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Non-Reproductive Host Killing Caused by *Aphelinus asychis* (Hymenoptera: Aphelinidae), A Parasitoid of Cotton Aphid, *Aphis gossypii* (Homoptera: Aphididae)

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Aphelinus asychis Walker (Hymenoptera: Aphelinidae) is a solitary endoparasitoid that parasitizes and host–feeds more than 40 aphid species, and has been considered a potential biological control agent of aphids. In many species of hymenopterous parasitoid, female wasps cause important additional mortality by non–reproductive killing (e.g., host–feeding and stinging behaviour). Host mortality caused by non–reproductive host killing of *A. asychis* was studied on cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) when infesting cucumber (*Cucumis sativus* L.), in the laboratory at 25 °C and photoperiod of 16L: 8D. The females killed 3.3 aphids per day and 73.9 aphids until they died by host–feeding and stinging during an average of 21.3 days. Peak age–specific aphid mortality (5.2 aphids per day) was observed on the fifth day after parasitoid emergence. *A. asychis* females continued non–reproductive host killing until died.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is one of the main insect pests on several vegetables including cucurbitaceous and solanaceous crops. This aphid sucks plant sap on underside of leaves and shoots, and transmits viruses resulting in reduced fruit production (Rabasse and Wyatt, 1985; Minks and Harrewijn, 1987; van Lenteren, 1991; Parrella *et al*., 1999; Rabasse and van Steenis, 1999). Insecticides have generally been used for control of cotton aphid (van Lenteren and Woet, 1988; Goh *et al*., 2002). Unfortunately, owing to continuous use of insecticide, cotton aphid has developed resistant against insecticides. For these reasons, several attempts were tried to control cotton aphids with aphidiine parasitoid such as *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and *Aphidius matricariae* Haliday (Hymenoptera: Braconidae), and gall midge, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). However, more studies are needed for other biological control agents of cotton aphid because control efficacy of aphidiine parasitoids and predatory gall midge against aphids was not always high in the greenhouses (van Steenis and El–Khawass, 1995; Sengonca *et al*., 2008; van Driesche *et al*., 2008).

Aphelinus asychis Walker (Hymenoptera: Aphelinidae) is native to Old World (e.g., Europe, Asia and Africa) and a solitay endoparasitoid of aphid. These species were imported into the United States from Asia, Europe and Africa for classical biological control of the Russian wheat aphid (Elliott *et al*., 1999). *A. asychis* female parasitizes and host–feeds on more than 40 aphid species including *A. gossypii* (Japoshvili and Abrantes, 2006; Li *et al*., 2007) and was also found in new countries (e.g., Nepal, Portugal, Japan and Republic of Korea: Hayat, 1991; Japoshvili and Abrantes, 2006; Takada, 2002; Li *et al*., 2007). Recently, Tatsumi and Takada (2005) have suggested that *A. asychis* is potential biological control agent of four aphid species: cotton aphid, *Aphis gossypii*; green peach aphid, Myzus persicae (Sulzer); foxglove aphid, *Aulacorthum solani* (Kaltenbach); potato aphid, *Macrosiphum euphorbiae* (Thomas).

For pre–introductory evaluation of biological control agent, studies on biological characteristics such as temperature–dependent development of immature stages, age–specific fecundity and host mortality caused by host–feeding and stinging behaviour of parasitoid species are principally required for understanding its potential as a biological control agent. Many of the studies have focused on parasitoid fecundity and searching behaviour (Schlinger and Hall, 1959; Force and Messenger, 1968; Raney *et al*., 1971; Bai and Mackauer, 1990; Tatsumi and Takada, 2005). However, in many species of hymenopterous parasitoid including *A. asychis*, female wasps cause important additional mortality by host–feeding and stinging behaviour, and moreover host–feeding plays a crucial role in egg maturation of synovigenic parasitoids (DeBach, 1943; Flanders, 1953; Cate *et al*., 1973; Sandlan, 1979; Walter, 1988)

Although host–feeding and stining behaviour is crucial for cause host mortality and egg maturation, there have been a few studies on non–reproductive host–killing by *A. asychis* in East Asian countries. Therefore, the

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present study aimed at describing age–specific host mortality caused by non–reproductive host killing behaviour of *A. asychis*. In addition, we discuss host–feeding parameter in comparison with geographical strains of *A. asychis* and other *Aphelinus* species.

MATERIALS AND METHODS

Insect culture

The aphid parasitoids, *A. asychis*, were collected from the mummies of green peach aphids, *Myzus persicae* Sulzer (Homoptera: Aphididae), on Chinese cabbage in Hoengseong, Republic of Korea and were identified as *A. asychis* (Li *et al*., 2007). Stock cultures of the parasitoids were reared on greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae), cultured on barley. The cotton aphids, *A. gossypii*, were maintained on cucumber (*Cucumis sativus* L.). All insects used in the present study were reared in air–conditioned insectaries at an average 24–27 °C, a photoperiod 16L: 8D and 50–70% RH. Potted barley (20–30 cm in height with about 50 barleys per one pot) had been infested with mixed stages of 200–300 greenbugs. About 100 parasitoids were introduced into barley infested with greenbugs in an acrylic cage (27×27×50 cm) for parasitization. After 24 h, parasitoids were removed and barleys infested with greenbugs were maintained until mummy formation. Newly emerged parasitoid adults were obtained by isolating aphid mummies singly in size 0 gelatin capsules (Bioquip, USA). Upon emergence, gender was determined by observation of genitalia and antennae. When mated female parasitoids were needed, one female was transferred into plastic tubes (26×67 mm) with two males for at least 4 h.

Age–specific non–reproductive host killing

Non–reproductive killing, i.e., host–feeding or stinging, of *A. asychis* was estimated by using a cohort of 12 *A. asychis* females that were 12 h old. Each female was caged with two males for 4 h, after which the female was transferred into experimental arena containing 7 cm cucumber leaf disks infested with 50 2nd or 3rd–instar cotton aphids. The experimental arena consisted of a 7 cm cucumber leaf disk placed ventral–side–up on water–saturated cotton in Petri dish (9.3×4 cm) with 4 cm diameter gauze on the lid for ventilation. The Petri dishes were replaced with new ones containing 50 cotton aphids every 24 h until parasitoid died and were kept until aphid mummified at 25 °C, photoperiod 16L: 8D in an incubator.

RESULTS

Age–specific non–reproductive host killing

When *A. asychis* female encountered host aphid, *A. gossypii*, it touched *A. gossypii* with its antennae before inserting its oviopsitor for host–feeding or oviopsiting. When 50 2nd or 3rd instar cotton aphids were supplied with *A. asychis* everyday, the number of aphid killed by non–reproductive activities (i.e., host–feeding and stinging) of *A. asychis* was 3.3 aphids per day and 73.9 aphids in its life span (Table 1).

Table 1. Aphid mortality caused by non–reproductive killing (number of dead aphids) of *A. asychis* females on *A. gossypii* at 25 °C (n=12)

	Mean ± SE	Range
Host mortality per day	3.3±0.2	2.5–4.2
Host mortality until parasitoid died	73.9±4.8	35–95
n	12	

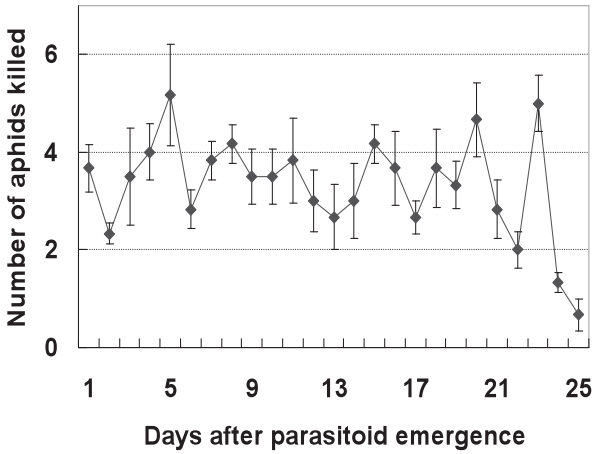

Fig. 1. Daily aphid mortality caused by non–reproductive killing of *A. asychis* female when provided with 50 *A. gossypii* (2nd or 3rd–instar) per day at 25 °C.

Peak age–specific host mortality caused by non–reproductive killing (5.2±0.7 aphids/female/day) was observed on the fifth day after parasitoid introduction and declined gradually thereafter (Fig. 1). After *A. asychis* females emerged, they continued non–reproductive host killing until died (Fig. 1).

DISCUSSION

Many of the studies have focused on parasitoid fecundity and searching behaviour (Force and Messenger, 1968; Raney *et al*., 1971; Schlinger and Hall, 1959; Bai and Mackauer, 1990; Perng and Liu, 2002; Tatsumi and Takada, 2005). However, in many species of hymenopterous parasitoid, female wasps cause important additional mortality by non–reproductive killing behaviour (e.g., host feeding and stinging behaviour) (DeBach, 1943; Flanders, 1953; Cate *et al*., 1973; Sandlan, 1979; Walter, 1988; Tran and Takagi, 2006). Our observations showed that host mortalities caused by non–reproductive killing of *A. asychis* were 3.3 aphids per day and 73.9 aphids until parasitoid died. These mortalities may be caused by host feeding and stinging behaviour of *A. asychis*. Host mortality due to non–reproductive killing in the present study was higher than two geographic strains of *A. asychis* (1.5 aphids/female/day at 27 °C, 1 aphid/female/day at 25 °C) reported by Cate *et al.* (1973), and Bai and Mackauer (1990) (Table 2). Also, host mortality by *A.*

Table 2. Comparison of longevity of adult female, numbers of aphids killed by non–reproductive activities of five *Aphelinus* species

Aphelinus species	Temp.	Longevity of female	Mean number of aphids killed		References
			daily	total	
A. flavus	–	27	1.2	28	Hamilton (1973)
A. asychis	26.7 °C	23.4	ca. 1.5	30.4	Cate *et al*. (1973)
A. asychis	25 °C	–	ca. 1	–	Bai and Mackauer (1990)
A. abdominalis	20 °C	51	1.3	–	Hoeller and Haardt (1993)
A. spaericolae	24 °C	17.8	1.9	27.1	Tang and Yokomi (1996)
A. gossypii	18 °C	8	1.5	10.7	Tokumaru and Takada (1996)
A. gossypii	25 °C	26.3	ca. 3–4	87.9	Perng and Liu (2002)
A. asychis	25 °C	32.8	7.5	161.2	Sengonca *et al*. (2008)
A. asychis	25 °C	21.3	3.3	73.9	Present study

asychis in this study was higher than other aphelinid wasps, *A. thomsoni*, *A. abdomonalis* and *A. gossypii* (Table 2). However, aphid mortality caused by non–reproductive host killing was much higher in Kyoto strain of Japan (7.5 aphids/female/day at 25 °C, Sengonca *et al*., 2008) than other geographic strains of *A. asychis* (Table 2). These differences among geographic strains of *A. asychis* may attribute to different biological characteristics because geographic strains of parasitoids have different fecundity, thermal requirement and host mortality (Elliott *et al*., 1999; Tokumaru and Takada, 1996).

In our observation, when *A. asychis* encountered a cotton aphid, it touched the host aphid with its antennae before inserting ovipositor for oviopsition or host–feeding. Host feeding behaviour is commonly observed in aphelinid wasps, *A. asychis*, *A. gossypii*, *A. spireacolae* and *A. albipodus* (Cate *et al*., 1973; Hamilton, 1973; Stary, 1988; Bai and Mackauer, 1990; Tang and Yokomi, 1996; Zhishan and Heimpel, 2007). Various studies have shown that positive relations between host–feeding and oviposition in synovigenic aphelinid wasps (DeBach, 1943; Bai and Mackauer, 1990; Zhishan and Heimpel, 2007). Host–feeding by an adult female wasp can have a strong influence on her egg maturation, with proteinaceous foods being important for maturing egg (Jervis and Kidd, 1986; Heimpel and Collier, 1996; Wheeler, 1996). In our experiment, *A. asychis* showed host feeding and stinging behaviours, and these behaviours caused host mortality.

In the present study, we report the age–specific aphid mortality caused by non–reproductive killing behaviour of *A. asychis* at 25 °C under laboratory conditions. South Korean population of *A. asychis* has caused high mortality by non–reproductive host killing behaviour. These findings imply that this geographical strain of *A. asychis* has potential as a biological control agent of cotton aphid. In addition, for developing successful biological control program for *A. gossypii* or other aphid species using *A. asychis*, additional studies, both experimental and theoretical (c.f., Tuda and Shimada, 1995; Tuda, 1996; Tuda and Shimada, 2005), would be performed on intrinsic rate of increase, searching efficiency, spatial distribution and population dynamics under both laboratory and field (c.f., Tuda and Shima, 2002; Tuda *et al*., 2006) conditions.

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