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https://doi.org/10.5109/25202

出版情報:九州大学大学院農学研究院紀要. 57 (2), pp. 431-439, 2012-09-20. Faculty of

Agriculture, Kyushu University

バージョン:

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Laboratory Studies of *Aphelinus asychis*, a Potential Biological Control Agent for *Myzus persicae*

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In order to effectively control *Myzus persicae* using the parasitoid *Aphelinus asychis* as a biological control agent in the greenhouse, biological characteristics such as the effect of temperature, host stage and host plant on parasitization, fecundity, longevity, sex ratio, development time, behavioral response on host, host plant and oviposition and host feeding behavior of *A. asychis* were evaluated. When *A. asychis* exposed to 40 individuals of each instar of *M. persicae* for 24 h at 25°C, 1st, 2nd, 3rd, 4th instar nymphs and adult were parasitized at 47.8%, 70.0%, 32.2%, 14.4% and 7.8%, respectively. There was a difference in the amount of parasitism depending on the host plant utilized by *M. persicae*. Aphids were most heavily parasitized on Chinese cabbage (44.1%) followed by pepper (34.2%). The lowest parasitization occurred on aphids reared on radish (23.3%). Second instar nymphs were most heavily parasitized and adult aphids were rarely successfully utilized.

Keywords: Aphelinus asychis, biological characteristic, Myzus persicae, parasitization

INTRODUCTION

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is an insect pest distributed world–wide causing both direct and indirect damage on many crops (Blackman and Eastop, 2000). *M. persicae* extracts sap from plants and excretes a sweet stick substance known as honeydew. Black sooty mold grows on the honeydew and, though not directly harming the plant, may block out sufficient light to reduce yield. Furthermore, green peach aphids can transmit many virus diseases in crops (Hertel and Bambach, 2004).

The control of *M. persicae* remains almost exclusively based on insecticides. The extensive use of chemical insecticides for more than five decades has brought serious resistance problems. The aphid has developed multiple mechanisms of insecticide resistance (Field *et al.*, 1988), and high levels of insecticide resistance have been observed in many parts of the world. Also, this use of chemical insecticides has brought about environmental pollution and risks to human health. Because of these problems, there is a need for the use of environmentally compatible control methods such as biological control using the natural enemies.

Many natural enemies of aphids have been reported including predators such as lady beetles (Coleoptera: Coccinellidae), flower flies (Diptera: Syrphidae) and lacewings (Neuroptera: Chrysopidae) and Hymenopteran parasitoids in the families Braconidae and Aphelinidae.

Detailed studies of the biology of several species, including the effects of pesticides have been conducted. For example; the effect of pesticides on Aphidius colemani was studied as well as the compatibility of biological and insecticidal control (Kim et al., 2006). Seo and Youn (2000) studied the predacious behavior and feeding ability of ladybird beetle (Harmonia axyridis) on the green peach aphid and the cotton aphid. The biological traits of aphid-eating gall-midge (Aphidoletes aphidimyza) were investigated (Jeoung et al., 2003). Many of these natural enemies have been reared and commercialized by companies like Sesil, Biobest and Korppert in the world. Especially, A. colemani is a koinobiont parasitoid that is considered to be an effective biological agent for various economically-important aphid species, including M. persicae (Messing and Rabasse, 1995) and is reared commercially for use on glasshouse crops in many countries (van Lenteren, 2003) including Korea.

Aphelinus spp. parasitoids have been used to control aphid's species such as A. abdominalis for the potato aphid (Macrosiphum euphorbiae) and A. mali for the woolly apple aphid, Eriosoma lanigerum (Nikol'skaya and Yasnosh, 1966; Yasnosh, 2002). General characteristics of Aphelinus spp. are that these female parasitoids behave like predators and prefer to feed on their host fluids (Stary, 1988). These behaviors are beneficial as a biological control agent since the parasitoids kill aphids for feeding in addition to parasitization (Takada and Tokumaru, 1996). Also Aphelinus spp. have a long longevity and oviposition period compared with other species of parasitoids.

Aphelinus asychis (Hymenoptera: Aphelinidae) is well–known species of Aphelinus and together with A. abdominalis have been used to control of aphids in Europe and the USA. A. asychis is widely distributed throughout the world but was reported in Korea for the

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first time in 2007 (Li et al., 2007). At least 42 species of aphids have been recorded as hosts of this parasitoid from Europe (Zuparko, 1997) and six species are known in Japan (Takada, 2002). A. asychis has been successfully used for biological control of the cotton aphid (Aphis gossypii) in greenhouses (Wyatt, 1969).

Aphelinus asychis was commercially applied as a biological control of M. persicae in Europe (van Lenteren et al., 1997). A. asychis is suitable to control pest aphids on greenhouse vegetable crops in Japan and M. persicae is a suitable host for A. asychis (Mackauer, 1968). Otherwise, Rao et al. (1999) reported that A. asychis depend on chemical cues from the host-plant complex to orient themselves and locate their host. And interactions between entomopathogenic fungus and A. asychis were studied by Mesquita and Lacey (2001). Also research about complementary biological control of aphids by Harmonia axyridis and A. asychis were conducted (Snyder et al., 2004). The research about Aphelinus, however, has been studied in only a few species in Korea (Choi, 2007).

In order to use effectively A. asychis as biological control agent, biological traits of this parasitoid must be studied. And among the ecological factors influencing parasitoid and their hosts in the field and greenhouse, especially temperature affects longevity and fecundity of parasitoid (Pandey and Tripathi, 2008). Therefore, the present study evaluated effect of temperature on biological characteristics such as fecundity, longevity, killing aphids and developmental time. Also age-specific sex ratio and longevity of male and female of A. asychis were investigated at 25°C. And responses of A. asychis to host and host plant were investigated by using Y-tube olfactometer experiment. Also oviposition and host feeding behavior of this parasitoid were compared and examined by using digital microscope.

MATERIALS AND METHODS

Host plants and aphid

Pepper plants (Capsicum annuum), Chinese cabbage (Brassica rapa) and radish plants (Raphanus sativus) were grown in the greenhouse with 25±5°C and natural light. All plants were grown for 3 to 5 weeks in the greenhouse and then moved to laboratory. The green peach aphid, Myzus persicae, were reared on these plants in acrylic insect rearing cages (L×W×H: 30×30× 50 cm) under laboratory conditions with 25±2°C, 50~70% R.H. and 16L:8D.

Parasitoids

Identification of A. asychis parasitoid was conducted by a classified table of Takada (2002) and Li et al. (2007). This species was collected from the culture of green peach aphid which it had accidently infested at Chungnam National University in 2007. Parasitoids collected were transferred to insect rearing cage with M. persicae. A. asychis was also reared continuously with pepper plants infesting M. persicae under laboratory conditions.

Parasitization of A. asychis

Effect of temperature on parasitization of A. asychis

To evaluate parasitization of parasitoids at 6 different temperatures (10, 15, 20, 25, 30 and 35°C), thirty 2nd or 3^{rd} instar nymphs of M. persicae were placed in a Petri dish (\emptyset 10×4 cm) on an excised pepper leaf with a piece of wet paper towel to maintain moisture. Two mated female A. asychis were introduced into Petri dish. These Petri dishes were transferred to an incubator set for each temperatures (10, 15, 20, 25, 30 and 35°C). Parasitoids were removed 12 hours later and each Petri dish was held in the incubator until aphid mummy formation was complete. Ten replicates were examined. Effects of host stage and host plant on parasitization

of A. asychis

These experiments were conducted as described by Rohne (2002). To obtain each instar of aphids, over 10 adult aphids were placed in a Petri dish with host material and wet paper toweling for 24h. When the nymphs had reached the desired stage, nymphs were removed from each dish until 40 nymphs remained. Forty adults were also placed in a Petri dish. Each dish was replicated 5 times. One mated A. asychis female was introduced into each dish, and the dishes were placed in the incubator at 25°C and removed after 24 hours. Aphid mummies were counted after formation was complete. To assess effect of host plant on parasitization, each plant (e.g., radish, Chinese cabbage, pepper) was placed in a separate cage with forty 2^{nd} or 3^{rd} instar of M. persicae. One mated female A. asychis was also introduced and removed after 24 hours. This was replicated 10 times. Aphid mummies were counted upon completion of mummy formation.

Effects of temperature on fecundity and longevity of A. asychis

M. persicae mummies containing A. asychis were collected from the laboratory colony and were maintained in a small Petri dish (60×15 mm, 10060, SPL, Pochean, Korea) until emergence. The emerged parasitoids were sorted by sex and one male and female were introduced into a same Petri dish with 5 aphids (2-3rd instar) and wet absorbent cotton for 12 hrs. The mated female together with male was transferred into a Petri dish (10×4 cm) that contained 40 mixed 2-3 instar aphid nymphs aphids on excised pepper leaf. These dishes were placed in incubators set at 15, 20, 25 and 30°C with 16L:8D. After 24 hrs, the female was transferred to a new Petri dish and the male was removed, dead and remaining aphids were counted and reared in the same dish at each incubator until mummification. The dead aphids were presumed to have died from adult feeding. This procedure was repeated continually carried out until the parasitoid was dead. There were 3 replications for each temperature. The total fecundity of the female parasitoids was determined daily and recorded as the total of mummies produced during their lifetime. To investigate the sex ratio of the progeny of A. asychis at 25°C, each mummified aphid transferred into small Petri dish. The adult parasitoids they were sort by sex and counted upon emergence.

Longevities of A. asychis

To investigate the longevity of A. asychis, 5 newly emerged pairs of the parasitoid were transferred into a Petri dish contained 100 mixed instar aphids on an excised pepper leaf. The Petri dish was transferred to incubator set at $25\pm2^{\circ}\mathrm{C}$ with 16L:8D. The mortality was checked daily and 100 fresh mixed stage aphids were provided every 3 days. This was replicated 10 times. Developmental periods

Four mated female *A. asychis* were introduced into Petri dish contained fifty *M. persicae* (2nd and 3rd instar) on an excised pepper leaf at 25°C. The parasitoids parasitized aphids for 6 hrs and then were removed. The Petri dishes were then moved into each incubator at 15, 20, 25 and 30°C under L16:D8. This was replicated 10 times for each temperature. Developmental times from egg to mummy were recorded. To estimate the developmental time from mummy to adult one hundred mummies were randomly collected at each temperature transferred to a small Petri dish. These dishes were held in the incubator at the temperature they were collected from. The dishes were monitored daily and any emerging adults were sexed and the day of emergence recorded. *Population dynamics of M. persicae and A. asychis*

To observe the aphid–parasitoid population dynamics, cages with pepper plants infested with 250–350 aphids were placed in an incubator at 25°C. One, 5, 10 and 25 female parasitoids were introduced into each cage. A separate cage received no parasitoids so that the population changes of *M. persicae* alone could be observed. There were 10 replicated cages for each level of parasitoid introduction. The number of aphids and mummies were counted at 7, 10 and 14 days after parasitoids were placed in the cages.

Effects of parasitization of A. asychis combined with A. colemani

In order to understand the interaction between *A. asychis* and *A. colemani*, Petri dishes were prepared containing 60 2–3rd instar nymphs of *M. persicae*. Each dish then received one of the following, 1) two mated females of *A. colemani* (2–3 days old), 2) two mated females of *A. asychis* (3–4 days old), or 3) one mated female each of *A. colemani* and *A. asychis*. Each treatment combination was placed in incubators at 4 different (15, 20, 25 and 30°C). After 24 hs, the parasitoids were removed and then all Petri dishes were stored in an incubator (25±2°C, 16L:8D). There were 10 replicates for each treatment at each temperature. Aphid mummies were counted for each species of parasitoid after completion of mummy formation.

Y-tube olfactometer bioassays

In order to know whether *A. asychis* respond to volatile chemicals from the host plant and host–aphid complex, a glass Y–tube olfactometer 1cm in diameter, with 10 cm stem and 8 cm arms at a 100° angle, was used. Incoming air was filtered through activated charcoal and humidified through doubly distilled and deionizer water. The filtered air was spilt through Teflon tubing and divided by a T–junction. In experiment 1 one of the arms

of the olfactometer had no treatment (air control) and the other contained a 1 by 1 cm piece of the pepper host plant infested with M. persicae. In experiment 2, both arms of the olfactometer contained plant material but one was un-infested plant and the other was infested with M. persicae. The airflow in each arm was regulated to 40 ml/min. Bioassays were conducted at 25°C and under 16 hrs light conditions. Ten mated female parasitoids were introduced to the stem of the Y-tube. Wasps were given for 10 min to make a choice. The olfactometer was divided two sections. The first section was the "no choice zone", defined as from the first 10 cm of the stem to the first 3.5 cm of each arm of the olfactometer from the end of the stem. The last section was the "choice zone"; the area in which the parasitoid made its "choice" between the first 3.5 cm of each arm of the olfactometer and the end of the arms. These experiments were replicated 10 times.

Oviposition and host-feeding behavior of A. asychis

Three to five day old mated female parasitoids were used. One parasitoid starved for 12 hrs was introduced into a Petri dish (35×10 mm) which contained one *M. persicae* on an excised pepper leaf with moist absorbent cotton. The behaviors associated with oviposition and host feeding of parasitoids were observed using a digital microscope (SIWON, DIMIS–M, Seoul, Korea) and compared. Observation of oviposition behaviors of *A. asychis* on different host stage: Oviposition behaviors of *A. asychis* on each instar of 1st, 2nd, 3rd and 4th nymphs and adults were observed by using above–described method. Oviposition success rates, time to first oviposition and oviposition times were recorded. These data compared with host preference results.

Statistical analyses

All data obtained from each experiment were analyzed by completely randomized one–way analysis of variance (ANOVA) at P<0.05 and then differences among treatments were compared using Duncan's Post Hoc Test HSD. All analyses were conducted using the SPSS ver. 18.0 (SPSS Inc., 2010).

RESULTS

Parasitization of A. asychis

The parasitization of M. persicae by A. asychis is shown in Fig. 1 at 5 different temperatures (10, 15, 20, 25, and 30°C). Because the most of the aphids tested 35°C did not survive, the data for 35°C is not shown. At 30°C, parasitization was the highest than at any other temperature (76.7%) and mean number of mummies produced was 23. The percent parasitism decreased with temperature (62.2, 57.8, 34.4% at 25, 20 and 15°C, respectively). The lowest percent parasitization was 14.4% at 10°C where only 4 mummies were produced.

The percent of parasitization of A. asychis against M. persicae is shown in Fig. 2. These results show that the 2^{nd} instar nymphs of M. persicae are most suitable

for parasitization of *A. asychis*. Percent parasitization on 1st to 4th instar nymphs and adult aphids were 47.8, 70.0, 32.2, 14.4 and 7.8%, respectively. These results show that this parasitioid prefers to parasitize younger aphids and also 4th nymph and adult were not easily parasitized by females. Figure 3 shows that parasitization of *M. persicae* by *A. asychis* is affected by the aphid host plant. Greater parasitization was observed on Chinese cabbage than on radish and pepper.

A. asychis attacked aphids on the first day after emergence. The parasitoids produced the lowest mummies at 15°C. This parasitoid produced similar number of mummies at every temperature tested except for 15°C (Fig. 4a and Table 1). The trend of mummy production

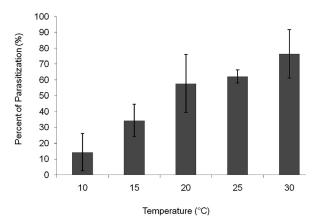


Fig. 1. The parasitization rate of *A. asychis* against 2nd and 3rd instars of *M. persicae* at different temperatures.

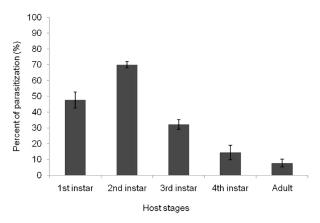


Fig. 2. The parasitization rate of *A. asychis* against different stage of *M. persicae* at 25°C.

by females at 25°C and 30°C was not different during their entire reproductive period (Fig. 4c and d). The longevity of parasitoids was increased with decreasing temperature (Table 1). Wasp survival was about 40 days at 15°C (Fig. 4a) and was less at 20°C, 25°C and 30°C (29, 24 and 25 days, respectively) (Fig. 4b, c and d). The number of aphids killed by host feeding was fairly constant over the temperatures tested. Female wasps produced more female progeny initially for about 5 days and thereafter more male progeny were produced (Fig. 5). Over the entire reproductive period, however, fewer females were produced (43.6%) than males.

At 25°C, the longevity of *Aphelinus asychis* is shown in Fig. 6. The mean survival time of males was about 12.06±3.03 days (n=15), which significantly shorter than the mean survival time of females (23.00±9.09 days, n=15).

Development time increased with decreasing temperature. The average development time from egg to adult was significantly shorter at 25°C and 30°C than at 20°C (Table 2). The total developmental time of female *A. asychis* was 19.26 days at 20°C, 12.69 days at 25°C, 10.02 days at 30°C. Total developmental period of male was slightly shorter than female at every temperature. At 15°C, most of the parasitoids were unable to complete development to adults and the data is not shown.

The cage studies indicated that there was an optimum ratio of parasitoids to aphids in order to decrease the aphid populations on the plants. When 1 female parasitoid was introduced into the cage, the aphid population continued to increase (Fig. 7). Mummy production

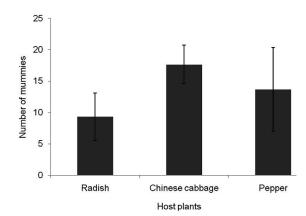


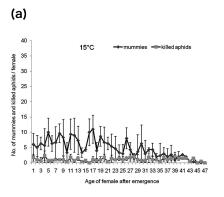
Fig. 3. Number of aphids parasitized by mated female on different host plants.

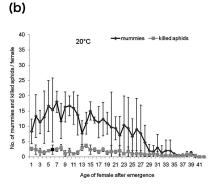
Table 1. Number of *Myzus persicae* nymph (mixed 2nd and 3nd instar) parasitized or killed by *A. asychis* and longevity and oviposition period of *A. asychis* at different temperatures

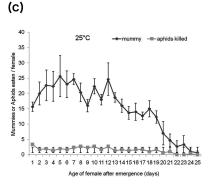
| Temperature (°C) | Longevity of female A. asychis (n=3) | Oviposition period of female A. asychis (n=3) | No. of <i>M. persicae</i> mummies (A) | No. of 2nd& 3rd nymph killed by host feeding (B) | Mortality of M. persicae (A+B) |
|------------------|--------------------------------------|---|---------------------------------------|--|--------------------------------|
| 15 | 43.33±5.51 | 41.00±4.36 | 219.67±56.19 | 45.33±6.03 | 265.00±58.51 |
| 20 | 32.67 ± 9.02 | 30.33 ± 9.01 | 332.33 ± 126.67 | 53.67±10.69 | 386.00 ± 137.27 |
| 25 | 24.33±3.06 | 22.00 ± 3.00 | 369.33±93.52 | 38.67 ± 8.02 | 408.00 ± 99.45 |
| 30 | 25.00 ± 2.65 | 21.67 ± 2.08 | 341.00±56.03 | 46.00±8.19 | 387.00 ± 61.65 |

Table 2. Development period of M. persicae parasitized A. asychis

| Temperature | Egg–mummy | | Mummy–adult | | Total period | |
|-------------|-----------------|------------------------|------------------------|---------------------|------------------|------------------|
| (°C) | male | female | male | female | male | female |
| 20 | 8.01±0.90(n=46) | 8.33±0.89(n=48) | 10.65±0.43(n=46) | 10.93±0.37(n=48) | 18.66±0.90(n=46) | 19.26±1.02(n=48) |
| 25 | 5.23±1.10(n=44) | $5.59 \pm 0.87 (n=45)$ | $6.78 \pm 0.56 (n=44)$ | $7.10\pm0.68(n=45)$ | 12.01±1.28(n=44) | 12.69±0.91(n=45) |
| 30 | 4.60±0.56(n=35) | 4.73±0.61(n=63) | $5.09\pm0.69(n=35)$ | 5.28±0.71(n=63) | 9.69±0.83(n=35) | 10.02±0.77(n=63) |







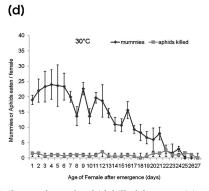


Fig. 4. Daily number of aphid killed by parasitism and aphid killed by host feeding of *A. asychis* at 15° C (a), 20° C (b), 25° C (c) and 30° C (d).

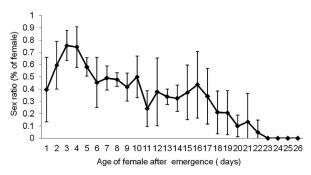


Fig. 5. Sex ratio (% of female) of emerging progeny of *A. asy-chis* at 25°C.

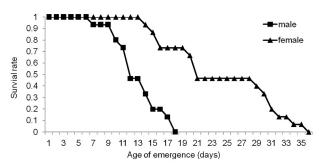


Fig. 6. Age–specific survival rate (%) for mated females and males of A.~asychis at 25°C.

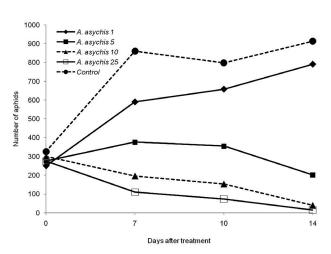


Fig. 7. Aphid population changes after each $A.\ asychis$ treatment.

was slowly increasing (Fig. 8), but not at a rate sufficient to reduce aphid numbers. At 5 female parasitoids per cage, parasitization by *A. asychis* was sufficient to keep aphid populations from increasing. In cages where 10 and

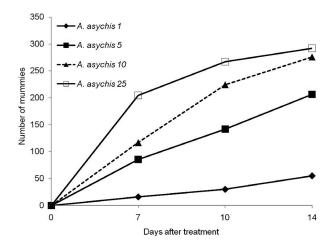


Fig. 8. Mummy population changes after each *A. asychis* treatment.

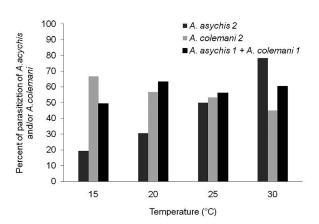


Fig. 9. Percent of parasitization of *M. persicae* by *Aphelinus asychis* and/or *Aphidius colemani* at different temperatures

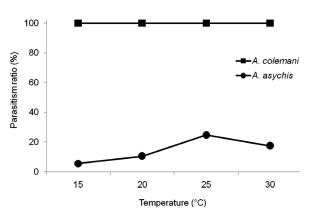
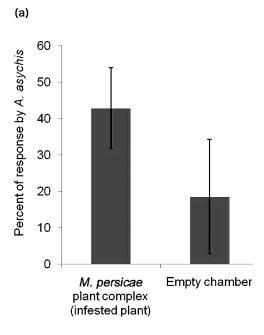


Fig. 10. Percent of parasitization of *M. persicae* by *Aphelinus asychis* combined with *Aphidius colemani* at different temperatures.

25 female parasitoids were introduced, aphid population levels decreased by 7 days and for the 25 parasitoid treatments were almost eliminated by 14 days (Fig. 7).

The effect of introduction of a second parasitoid at different temperatures is shown in Figs. 9 and 10. When A. asychis were exposed to $60\ 2^{nd}$ – 3^{rd} instar of aphids alone, the highest parasitism rate was observed (78%) at 30° C. As noted previously, parasitism by A. asychis increases with increasing temperature. Parasitization by $Aphidius\ colemani$ showed no such effect and parasitism was approximately the same over all temperatures tested even at 15° C where A. asychis showed a relatively low percent parasitism (Fig. 9). The treatment where both parasitoids were introduced to the dishes with



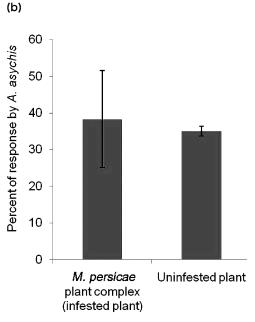


Fig. 11. Percent of response by *A. asychis* (a) to host–plant complex vs. no plant; (b) host–plant complex vs. uninfested plant.

aphids showed that most of the mummies were from successful parasitization by *A. colemani*. This could suggest that *A. colemani* has a higher fecundity or shorter host handling time or both than *A. asychis*.

Y-tube olfactometer bioassay

The olfactometer bioassays showed that *A. asychis* were attracted by volatiles from pepper plants whether infested with *M. persicae* or not. When presented with a choice between a blank chamber and one with *M. persicae*—infested pepper plants, 43% of the parasitoids chose the arm with the host/host—plant material 18% chose the blank chamber and the remaining 38% remained either the no choice area (Fig. 11a).

When presented with the choice between *M. persicae* infested pepper plants and uninfested pepper plants a similar number of parasitoids were responded to uninfested (38.3%) and infested (35%) pepper plants (Fig. 11b). However, about 26.6% female wasps remained in the no choice area of the olfactometer.

Oviposition and host-feeding behavior characteristics of *A. asychis*

Behavioral comparison of oviposition and host feeding behavior: When female A. asychis encounters a host aphid for oviposition, she palpates the aphid by using her antennae (Fig. 12a), then turns away from the aphid (Fig. 12b). The ovipositor is extended and inserted into aphid's body (Fig. 12c). Parasitized aphids form a distinctive a black mummy (Fig. 12d). The behavior of A. asychis for during host feeding is somewhat similar to oviposition. However, during host feeding, the ovipositor is inserted deeply and a meta-thoracic leg is placed on the aphid's body (Fig. 13a). The female repeatedly inserts and withdraws the ovipositor. The female then withdraws her ovipositor one final time and palpates the wound formed with her antennae (Fig. 13b). She then feeds on body fluids of the aphid (Fig. 13c) causing the aphid to become shriveled in appearance (Fig. 13d). We measured the amount of time that parasitoids take for oviposition and host feeding behaviors. Ovipositor insertion for oviposition takes 119.1 +/- 68.63 sec (N=30)



Fig. 12. Behaviors of host searching and oviposition of *A. asychis*; (a) examining aphid, (b) turning backward, (c) insertion ovipositor into aphid and (d) mummy.

Table 3. Oviposition success rate, first searching time, oviposition time and total time from searching to laying eggs of *A. asychis* at each host stage

| Host stage | Oviposition success (%) | First searching time | Oviposition time | Total time (searching- oviposition success) |
|------------------|-------------------------|----------------------|------------------|--|
| 1st instar nymph | 78.57 (7) | 14.17±5.60 (7) | 72.00±41.28 (6) | 88.83±41.28 (6) |
| 2nd instar nymph | 85.71 (7) | 39.43±40.35 (7) | 71.33±25.55 (6) | 114.33±30.07 (6) |
| 3rd instar nymph | 64.81 (9) | 45.11±38.52 (9) | 142.57±54.71 (7) | 531.86±807.30 (7) |
| 4th instar nymph | 64.28 (7) | 67.71±126.9 (7) | 99.00±22.66 (5) | 189.6±165.52 (5) |
| Adult | 20.42 (14) | 65.57±147.88 (14) | 203.33±76.59 (6) | 813.17±544.63 (6) |



Fig. 13. Behaviors of host feeding of *A. asychis* (a) insert ovipositor into aphid and place hind legs on aphid, (b) searching for wound by ovipositor, (c) fed on aphid's fluid and (d) killed aphid.

while ovipositor insertion for host feeding takes 618.88 + -153.57 sec (N=8). The amount of time actually spent feeding is 479.13 + -288.95 sec (N=8).

Effect of host stage on oviposition of A. asychis: Table 3 shows that oviposition success rate decreases with increasing age of aphid. It is difficult to parasitize adult aphids and the female parasitoids fail in parasitization against the adult aphids frequently. While aphids are actively feeding, the female parasitoids easily can utilize both younger and older aphids. But when aphids were walking the parasitoid rarely attacked. Because $1^{\rm st}$ instar aphids are the smallest in size A. asychis sometimes fail to successfully develop in this stage.

DISCUSSION

Research and application of environmentally friendly control of aphids has conducted over the world including Korea. Many natural enemies have been used for biological control of aphid species. Because different environmental conditions can impact the efficacy of natural enemies, it is important to study the biological, ecological and physiological traits of natural enemies under the conditions where they will be utilized. This study has investigated biological characteristics such as parasitization, longevity, fecundity, host location behavior and oviposition behavior of *A. asychis*.

The parasitization of parasitoid depends on various factors, among which host stage and temperature are of special importance. Parasitization and host feeding of A. asychis increase with increasing temperature and decreasing aphid age. This result is similar to that reported by Choi (2007) for A. varipes and it was sug-

gested that this parasitoid would control of M. persicae more effectively at temperatures above 30°C than Aphidius colemani. Our experiments indicate that A. asychis, like other aphelinid species, have the highest parasitization rate for the earlier host instar (Stary, 1988; Tang and Yokomi, 1996; Rohne, 2002). The lower rate of parasitization in the later stages is most likely due to a more effective defense of larger instars against parasitoids (Rohne, 2002). In our observations of A. asychis oviposition behavior, the older aphids can defend against parasitoids more effectively than younger aphids. They actively walk, jump and push with their legs to escape oviposition by the parasitoid. The longevity of A. asychis observed in this study is comparable to that of other Aphelinus species reported by various authors (Tang and Yokomi, 1996; Bernal et al., 1997; Perng and Liu, 2002). Our results are similar to the results of Bernal et al. (1997) where the longevity and fecundity of A. albipodus was measured. A. asychis had an increased longevity at the lowest temperature investigated but a higher fecundity at the higher temperatures. But overall fecundity for all temperatures except 15°C was the same for the other higher temperatures tested (Table 1). This result suggests that A. asychis has the ability to adapt easily to temperature. The age-specific sex ratio of progeny is similar to that reported for A. gossypii (Perng and Liu, 2002). Females lived significantly longer than males at 25°C.

The olfactometer studies showed that *A. asychis* responded to host volatiles from the host plant alone as well as volatiles from aphid–infested host plants. This data suggests that host plant volatiles are important for long–range attraction. Other cues from the host insect

may play a role in short range orientation and oviposition acceptance. Although *Aphidius colemani* was a more effective parasitoid for *M. persicae* when the two species were combined, the effectiveness of *A. asychis* at higher temperatures combined with the mortality associated with host feeding may make it a better choice for biological control programs. To use effectively utilize *A. asychis* for control of aphids including *M. persicae*, we must investigate the effects of other environmental parameters such as photoperiod and humidity.

ACKNOWLEDGEMENTS

The present research has been performed according to a planning project of Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries and the authors are grateful to financial support for this project.

REFERENCES

- Bernal, J. S., M. Waggoner and D. Gonzalez 1997 Reproduction of *Aphelinus albipodus* (Hymenoptera: Aphelinidae) on Russian wheat aphid (Hemiptera: Aphididae) hosts. *Eur. J. Entomol.* **94**: 83–96
- Blackman, R. L. and V. F. Eastop 2000 Aphids on the World's Crop: An Identification and Information Guide, 2nd eds. Wiley & Sons Ltd. London
- Choi, Y. S. 2007 Pest control for environmental friendly green perrilla leaves in polyvinyl houses. Ph. D. Thesis, Chungnam National University. Korea
- Field, L. M., A. L. Devonshire and B. G. Forde 1988 Molecular evidence that insecticide resistance in peach–potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. *Biochem. J.*, **251**: 309–312
- Hertel, K. S. M. and R. Bambach 2004 Virus Diseases in canola and mustard. Agnote DPI 495, NSW DPI–NSW Agriculture, Dubbo, Australia. p. 6
- Jeoung, Y. S., Y. S. Choe, I. S. Oh, K. H. Han, M. J. Seoand Y. N. Youn 2003 Biological characteristics of the aphid–eating gall–midge, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) as a biological control agents of aphids. *Korean J. Appl. Entomol.*, **42**: 241–248
- Kim, J. J., D. K. Seo and G. H. Kim 2006 Evaluation of toxicity of 83 pesticides against aphid parasitoid, *Aphidius colem*ani (Hymenoptera: Braconidae) and control effects of the green peach aphid, *Myzus persicae* with a combination of aphid parasitoid and pesticides. *Korean J. Appl. Entomol.*, 45: 217–226
- Li, C. D., Y. W. Byeon and B. R. Choi 2007 An aphelinid species, Aphelinus asychis Walker (Hymenoptera: Aphelinidae) new to Korea. J. Asia-Pacific Entomol., 10: 13–15
- Mackauer, M. 1968 Insect parasites of the green peach aphid, Myzus persicae Sulz., and their control potential. Biocontrol, 13: 91–106
- Mesquita, A. L. M. and L. A. Lacey 2001 Interactions among the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes), the parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae) and their aphid host. *Biol. Con.*, **22**: 51–59
- Messing, R. H. and J. M. Rabasse 1995 Oviposition behavior of

- the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera, Aphidiidae). *Agric. Ecosystems & Environ.*, **52**: 13–17
- Nikol'skaya, M. N. and V. A. Yasnoshl 1966 Aphelinidae of the European part of U.S.S.R. and Caucasus. *Izdatel. Akad. Nauk. SSSR* No. 91: 1–296 Academy of Sciences, USSR, Moscow and Leningrad (in Rus)
- Pandey, A. K. and C. P. M. Tripathi 2008 Effect of temperature on the development, fecundity, progeny sex ratio and life– table of *Campoletis chlorideae*, an endolarval parasitoid of the pod borer, *Helicoverpa armigera*. Biocon., 53: 461–471
- Perng, J. J. and Y. C. Liu 2002 Age-specific survival and fecundity and their effects on the intrinsic rate of increase of Aphelinus gossypii (Hym., Aphelinidae), a parasitoid of Aphis gossypii (Hom., Aphididae). J. Appl. Entomol., 126: 484-489
- Rao, A., S. B. Vinson, F. E. Gilstrap and G. J. Michels, Jr. 1999 Response of an aphid parasitoid, *Aphelinus asychis* to its host, plant, host–plant complex and to malathion. *Entomol. Exp. Appl.*, 91: 449–457
- Rohne, O. 2002 Effect of temperature and host stage on performance of *Aphelinus varipes* Foster (Hym., Aphelinidae) parasitizing the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae). *J. Appl. Entomol.*, **126**: 572–576
- Seo, M. J. and Y. N. Youn 2000 The Asian ladybird, Harmonia axyridis, as biological control agents: I. Predacious behavior and feeding ability. Korean J. Appl. Entomol., 39: 29–71
- Snyder, W. E., S. N. Ballard, S. Yang, G. M. Clevenger, T. D. Miller, J. J. Ahn, T. D. Hatten and A. A. Berryman 2004 Complementary biocontrol of aphids by the ladybird beetle Harmonia axyridis and the parasitoid Aphelinus asychis on greenhouse roses. Biol. Con., 30: 229–235
- Stary, P. 1988 Aphelinidae. In aphids, Their Biology, Natural Enemies and Control, Vol. B (A. K. Mingks and P. Harrewijn, Eds.). Elsevier, Amsterdam, pp. 185–188
- Takada, H. 2002 Parasitoids (Hymenoptera: Braconidae, Aphidiinae; Aphelinidae) of four principal pest aphids (Homoptera: Aphididae) on greenhouse vegetable crops in Japan. Appl. Entomol. Zool., 37: 237–249
- Takada, H. and S. Tokumaru 1996 Observations on oviposition and host–feeding behavior of *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae). *Appl. Entomol. Zool.*, **31**: 263–270
- Tang, Y. Q. and R. K. Yokomi 1996 Biology of Aphelinus spiraecolae (Hymenoptera: Aphelinidae), a parasitoid of the spirea aphid (Homoptera: Aphididae). Environ. Entomol., 25: 519– 523
- van Lenteren, J. C. 2003 Quality Control and Production of Biological Control Agents: Theory and Testing Procedures (ed.). CABI Publishing, Wallingford, UK. p. 331
- van Lenteren, J. C., Y. C. Drost, H. J. W. van Roermund and C. J. A. M. Posthruma–Doodeman 1997 Aphelinid parasitoids as sustainable biological control agents in greenhouses. *J. Appl. Entomol.*, **121**: 473–485
- Wyatt, I. J. 1969 Parasite control of *Aphis gossypii* on cucumbers. *Glasshouse Crops Res. Inst. Littleampton. Annu. Report 1968*, 86–87
- Yasnosh, V. A. 2002 Annotated check list of Aphelinidae (Hymenoptera: Chalcidoidea), parasitoids of aphids (Homoptera: Aphidoidea) in Georgia. Proceedings of the Institute of Zoology, Georgian Academy of Sciences, 21: 169–172
- Zuparko, R. L. 1997 A review of the host ranges of aphidophagous Aphelinidae (Hymenoptera: Chalcidoidea). J. Hym. Res., 6: 99–106