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Host-age preference of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae)

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DOI 10.5073/jka.2018.463.100

Abstract

The pteromalids (Hymenoptera: Pteromalidae) *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani), *Lariophagus distinguendus* (Förster), *Pteromalus cerealellae* (Ashmead) and *Theocolax elegans* (Westwood) are solitary larval ectoparasitoids used to suppress several species of stored-product insects that infest storage grains. We investigated host-age preference of *T. elegans* using no-choice laboratory experiments. Lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) larvae (9, 11, 13, 15, 17, 19, 21 and 23 days-old) in wheat grain kernel and cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) larvae (5–19 days-old) in cowpea beans were exposed to neonate *T. elegans* mated females to lay their eggs for two days. Our results showed that the highest number of parasitoids emerged from 23 days-old *R. dominica* larvae. The numbers of parasitoids emerged from 19, 21 and 23 days-old *R. dominica* larvae were statistically significantly different in experiments (F-test, 0.05). Progeny of *T. elegans* reared from *R. dominica* and *C. maculatus* larvae were either fully-winged (macropterous), short-winged (brachypterous) or wingless (apterous). Female *T. elegans* were rarely host-feeding on *C. maculatus* larvae. *Theocolax elegans* progeny were emerging from 14 days-old *C. maculatus* larvae only. We discussed insectary mass production of *T. elegans* for biological control.

Keywords: Biological control, *Callosobruchus maculatus*, host-age preference, *Rhyzopertha dominica*, stored-product insects

Introduction

Stored products such as grain, flour, legumes, tobacco and dried fruits have a value of more than one billion United States Dollar (USD) in developing countries (Eliopoulos *et al*., 2002). However, these commodities frequently lose quality and quantity due to the action of stored-product insect pests. The important problems are damages by stored-product insect pests (Coleoptera) such as the larger grain borer *Prostephanus truncatus* Horn (Bostrichidae), rice weevil *Sitophilus oryzae* (L.) (Curculionidae), maize weevil *Sitophilus zeamais* Motschulsky (Curculionidae), khapra beetle *Trogoderma granarium* Everts (Dermestidae), rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Laemophloeidae) and saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Silvanidae) and some moths such as Angoumois grain moth *Sitotroga cerealella* (Oliver) (Gelechiidae), rice moth *Corcyra cephalonica* (Stainton) and Indian meal moth *Plodia interpunctella* (Hübner) (Pyralidae) (Arthur, 2010; Flinn*et al*., 2006; Hayashi *et al*., 2004; Johnson *et al*., 2000; Plague *et al*., 2010). Many countries have controlled stored-product insect pests using fumigation with methyl bromide and phosphine. However, the first mentioned compound has been banned (Credland, 2010) and resistance towards the second mentioned have been detected (Nayak*et al*., 2003).

Biological control is an alternative, environmentally friendly method of insect pest management. Parasitoids and predators are used to reduce stored-product insect pests because the natural enemies are not harmful to the environment or the user (Schöller*et al*., 2006). Nevertheless, natural enemies such as the hymenopterous parasitoids *Anisopteromalus calandrae* (Howard) and *Lariophagus distinguendus* (Förster) (Pteromalidae), *Cephalonomia tarsalis* (Ashmead) and *Holepyris sylvanidis* (Bréthes) (Bethylidae), and *Venturia canescens* (Gravenhorst) (Ichneumonidae) are well-known to suppress stored-product insect pests.

Theocolaxelegans (Westwood) (Pteromalidae) is a solitary ectoparasitoid used for biological control. *Theocolax elegans* attacks several stored-product insect pests that develop inside the grain kernel such as *S. zeamais* (Wen *et al*., 1994), cigarette beetle *Lasioderma serricorne* (Fabricius) (Anobiidae) (Hayashi *et al*., 2004) and lesser grain borer, *Rhyzopertha dominica* (F.) (Bostrichidae) (Flinn *et al*., 2006). The female parasitoid parasitizes host larvae within infested grain by using her ovipositor (Sharifi, 1972). Gordh (1979) found different morphs in *T. elegans*, i.e. winged and wingless morphs both in males and females. However, little is known concerning host-age preference of *T. elegans* with *R. dominica* and *C. maculatus* host larvae.

Materials and Methods

Mass rearing of insects

Callosobruchus maculatus, R. dominica, *S. zeamais* and *T. elegans* were reared at the Post-harvest and Processing Research and Development Division, Department of Agriculture, Chatuchak, Bangkok, Thailand under conditions of 24–30°C, 70–73% RH and 12L:12D/natural photoperiod. The mass-rearing method used a glass container holding 50 g of brown rice (Poaceae). One hundred unsexed adults of *S. zeamais* were placed in a glass bottle (5.5 cm diameter and 15 cm height) for oviposition on brown rice. Then the glass bottle was covered with a filter paper. After five days *S. zeamais* adults were sieved off from the brown rice. *Sithophilus zeamais* larvae (21 d old) were used as hosts for *T. elegans*. *Callosobruchus maculatus* and *R. dominica* larvae developed on cowpea bean (Fabaceae) and wheat grain (Poaceae), respectively, they were used as hosts in our further laboratory trials.

Host-age

Callosobruchus maculatus and *R. dominica* were mass reared on cowpea bean and wheat grain, respectively 50 g in each glass bottle as host species. Twenty unsexed adults of *C. maculatus* and *R. dominica* were sustained on grains in our no-choice experiment. The adults of two host species were mated and laid eggs for five days. *Theocolax elegans* parasitized different ages of *C. maculatus* larvae (5–19 d old) and *R. dominica* larvae (9, 11, 13, 15, 17, 19, 21 and 23 d old). A neonate mated *T. elegans* female was released into a glass bottle for parasitism. The experiment was replicated 30 times. The number of progeny and sex ratio of wasps in the bottle were recorded.

Statistical Analysis

Analysis was performed using the software IBM SPSS version 20.0. The numbers of *T. elegans* progeny were fully-winged, short-winged or wingless morphs that emerged from different host-ages depending on host species was estimated using the mean±SD. The total numbers of *T. elegans* progeny were determined using one-way analysis of variance to compare mean via an F-Test in completely randomized design (CRD).

Results

Our experiments showed that *T.elegans* progeny were either fully-winged, short-winged or wingless morphs (Tab. 1). The numbers of progeny produced on *R. dominica* (9, 11, 13, 15, 17, 19, 21 and 23 d old) and on *C. maculatus* larvae (5–19 d old) was different. The highest number of *T. elegans* fully-winged progeny emerged from 23 d old *R. dominica* larvae including both females and males (Tab. 1), suggesting that ovipositing females were fertilized.

Tab. 1 *Theocolax elegans* (mean±SD) progeny emerging from different host-ages on *C. maculatus* and *R. dominica* larvae.

Host-ages in different host species	***T. elegans* ♀**			***T. elegans* ♂**		
	Fw	**Sw**	**Wl**	**Fw**	**Sw**	**Wl**
14 d *C. maculatus*	0.5±0.9	0.0±0.0	0.1±0.3	0.1±0.4	0.0±0.0	0.0±0.0
19 d *R. dominica*	1.7±2.5	0.0±0.0	3.1±4.2	0.3±1.3	0.0±0.0	1.0±2.1
21 d *R. dominica*	3.3±4.1	0.1±0.3	1.6±1.9	1.0±1.7	0.0±0.0	0.4±0.8
23 d *R. dominica*	8.3±6.7	0.1±0.4	1.1±1.5	1.9±1.4	0.1±0.4	1.3±1.2

Fw = Fully-winged, Sw = Short-winged, Wl = Wingless

The number of *T. elegans* progeny was statistically significantly different (P value<0.05) depending on host species. The highest number of progeny emerged from 23 d old *R. dominica* larvae (Fig. 1). Sex ratio of *T. elegans* progeny produced from 19, 21 and 23 d old *R. dominica* larvae was 3.7:1.0, 3.5:1.0 and 2.9:1.0, respectively. However, the results showed no progeny on 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18 and 19 d old *C. maculatus* larvae. Female *T.elegans* preferred to parasitize *C. maculatus* larvae only at an age of 14 d (Fig. 2). Sex ratio of *T. elegans* progeny emerged from *C. maculatus* larvae was 5.7:1.0.

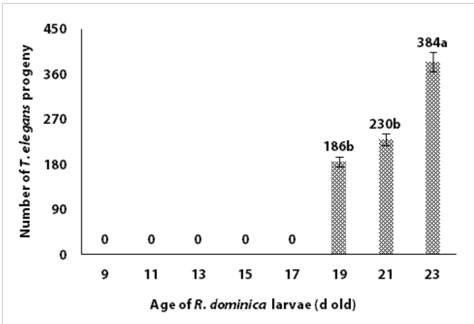

Fig. 1 Progeny of Theocolax elegans emerging from Rhyzopertha dominica larvae at different host-ages.

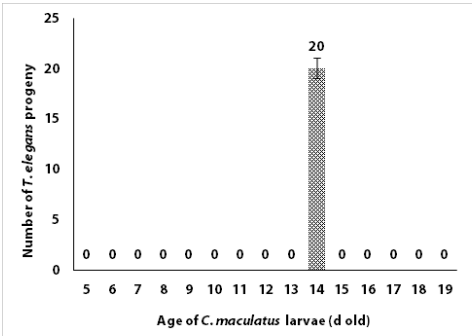

Fig. 2 Progeny of Theocolax elegans emerging from Callobruchus maculatus larvae at different host-ages.

Discussion

Theocolax elegans was reared on larvae of *R. dominica* and *C. maculatus*, respectively at different larval ages. Godh (1979) reported that fully-winged, short-winged and wingless morphs exist both in female and male. We found the number of *T. elegans* progeny produced on *R. dominica* larvae was higher than on *C. maculatus* larvae (Tab. 1). In this study, we infer that *T. elegans* prefers to parasitize *R. dominica* larvae compared to *C. maculatus* larvae. Similarly Shin et al. (1994) found that the Pteromalidae parasitoid *L. distinguendus* parasitized more *S. oryzae* larvae than *Callosobruchus chinensis* (L.) (Chrysomelidae) larvae. Odours may have different influence on parasitoid activity. Steidle *et al*. (2001) reported that parasitoids were affected by odours which emanate from host plants. Volatiles from faeces originating from *C. maculatus* were different to those from *Sithophilus granarius* (L.) (Dryophthoridae) and *R. dominica* (Steidle *et al*., 2001).

The results showed that the number of *T. elegans* progeny emerged from 23 d old *R. dominica* larvae was highest (Fig. 1). The optimal host-age for *T. elegans* to parasitize was 23 d old *R. dominica* larvae. Similarly, Choi and Ryoo (2002) reported that *A. calandrae* preferred to parasitize older host larvae more than young hosts. However, our no-choice test showed that *T. elegans* females prefer to parasitize *C. maculatus* larvae at 14 d only. The other ages of *C. maculatus* larvae were never parasitized (Fig. 2). Host numbers per kernel decreased with increasing host size (Wen *et al*., 1995). The larval instar of *S. oryzae* affects the progeny sex ratio of *A. calandrae* (Choi and Ryoo, 2002). Charnov (1982) and Ji *et al*. (2004) reported that female parasitoids assign daughters on large hosts and sons on small hosts. Host-age or host size also influenced the sex ratio (Wen *et al*., 1995). Type of grain also influences pteromalid parasitoid growth (Smith *et al*., 1995). The resource of food as nutritient is important to parasitoid progeny (Godfray 1994).

Acknowledgement

Fund was provided by the Post-harvest and Processing Research and Development Division, Department of Agriculture, Bangkok Thailand.

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Phytochemical-Based Nano Emulsions for Stored Grain Protection

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Abstract

Stored grain losses caused by pest insects contribute significantly to the global food crisis. Currently, there are two main chemical control methods against stored product insect pests: fumigation with very toxic gases and grain protection by residual contact insecticides. Today, the global tendency is to prevent/reduce the wide use of insecticides, which have high toxicity to humans and harm the environment. Therefore, there is an urgent need to develop alternative eco-friendly approaches for stored insect pest control.

Essential oils from *Micromeria fruticosa* and *Mentha rotundifolia* (Fam. Labiatae) and their main constituent pulegone which previously were shown by us as very active against stored product insect pests, were encapsulated into coarse and nano emulsions. The insecticidal activity of the developed formulations against primary internal insect rice weevil (*Sitophilus oryzae* L.) and secondary external pest red flour beetle (*Tribolium castaneum* Herbst) was evaluated in laboratory and pilot experiments.

It was found that the phytochemical-based nano emulsions offered significant advantages and provided powerful and prolonged biological activity compare with the coarse formulations. The developed nano emulsions could serve as a natural, effective, low-toxify for human, and environmentally preferred method for protection stored grain and dry food products from pest insects.

Keywords: essential oils**,** nano emulsions, pulegone, stored product insects, stored product protection.

Introduction

Insect damage in stored grain contributes significantly to the global food crisis (Philips and Throne, 2010, Nopsa et al, 2015). Today, the use of fumigants and protectants are common chemical control methods for stored product protection against pest insects. In spite of their high efficacy, both these methods have well known disadvantages (Kostyukovsky and Shaaya, 2012, Opit et al, 2012, Nayak et al, 2013, Daglish et al, 2014). The use of plant essential oils (EOs) and their constituents may be one of alternative eco-friendly approaches for stored insect pest control (Shaaya et al., 1991, 1993, Shaaya and Kostyukovsky, 2006, Kostyukovsky and Shaaya, 2011, 2012). However, for the implementation of the essential oils, suitable formulations are needed.