeamais* larvae in brown rice was greater than in maize, sorghum and wheat. Sharifi and Mills (1971) reported that the width of the head capsule of *S. zeamais* larvae in stages 1–4 was 0.20 mm, 0.29 mm, 0.43 mm and 0.63 mm, respectively. In the current study, it was assumed that the *T. elegans* parasitoids preferred to parasitize *S. zeamais* developing in brown rice grain kernel because of the host size. The effect of host substrates on parasitoids was significant ($p < 0.05$) in the choice and no-choice tests, where the results showed that both sexes of *T. elegans* adults were fully winged (macropterous), short winged (brachypterous) and wingless (apterous) (Gordh, 1979). Gao et al. (2004) similarly reported that *T. elegans* shown winged, shorted winged and wingless morphs. More progeny of *T. elegans* adults were fully winged when emerging from brown rice compared with maize, sorghum and wheat (Tables 2 and 3). Various factors may influence these phenomena. Godfray (1994) reported that parasitoids used their host for ovipositing and also as a food source, which may relate to the development of *S. oryzae* on the different types of grain. Harydi and Fleurat-Lessard (1994) demonstrated that the host developmental time was shortest on brown rice and this substrate produced more progeny than polished rice. Smith et al. (1995) reported that the type of grain affected the host size and host suitability for parasitism by the pteromalid *A. calandrae*. The current study suggested that the host developmental time may depend on the type of grain or on nutrition for host suitability. Parasitoids choose the “best” (most suitable) host for ovipositing. These results may be explained by *T. elegans* females preferring to parasitize hosts in brown rice because larger hosts have more nutrients available for the parasitoid's progeny. The current study suggested both qualitative and quantitative development of host larvae on different host substrates. A similar explanation was given by Godfray (1994), namely that increased ovipositing on larger hosts could be deliberate in

Table 2
Mean \pm SD of *Theocolax elegans* emerging from *Sitophilus zeamais* larvae in different grains in choice tests.

Grain	<i>T. elegans</i> (female)			<i>T. elegans</i> (male)		
	Winged	Short winged	Wingless	Winged	Short winged	Wingless
Brown rice	$5.0 \pm 5.3^{a*}$	0.4 ± 0.8^a	0.2 ± 0.6^a	2.6 ± 2.3^a	0.3 ± 0.7^a	0.2 ± 0.6^a
Maize	0.1 ± 0.4^b	0.0 ± 0.0^a	0.0 ± 0.0^a	0.3 ± 0.8^b	0.0 ± 0.0^a	0.0 ± 0.0^a
Sorghum	0.3 ± 1.3^b	0.0 ± 0.0^a	0.0 ± 0.0^a	0.1 ± 0.2^b	0.0 ± 0.0^a	0.2 ± 0.7^a
Wheat	0.1 ± 0.4^b	0.2 ± 0.7^a	0.0 ± 0.0^a	0.1 ± 0.2^b	0.0 ± 0.0^a	0.0 ± 0.0^a

* Values followed by the same lowercase superscript letter in a column are significantly different at the 95% level of confidence (Tukey's test).

Table 3
Mean \pm SD of *Theocolax elegans* emerging from *Sitophilus zeamais* larvae in different grains in no-choice tests.

Grain	<i>T. elegans</i> (female)			<i>T. elegans</i> (male)		
	Winged	Short winged	Wingless	Winged	Short winged	Wingless
Brown rice	$6.7 \pm 3.2^{a*}$	0.1 ± 0.2^b	0.4 ± 0.7^a	3.0 ± 1.7^a	0.3 ± 0.7^a	0.6 ± 0.9^a
Maize	0.7 ± 0.7^b	0.4 ± 0.5^a	0.4 ± 0.6^a	0.3 ± 0.6^b	0.1 ± 0.2^a	0.5 ± 0.8^a
Sorghum	2.2 ± 1.8^b	0.1 ± 0.3^{ab}	0.4 ± 0.6^a	1.1 ± 1.4^b	0.2 ± 0.4^a	0.5 ± 0.6^a
Wheat	1.5 ± 1.4^b	0.3 ± 0.6^{ab}	0.6 ± 0.6^a	0.7 ± 1.3^b	0.1 ± 0.4^a	0.6 ± 0.7^a

* Values followed by the same lowercase superscript letter in a column are significantly different at the 95% level of confidence (Tukey's test).

terms of parasitoid fitness. The use of pteromalid parasitoids as biological control agents might improve the control of stored-product insect pests, especially *Sitophilus* sp.

In conclusion, *T. elegans* were mass-reared on 21-day-old *S. zeamais*. The head capsule width was used as an indicator of development (growth) by *S. zeamais* larvae on different host substrates. The greatest head capsule width of *S. zeamais* was on larvae consuming brown rice grain kernels. Female *T. elegans* preferred to parasitize *S. zeamais* larvae reared on brown rice in the choice and no-choice tests. The number of *T. elegans* progeny emerging from *S. zeamais* developed in different host substrates was significantly different ($p < 0.05$) based on an F test. The most progeny were produced from *S. zeamais* in brown rice grain kernels. The *T. elegans* progeny were fully winged, short winged and wingless.

Conflict of interest

None.

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