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Comparison of the suitability of *Liriomyza chinensis* and *L. trifolii* (Diptera: Agromyzidae) as hosts for *Neochrysocharis okazakii* (Hymenoptera: Eulophidae)

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Abstract

Effective parasitoid production or mass-rearing is one of the key components in practicing biological control with parasitoids. Selection of host species for rearing parasitoids is a major approach to improve the production efficiency. The stone leek leafminer *Liriomyza chinensis* (Kato) is a destructive pest of onion crops in many Asian countries, which is difficult to control with chemicals. The present study examined whether the production of *Neochrysocharis okazakii* Kamijo, a promising native biological agent of *L. chinensis*, could be effective if kidney bean-*Liriomyza trifolii* (Burgess) system was used as an alternative rearing procedure. The suitability of *L. chinensis* and *L. trifolii* as hosts of *N. okazakii* was investigated in the laboratory. *Neochrysocharis okazakii* completed its development on both two host species, and the total development time was similar on these hosts. Longevity of *N. okazakii* females provided with *L. chinensis* was longer than those on *L. trifolii*. There were no significant differences in fecundity and offspring sex ratio when females had been provided with these two host species. The adult parasitoids emerged from *L. chinensis* were significantly larger than those from *L. trifolii*. However, the intrinsic rate of increase was higher, and mean generation time was lower, for *L. trifolii* than *L. chinensis*. Thus, mean net reproductive rate (*Ro*) was higher when *L. trifolii* was used as host. Although the two host species were equally suitable as host for *N. okazakii*, our findings suggested that *L. trifolii* and kidney bean is an ideal system for *N. okazakii* production.

Keywords: Leafminer; Parasitoid; Daily fecundity; Longevity; Host-feeding; Host suitability; Mass-rearing

1. Introduction

The American serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) is a serious pest of numerous ornamental and vegetable crops worldwide (Parrella and Jones, 1987; Spencer, 1990). The stone leek leafminer *Liriomyza chinensis* (Kato) (Diptera: Agromyzidae) has become the most serious pest on *Allium* spp. in many Asian countries (Spencer, 1973; Hwang and Moon, 1995; Andersen et al., 2002; Chen et al., 2003; Shiao, 2004; Tran and Takagi, 2005a). Recently outbreaks of *L. chinensis* have been found in onion crops in Vietnam. Farmers applied a wide range of conventional insecticides, but these proved largely ineffective (Tran and Takagi, 2005a). Frequent applications of these insecticides can adversely affect parasitoid abundance in the vegetable agro-ecosystem (Johnson et al., 1980; Saito et al., 1996), can promote the development of pesticide resistance within fly populations (Keil et al., 1985; Johansen et al., 2003) and frequently lead to an increase in leafminer density (Oatman and Kennedy, 1976; Murphy and LaSalle, 1999). These findings emphasize the need for a biological control program based on the use of parasitoids against these pests.

Agromyzid leafminers are known to have rich natural enemy communities, particularly insect parasitoids in both

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their native and invaded ranges. Over 40 species of parasitoids have been recovered worldwide from *Liriomyza* spp. (Waterhouse and Norris, 1987) including 27 species in Japan (Konishi, 1998), 14 species in China (Murphy and LaSalle, 1999; Chen et al., 2003), 11 species in Indonesia (Rauf et al., 2000), 8 species in Malaysia (Murphy and LaSalle, 1999) and 18 species in Vietnam (Tran et al., 2006). These communities of parasitoids have been recognized for their potential contribution to the integrated pest management (IPM) of leafminers in both glasshouses and open fields (Waterhouse and Norris, 1987; Minkenberg, 1990).

Two parasitoid species, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae), which are indigenous to Europe, are commercially available and have been used as biological control agents against pest *Liriomyza* leafminers in many countries (Chow and Heinz, 2004). However, use of the two commercially available parasitoids is not acceptable for Vietnamese (and other Southeast Asian) farmers because they are too costly. Conservation of natural enemies would be most ideal if effective native agents are present. Augmentation and release of such agents with relatively small numbers can help build-up the population of effective natural enemies in an agricultural field if natural control by the agents is not always enough. In Vietnam, it is thus important to establish the mass production system of native natural enemies with economically cheaper procedures and to produce cheep natural enemies.

Neochrysocharis okazakii Kamijo (Hymenoptera: Eulophidae) is dominant in warm regions of many Asian countries including China, Japan and Vietnam (Murphy and LaSalle, 1999). This endoparasitoid is capable of developing on several *Liriomyza* leafminer species, including *L. trifolii*, *L. sativae*, *L. brassicae* and *L. chinensis* (Saito et al., 1996; Arakaki and Kinjo, 1998; Konishi, 2004; Bjorksten et al., 2005; Tran et al., 2006). This wasp species is also predominant among the parasitoids attacking *L. chinensis* in onion crops, and is likely to be useful for leafminer control in Vietnam (Tran et al., 2006).

Since the host plants of *L. chinensis* are *Allium* spp. (Spencer, 1973), Welsh onion (*Allium fistulosum* L.) would be an ideal host plant in a rearing system for *N. okazakii* to ensure that the parasitoids are well adapted to *L. chinensis*. However, because onion plants are slow-growing, their use in a mass-rearing system is not conducive to producing high numbers of parasitoids at an economical price. Production costs could be reduced by using kidney been (*Phaseolus vulgaris* L.) and *L. trifolii* as the host plant and host leafminer. However, *N. okazakii* immature developmental time, host-feeding activity, fecundity, and longevity may be conditioned by host species. The objectives of the present study were to evaluate longevity, reproductive capacity, host mortality caused by non-reproductive killing, and progeny immature development, sex ratio and body size of *N. okazakii* females provided with *L. chinensis* or *L. trifolii*. The results are to contribute to the knowledge of the biology of this parasitoid and to optimize biological control program against *L. chinensis*.

2. Materials and methods

2.1. Insect rearing

Liriomyza chinensis and *L. trifolii* used for the present study originated from a culture kept at the Fukuoka Agricultural Research Center, Fukuoka, Japan. Colonies of the two leafminers were maintained separately in MIR-253 Sanyo incubator chambers at 25 ± 0.5 °C, 60–70% humidity and a photoperiod of 16L:8D. *Liriomyza trifolii* had been reared on kidney bean, *P. vulgaris* (Tran et al., 2004), whereas *L. chinensis* had been maintained on Welsh onion, *A. fistulosum* (Tran and Takagi, 2005b).

The colony of *N. okazakii* originated from Hue City, Vietnam. This parasitoid was reared on larvae of *L. chinensis* in MIR-253 Sanyo incubator chambers at 25 ± 0.5 °C, 60–70% humidity and 16L:8D photoperiod at the Institute of Biological Control, Faculty of Agriculture, Kyushu University, Japan. Each leaf of the infested onion plants (30–40 cm in height with 2–3 leaves) had 20–40 of second and third instars *L. chinensis*. For parasitism, the 4 host-infested plants were placed in a plastic cage (45 cm × 30 cm × 32 cm) covered with a fine nylon mesh and contained a piece of tissue paper (2 cm × 2 cm) saturated with a 30% honey solution. About 100–200 parasitoids were introduced into the cage. After an exposure of 24 h, these plants were replaced into a vented plastic container (60 cm × 50 cm × 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). Parasitoid emergence was checked daily. Females were provided with honey immediately after emergence.

2.2. Immature development

The development of *N. okazakii* reared on *L. chinensis* and *L. trifolii* was investigated in parallel. Four potted plants of Welsh onion (2–3 leave stage and 30–40 cm in height) and four potted kidney bean plants (with well developed first post-cotyledon leaves) were exposed to 50 mixed sex *L. chinensis* and *L. trifolii* adults, respectively, in plastic cages (45 cm × 30 cm × 25 cm) covered with a fine nylon mesh for an oviposition access period of 2–4 h. The plants were then removed and held at 25 ± 0.5 °C, 60–70% humidity and a photoperiod of 16L:8D until all leafminer larvae reach the final instar.

Welsh onion and kidney bean plants infested with the last instars of the leafminers and approximately 100 mixed sex 2 day-old adults of *N. okazakii* were introduced into a plastic cage (45 cm × 30 cm × 32 cm) covered with a fine nylon mesh. Female parasitoids were allowed to attack and parasitized leafminer larvae for 6 h. After the parasitism period, the plants were removed from the cage. The leaves of onion plants were then dissected using a binocular microscope to check for paralyzed larvae. The paralyzed larvae were placed into Petri dishes (6 cm diameter). A piece of cotton saturated with distilled water was laid on each dish, and then a piece of filter paper (5.5 cm in diameter) was placed on the piece of the cotton. The paralyzed larvae were placed on the paper and then covered with another piece of the filter paper. The leaves of kidney bean plants were cut off and then placed in Petri dishes (9 cm in diameter) lined with a piece of water-saturated cotton and filter paper. These dishes with paralyzed larvae were maintained in the MIR-253 Sanyo incubator chambers at 25 ± 0.5 °C, 60–70% humidity and a photoperiod of 16L:8D until pupation of parasitoids. Parasitoid pupae were collected once a day in the afternoon. The development time of combined egg and larval stages was defined as the time from oviposition until pupa collection. The pupae were individually placed in Petri dishes (6 cm in diameter) lined with filter paper. These dishes were placed at the same experimental conditions and supplied daily with some drops of water for maintaining appropriate humidity in the Petri dishes. The emergence and sex of parasitoids were daily recorded for each pupa to determine mean development time.

2.3. Longevity, fecundity and host mortality due to non-reproductive killing

Parasitoid pupae were collected from the insect rearing cages and placed individually in microcentrifuge tubes (1.5 mL in volume), and maintained in the MIR-253 Sanyo incubator chamber at 25 ± 0.5 °C, 60–70% humidity and a photoperiod of 16L:8D. Welsh onion or kidney bean plants infested with approximately 30–40 last instars of *L. chinensis* or *L. trifolii* were cut, and the base of the stem was singly put in a 50 ml glass vial filled with water. Each vial with a host plant was placed in a PET bottle (1.5 L in volume), which had two holes (5 cm × 5 cm) covered with a fine nylon mesh for air circulation. A piece of Sealon film (Fuji Photo Film Co., Ltd.), onto which undiluted honey was streaked, was attached to the top of the bottles, and was replaced daily to provide wasps with fresh food. One pair of newly emerged wasps was released in each bottle for parasitism, and the bottles were kept at 25 ± 0.5 °C, 60–70% humidity and a photoperiod of 16L:8D. After exposure for 24 h, the plants were removed from the bottles. The leaves of onion plants were then dissected using a microscope to check the presence of paralyzed larvae. The paralyzed larvae were reared in the same methods as described above. The leaves of kidney bean plants were removed from the plant stems. After being checked for paralyzed, the leaves were individually placed in Petri dishes (9 cm in diameter) lined with piece of water-saturated cotton and filter paper. These dishes were maintained in the environmental chambers at the same conditions until pupation of the parasitoids. Plants were exchanged daily until the test females died. The number of parasitoid pupae was recorded as an index of parasitoid fecundity, and longevity of females was determined. Host mortality due to non-reproductive killing (e.g. host-feeding, host-stinging without oviposition) was calculated as the difference between the numbers of paralyzed larvae and parasitoid pupae. The pupae were individually placed in centrifugal tubes (1.5 mL in volume) and were 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). Two hundred wasps emerged from each of the host species during the experiment were randomly selected and used to examine the size of wasps. The hind tibial length was used as an idex of wasp size and was measured under a binocular microscope.

A total of 20 females (0.30 ± 0.004 mm in hind tibial length) were used for the test. No significantly difference was detected in the hind tibial lengths between test female groups (*L. chinensis* versus *L. trifolii* groups: *t*-test, $t = 0.43$, $n = 20$, $P = 0.34$).

2.4. Rate of population increase

The net reproduction rate (*Ro*), mean generation time (T) and intrinsic rate of natural increase (r_m) were calculated according to the equations given by Birch (1948)

$$Ro = \sum l_x m_x; T = \sum x l_x m_x / \sum l_x m_x; \sum (\exp(-r_m x) l_x m_x) = 1,$$

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

2.5. Statistical analysis

The effects of host species and wasp sex on parasitoid developmental time and body size were analyzed with two-way ANOVAs using the StatView (SAS Institute, 1998). The effects of host species on female fitness (e.g. the numbers of collected pupae and host mortality without oviposition) were analyzed using unpaired *t*-test. To evaluate the effects of host species on female longevity, parametric survival fit based on the Weibull distribution was used using JPM (SAS Institute, 2005).

3. Results

3.1. Immature development

Neochrysocharis okazakii successfully developed to adulthood on both *L. chinensis* and *L. trifolii*. Development time for immature stages is summarized in Table 1. The total development time was similar between host species or parasitoid sexes ($P > 0.05$), though pupae of male

Table 1
Developmental time (days; mean ± SE) of *N. okazakii* reared on *L. chinensis* and *L. trifolii* at 25 °C

Stage	*L. chinensis*		*L. trifolii*		Sources of variation (F)[a]		
	Male	Female	Male	Female	Sex	Species	Sex × species
Egg–larval	6.1 ± 0.07	6.3 ± 0.08	6.4 ± 0.12	6.3 ± 0.1	0.0004	1.78	1.56
Pupal	5.9 ± 0.05	5.9 ± 0.08	5.5 ± 0.11	5.9 ± 0.13	3.21	5.07[b]	3.07
Total	12.1 ± 0.50	12.2 ± 0.09	11.7 ± 0.16	12.2 ± 0.16	3.92[b]	1.62	0.64
N	19	42	33	55			

[a] Two-way ANOVAs were performed.
[b] The F values show a significance at $P < 0.05$ levels .

parasitoids reared on *L. trifolii* developed faster than those of females ($P < 0.05$).

3.2. Longevity, fecundity and host mortality caused by non-reproductive killing

Longevity of *N. okazakii* females differed significantly between female groups ($\chi^2 = 5.73$; $df = 1$; $P = 0.017$), and the females lived longer when *L. trifolii* had been provided (Table 2). Age-specific survival of *N. okazakii* on both hosts followed a type I survivorship curve (Fig. 1).

There was no effect of host species on female *N. okazakii* lifetime fecundity whether we measured it by the number of progeny reaching the pupal stage (t-test, $t = 1.511$; $n = 20$; $P = 0.074$) or the net reproductive output (Ro = the number of daughters reaching maturity) (t-test, $t = 1.12$; $n = 20$; $P = 0.138$). However, daily number of collected pupae was significantly higher on *L. trifolii* than those on *L. chinensis* (t-test, $t = 3.27$; $n = 20$; $P = 0.002$) (Table 2). Daily fecundity distribution showed an increase with a peak at day 3 (11.1 ± 1.74 and 14.6 ± 3.81 progeny/female/day on *L. chinensis* and *L. trifolii*, respectively), after which a slow decrease followed until females' death (Fig. 2). However, females reared on *L. trifolii* appeared to have a higher fecundity in early phase of adult life than those on *L. chinensis* (Fig. 2).

The total and daily numbers of host mortality of *L. trifolii* due to non-reproductive killing by *N. okazakii* females were significantly higher than those of *L. chinensis* (t-test, host mortality: $t = 3.18$; $n = 20$; $P = 0.003$, daily host mortality: $t = 8.93$; $n = 20$; $P < 0.00001$) (Table 2). The peak of host mortality caused by non-reproductive killing was on the 4th day after emergence, with the means of 5.3 ± 1.72 larvae for *L. chinensis* and 14.9 ± 1.72 larvae for *L. trifolii* (Fig. 3).

Table 2
Reproductive parameter of female *N. okazakii* presented with *L. chinensis* and *L. trifolii* during the lifetime at 25 °C

Parameter	*L. chinensis*	*L. trifolii*
Longevity	20.1 ± 3.68a	11.9 ± 1.78b
Fecundity	61.8 ± 5.59a	92.4 ± 17.65a
Adult daughters	42.0 ± 4.18a	55.9 ± 11.32a
Host mortality	36.4 ± 5.99a	122.8 ± 23.99b
Daily fecundity	3.9 ± 0.78a	7.8 ± 0.82b
Daily host mortality	2.3 ± 0.49a	9.7 ± 0.63b
Sex ratio	27.8 ± 2.84a	33.5 ± 2.36a
N	9	11

Data were shown as mean ± SE. Means with the same letter within a row are not significantly different by unpaired t-test, $P > 0.05$. Mean longevity was different between the groups by Parametric Survival Fit, $P < 0.05$.

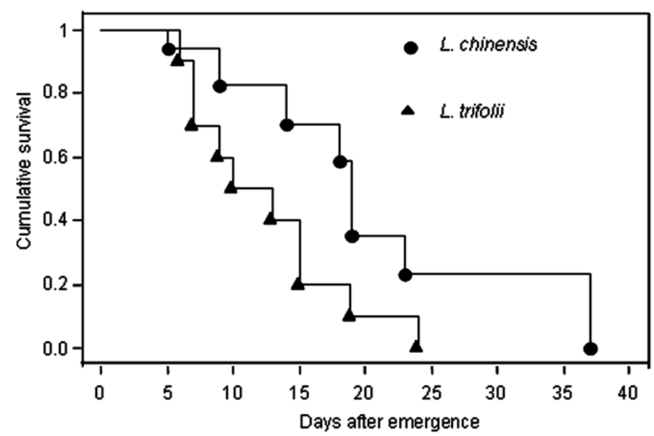

Fig. 1. Cumulative survival of *N. okazakii* females allowed accessing to *L. trifolii*and *L. chinensis* larvae.

3.3. Body size and sex ratio of offspring

The parasitoids reared on *L. chinensis* were significantly larger than those reared on *L. trifolii* ($F_{1,396} = 62.871$; $P < 0.0001$). Hind tibial lengths of females were significantly longer than males for both hosts ($F_{1,396} = 486.814$; $P < 0.0001$). The same sex attained a larger size when reared on *L. chinensis* than on *L. trifolii* ($F_{1,396} = 12.092$; $P < 0.001$) (Table 3, Fig. 4). The offspring sex ratio was female-biased and not significantly different between the two host species (t-test, $t = 1.58$; $n = 20$; $P = 0.066$) (Table 2).

3.4. Rate of population increase

The cumulative net reproduction curves ($l_x m_x$) were similar in shape between two host species (Fig. 5). However, females provided with *L. trifolii* tended to produce more daughters in early adulthood than those with *L. chinensis*. This caused a difference in the intrinsic rate of increase (r_m) on two host species. The intrinsic rates of increase values (r_m) were of 0.2197 and 0.2409 day^{-1} for *L. chinensis* and *L. trifolii*, respectively. Mean generation time (T) was

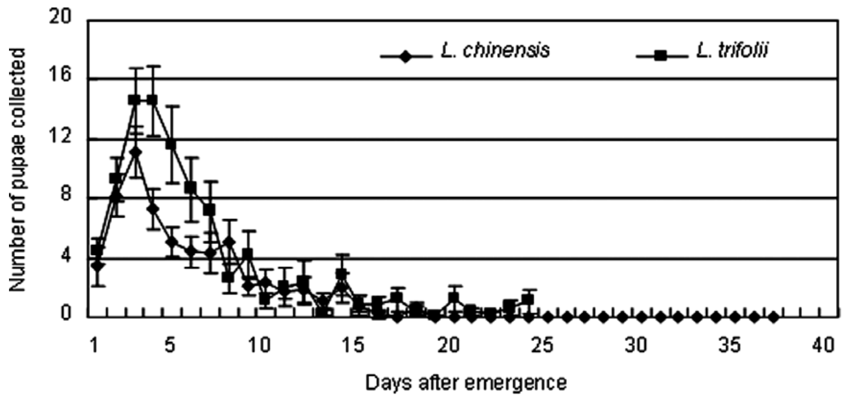

Fig. 2. Daily distribution of fecundity of *N. okazakii* females on *L. chinensis* and *L. trifolii* (Mean ± SE).

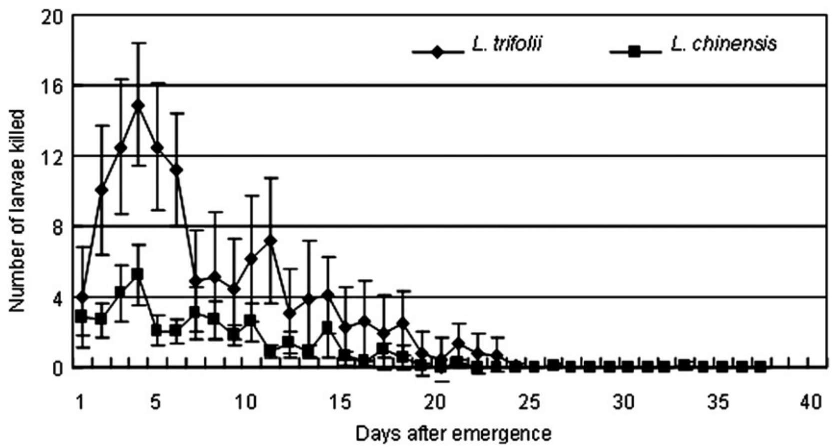

Fig. 3. Daily distribution of host mortality caused by non-reproductive killing of *N. okazakii* females given *L. chinensis* and *L. trifolii* (Mean ± SE).

Table 3
Summary of two-way ANOVA results for the effects of host species (*L. chinensis* and *L. trifolii*) on hind tibial length of male and female *N. okazakii*

Source of variation	*F*	*P*
Host species	62.871	<0.0001
Sex	496.814	<0.0001
Host species * sex	12.092	<0.001

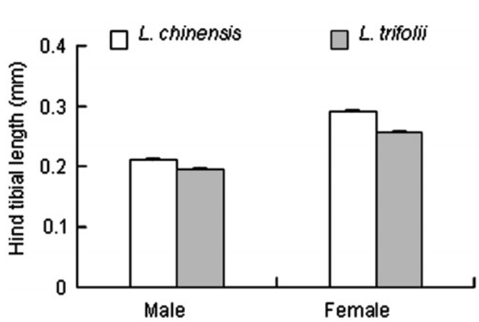

Fig. 4. Hind tibial lengths of male and female *N. okazakii* reared on *L. chinensis* and *L. trifolii* at 25 °C. Each column = Mean (+SE), $n = 100$.

17.7 days on *L. chinensis* and 16.9 days on *L. trifolii*. Mean net reproductive rate (*Ro*) was 40.3 and 48.3 on *L. chinensis* and *L. trifolii*, respectively.

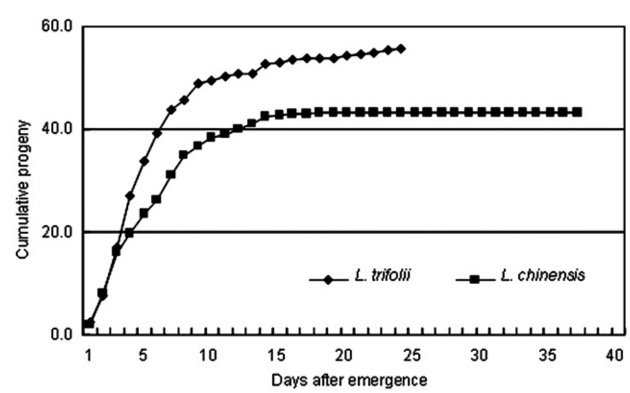

Fig. 5. Cumulative net reproduction of *N. okazakii* females emerged from *L. trifolii* and *L. chinensis*. Trends based on age-specific survival and fecundity ($l_x m_x$) showing fitness differences expected under synchronizing environmental condition.

4. Discussion

The lifetime offspring production of *N. okazakii* was not influenced by the host species. However, adult male and female *N. okazakii* emerged from *L. chinensis* were significantly larger than those from *L. trifolii*. Larvae of *L. chinensis* were larger than those of *L. trifolii* (Minkenberg, 1990;

Tran and Takagi, 2005b). The difference in the size of *N. okazakii* is probably due to the difference in host body size. Previous studies also indicated positive correlations between host size and adult size for leafminer parasitoids *D. sibirica* and *Gronotoma micromorpha* (Perkins) emerged from *Liriomyza bryoniae* (Kaltenbach) and *L. trifolii* (Abe et al., 2005; Abe, 2006). Adult size is one of the most frequently used indicators of parasitoid fitness. It has been shown that female fitness increased with body size (King, 1987; Godfray, 1994). Larger individuals have a higher potential fecundity, greater longevity, better searching capacity and higher dispersal ability (Godfray, 1994; Harvey et al., 1994; Visser, 1994; Ellers et al., 1998). Given a closely size–fitness relationship, female *N. okazakii* emerged from *L. chinensis* would have higher fitness than those from *L. trifolii*. Further study is needed to clarify the relationship between the size and fitness in female *N. okazakii*.

The average of net reproduction rate (*Ro*) suggests that *N. okazakii* population would increase 40.3 and 48.3 times during each generation on *L. chinensis* and *L. trifolii*, respectively. The average intrinsic rate of natural increase (r_m) was 0.22 and 0.24 per individual per day for *L. chinensis* and *L. trifolii*, respectively. Although such data are necessary to predict the reproductive potential of *N. okazakii* population in greenhouses or open fields, further experiments at different temperatures are required, since the net reproductive rates of leafminer parasitoids are variable with temperatures (Minkenberg, 1990; Hondo et al., 2006).

Non-reproductive killing behavior of leafminer parasitoids includes host-feeding and host-stinging without oviposition (Bernardo et al., 2006). Our results showed that the number of hosts killed by parasitoid feeding and stinging was greater on *L. trifolii* than on *L. chinensis*. The mean numbers of hosts killed were 112.4 and 36.4 larvae, respectively, accounting for 56.2% and 36% of total host mortality caused by the females. For synovigenic parasitoids, there is a tight link between host-feeding and subsequent reproduction. Since host blood is superior as a source of proteins, essential vitamins and salts for egg maturation, and a valuable source of nutrition for maintaining metabolism, host-feeding allows female parasitoids to enhance life time fecundity and longevity (Jervis and Kidd, 1986; Heimpel and Collier, 1996). However, host-feeding causes direct mortality of the host or reduces the quality of the host for oviposition (Jervis and Kidd, 1986; Jervis et al., 1996). As with other destructively host-feeding species, *N. okazakii* females feed preferentially on earlier host stages and to oviposit preferentially in later host stages (Tran, personal observations). Therefore, using the mixtures of small and large hosts for producing *N. okazakii* can increase mass-rearing efficiency.

Host-stinging without oviposition 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 hosts varies depending on the host size distribution (Heinz and Parrella, 1989), host density (Patel et al., 2003) and temperature (Patel and Schuster, 1991). Host stinging may be a mechanism for managing the density of leafminer larvae on invididual leaflets to ensure that a leaflet containing parasitized larvae will not be lost to the leafmining of surviving, non-parasitized larvae on the same leaflet (Patel et al., 2003). Also, since extensive leafmining can cause desiccation, necrosis and abscission of a leaf, host stinging can be potentially result in enhanced survival of parasitoid larvae (Patel et al., 2003). Although there is no individual data on host-stinging and host-feeding of *N. okazakii* on the hosts, total hosts killed with parasitoid feeding and stinging are also a source of host mortality due to the parasitoid.

There is little evidence that *Liriomyza* parasitoids belonging to Eulophidae display any high degree of host specificity (Murphy and LaSalle, 1999; Chen et al., 2003). These parasitoid females accept their hosts according to their suitability for progeny development (Olivera and Bordat, 1996; Bazzocchi et al., 2003; Abe et al., 2005; Abe, 2006). *N. okazakii* developed completely and similarly on *L. chinensis* and *L. trifolii*, and the females showed no significant difference in fecundity and offspring sex ratio between these hosts. Thus, the two host species are equally suitable as host for *N. okazakii*.

For many natural host-parasitoid rearing systems, in which the biological control agent is produced using the natural host or prey with natural food plants, labor cost for rearing plants and herbivores can make the mass-reared natural enemies uneconomic. The production costs can normally be lowered only by reducing costs of raw materials, largely by finding cheaper substitutes at either the plant or herbivore trophic level in a rearing system (Van Driesche and Bellows, 1996). Our findings suggested that *L. trifolii* and kidney bean could be used in the rearing system of *N. okazakii* for reducing the costs because planting kidney bean for rearing *L. trifolii* costs less time and labor. Given that host/plant systems used to rear natural enemies can affect its foraging behavior (Van Driesche and Bellows, 1996), it is appropriate to consider further research with respect to such potential behavioral effects on *N. okazakii*.

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