Biology of Neochrysocharis okazakii (Hymenoptera: Eulophidae), A Parasitoid of The Stone Leak Leafminer Lifiomyza chinensis (Diptera: Agromyzidae)

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**Biology of Neochrysocharis okazakii (Hymenoptera: Eulophidae), A Parasitoid of The Stone Leak Leafminer Liriomyza chinensis (Diptera: Agromyzidae)**

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Neochrysocharis okazakii Kamijo (Hymenoptera: Eulophidae) is endoparasitoid capable of developing on several Liriomyza leafminer species, and a dominant parasitoid associated with the stone leak leafminer Liriomyza chinensis (Kato) (Diptera: Agromyzidae) in Vietnam. Its biology on L. chinensis was studied in the laboratory at a constant temperature of 25°C and a photoperiod of 16L:8D. Total developmental time from egg to adult emergence was 12.1 and 12.2 days for males and females, respectively. Pupal development lasted slightly shorter than the combined egg and larval stages. The females laid a mean of 60.1 eggs and caused other 36.4 host larvae died during an average lifespan of 20.1 days. Fecundity peaked at age 3 days. The offspring sex ratio was female–biased as 27.8% males. The intrinsic rate of natural increase \( (r_e) \) (day \(^{-1}\)), net reproduction \( (R_0) \), and generation time \( (T) \) (day) were 0.219, 17.7 and 40.3, respectively.

**INTRODUCTION**

Agromyzid leafminers are known to have rich natural enemy communities, particularly insect parasitoids in both their native and invaded ranges (Waterhouse and Norris, 1987; Konishi, 1998; Murphy and LaSalle, 1999, Chen et al., 2003; Tran et al., 2006). Important factors that have encouraged the use of natural enemies for control of the leafminers are their development of resistance against pesticides and the interference of chemical control with biological control with other pests (van der Linden, 2004).

The stone leak leafminer Liriomyza chinensis (Kato) has become a serious pest on Allium spp. in many countries including China, Japan, Malaysia, Singapore, Thailand (Spencer, 1973, 1990, Chen et al., 2003), Korea (Hwang and Moon, 1995), Vietnam (Andersen et al., 2002, Tran and Takagi, 2005a), and Taiwan (Shiao, 2004). Recently, outbreak of the leafminer has been found in onion crops across Vietnam, and it is treated by a wide range of conventional insecticides, which are ineffective (Tran and Takagi, 2005a). It is necessary to consider a biological control program based on the use of parasitoids against this pest.

Neochrysocharis okazakii Kamijo is endoparasitoid capable of developing on several Liriomyza leafminer species, including L. trifolii (Saito et al., 1996; Arakaki and Kinjo, 1998; Konishi, 2004), L. sativae (Konishi, 2004, Tran et al., 2006), and L. brassicae (Bjorksten et al., 2005). This wasp species is also predominant among the parasitoids attacking L. chinensis in onion, and appears to be a good biological control agent against the leafminer in Vietnam (Tran et al., 2006).

Information on basic biology of a parasitoid species (e.g. developmental time, fecundity, sex ratio) is fundamentally necessary to evaluate its effectiveness as a biological control agent. However, the biology of N. okazakii developing on L. chinensis has not been studied. The objectives of the present studies are to determine development time for immature developmental stages, and longevity, fecundity, host mortality caused by non–reproductive killing and offspring’s sex ratio of female N. okazakii reared on L. chinensis. The results would contribute to the knowledge of the biology of this parasitoid and to optimize biological control program against L. chinensis.

**MATERIALS AND METHODS**

**Insect rearing**

Liriomyza chinensis used for the present study originated from a culture reared by the Fukuoka Agricultural Research Center, Fukuoka, Japan. Colonies of the leafminers was reared on Japanese bunching onion, Allium fistulosum L. in an environmental chamber at a constant temperature of 25°C and a photoperiod of 16L:8D (Tran and Takagi, 2005b).

The wasp parasitoid N. okazakii was originated from Hue city, Vietnam. This parasitoid was maintained with the final–instars of L. chinensis under the condition of 25°C, 60–70% humidity and 16L:8D photoperiod. Each leaf of onion plants (30–40 cm in height with 2–3 leaves) had been infected with 20–40 of second and third instars L. chinensis. For parasitization, the 4-host–infested plants and a piece of tissue paper (2 cm × 2 cm) saturated with a honey solution were placed in a plastic cage (45 cm × 30 cm × 32 cm) covered with a
fine nylon mesh. About 100–200 parasitoids were introduced into the cage. After the exposure for 24 h, these plants were replaced into a plastic container (60 cm $\times$ 50 cm $\times$ 40 cm) until pupation of the parasitoids (approximately 6 days after parasitism). The onion leaves with parasitoid pupae were removed from the plant stems and placed into a polyethylene terephthalate (PET) bottle (1.5 l in volume). Emergence of the parasitoid was checked daily. Females were provided with honey immediately after emergence.

**Immature development**

Four potted onion plants at 2–3 leaf stage, 30–40 cm in height were exposed to 50 mixed sex *L. chinensis* adults (approximately 1:1 sex ratio) in plastic cages (45 cm $\times$ 30 cm $\times$ 25 cm) covered with a fine nylon mesh for an oviposition access period of 2–4 h. After oviposition access period, the plants were replaced into a polyethylene terephthalate (PET) bottle (1.5 l in volume). The base of the stem of Japanese bunching onion plants infested with approximately 30–40 second and third instar larvae of *L. chinensis* was immersed in water in a 50 ml glass vials. The vials were covered with a fine nylon mesh for air circulation. Undiluted honey was streaked on a piece of Sealon film (Fuji Photo Film Co., Ltd.). The streaked honey film was attached to the top of the bottles, and replaced daily to provide wasps with fresh food. Thereafter, one pair of newly emerged wasps was released in the bottles. These bottles were kept in the environmental chambers. After the exposure for 24 h, the plants were removed and the leaves were then dissected under microscope to check for paralyzed larvae. The paralyzed larvae were maintained in the environmental chamber set at 25°C and a 16L:8D photoperiod in the same manner as described above. Plants were exchanged daily until the females died. The number of parasitoid pupae was recorded as fecundity capacity, and longevity of females was determined. Host mortality due to non-reproductive killing (e.g. host feeding, host stinging) was calculated as a difference between number of paralyzed larvae and number of parasitoid pupae. A total of 9 females (0.299 $\pm$ 0.0052 mm in hind tibial length) were used for test.

**Sex ratio and body size of offspring**

The pupae collected from the second experiment were individually placed in centrifugal tubes (1.5 ml in volume) maintained at the same experimental condition until wasp emergence. All offspring wasps were sexed. The sex ratio is expressed as the proportion of males among the offspring (Godfray, 1994). The hind tibial lengths (as indices of body size) of 100 randomly selected wasps of each sex were measured under a binocular microscope.

**Rate of population increase**

The wasp offspring emerged for each female at a defined age were daily recorded and sexed. The net reproduction rate (Ro), mean generation time (T) and intrinsic rate of natural increase (rₑ) were calculated according to the equations given by Birch (1948)

$$Ro = \frac{\prod l_m}{\prod l_x / \prod l_m; (\exp(-r_x)l_m)} \quad = 1$$

where, $x$ is female age, $l$ is the proportion of females surviving to age, $x$, $m$ is the expected number of daughters produced per female alive at age $x$.

**Data analysis**

Comparison of developmental time among immature stages (e.g. egg–larva, pupa), and hind tibial length between males and females were analyzed using unpaired t-test. A binomial test was conducted to determining whether the sex ratio parasitoid offspring differs from 1:1 ratio. All statistical procedures were carried out using SPSS ver. 12.0 (SPSS Inc., 2003)

**RESULTS**

**Immature development**

Developmental time for immature stages of *N. okazakii* is summarized in Table 1. Total developmental time from egg to adult emergence was not significant
Longevity, fecundity and host mortality caused by non-reproductive killing

The females produced a mean of 61.8 offspring pupae and caused other 36.4 host larvae died during an average lifespan of 20.1 days (Table 2). Age-specific survival and fecundity daily distribution was shown in Figure 1. The daily fecundity distribution showed an increase with a peak at day 3 (11.1 ± 1.74 progeny/female/day), after which a slow decrease followed until females’ death (Fig. 1). There was a similar tendency in activities of oviposition and non-reproductive host killing. The peak of host mortality caused by non-reproductive killing activities of adult females was attained at the 4th day from emergence, with 5.3 ± 1.72 host larvae killed per female (Fig. 2).

Table 2. Longevity (days), fecundity (no. pupae) and host mortality caused by non-reproductive killing (no. dead larvae) of females Neochrysocharis okazakii at 25°C

<table>
<thead>
<tr>
<th>Stage</th>
<th>Mean ± SE</th>
<th>Range</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg + larva</td>
<td>6.1 ± 0.07</td>
<td>6–7</td>
<td>6.3 ± 0.08</td>
<td>6–8</td>
</tr>
<tr>
<td>Pupa</td>
<td>5.9 ± 0.05</td>
<td>5–6</td>
<td>5.9 ± 0.08</td>
<td>5–7</td>
</tr>
<tr>
<td>Total</td>
<td>12.1 ± 0.05</td>
<td>12–13</td>
<td>12.2 ± 0.09</td>
<td>12–15</td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td></td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Among the members of Neochrysocharis, N. formosa has been reared on L. trifolii in the laboratory (Maryana, 2000, Tran et al., 2004; Hondo et al., 2006). However, no data on the developmental biology of N. okazakii on Liriomyza leafminers are available. This is the first report about rearing of N. okazakii on L. chinensis. The present study indicated that total developmental time from egg to adult emergence of N. okazakii was about 12 days. The development is faster than that recorded for N. formosa on L. trifolii (Maryana, 2000; Hondo et al., 2006).

Male N. okazakii developed faster and hence emerged earlier than females did. This phenomenon is called protandry, and protandry is known for N. formosa (Maryana, 2000) and other eulophid parasites reared on L. trifolii (e.g. Minkenberg, 1990; Ho and Ueno, 2002; Bazoochti et al., 2003; Hondo et al., 2006).

The females produced a mean of 61.8 offspring pupae during an average lifespan of 20.1 days. The fecundity of N. okazakii on L. chinensis was apparently smaller than other parasitoids of various leafminers.
(Minkenberg, 1990; Maryana, 2000), but our results could have been influenced by the experimental manipulation (e.g., dissection, placement), since parasitoid larvae could not pupated due to hatching mortality. Therefore, the realizable fecundity of *N. okazakii* females could be probably higher. The average of net reproduction rate (\( R_0 \)) suggests that *N. okazakii* population would increase 40.3 times during each generation. The average intrinsic rate of natural increase (\( r_m \)) was 0.219 per individual per day at 25°C. Although such data are necessary to predict the reproductive potential of *N. okazakii* population in greenhouses or open fields, further experiments at different temperature are required, since the net reproductive rates of various parasitoids of leafminers were variable with temperature (Minkenberg, 1990; Hondo et al., 2006).

Non-reproductive killing behavior of various parasitoids of leafminers includes host feeding and host stinging without oviposition (Bernardo et al., 2006). Our results showed that host mortality by adult females without parasitization was a mean of 36.4 larvae, accounting for 37.1% of total host mortality caused by the females. For synovigenic parasitoids, there is a tight link between feeding by adults and subsequent reproduction. Since host blood is superior as a source of proteinaceous material, essential vitamins and salts for egg development, and a valuable source of nutrition for maintaining metabolism in host-feeding species, host-feeding parasitoids generally have reduced life time fecundity and longevity without host-feeding (Lervis and Kidd, 1986; Heimpel and Collier, 1996). In our experiments, only mature host larvae were used both for ovipositing and host-feeding. Since greater consumption of younger instars may be expected simply because they provide less source to host-feeding female parasitoids, the mortality by host-feeding in nature habitats could be probably higher (Bernardo et al., 2006).

Host-stinging behavior has been frequently observed in parasitoids of leafminers (Heinz and Parrella, 1989; Patel and Schuster, 1991; Patel et al., 2003; Bernardo et al., 2006). Proportion of stung host varied depending on the host size distribution (Heinz and Parrella, 1989), host density (Patel et al., 2003) and temperature (Patel and Schuster, 1991). The hosts stung may be a mechanism for limiting the density of leafminer larvae on invididual leaves, thus ensuring the parasitized larvae will not be lost due to the leafmining of survival, non-parasitized larvae on the same leaflet (Patel et al., 2003). Although there is no individual data on host-stinging and host-feeding of *N. okazakii* on *L. chinensis*, total hosts killed by parasitoid feeding and stinging without oviposition should also be considered as a source of mortality due to the parasitoid.

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