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TECHNICAL NOTE

A method for mass-rearing *Liriomyza chinensis* (Diptera: Agromyzidae)

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Abstract
We established a method for mass-rearing the stone leek leafminer, *Liriomyza chinensis* (Kato) (Diptera: Agromyzidae), one of the most destructive pests of Welsh onion, *Allium fistulosum* L. We examined three methods that allow *L. chinensis* to produce eggs year-round. By recording the number of emerged adults for 4 years, we determined the optimal mass-rearing method as follows. *Allium fistulosum* plants used for oviposition are grown in pots and maintained in a growth chamber with temperature and photoperiod set to 22 ℃ and 14 L:10 D, respectively. *Allium fistulosum* plants are exchanged for a new set of plants three times in a 7-day period. On the first day, 300 adults are placed in the rearing cage. On the fifth day, another 100 emerged adults are placed in the rearing cage. Using this rearing method, it becomes possible to obtain more than 1,000 *L. chinensis* eggs in a day. Our goal is to select and breed a highly resistant line of *A. fistulosum* using a novel method for evaluating egg killing defenses via artificial inoculation of plants with *L. chinensis* eggs. Here we present our mass-rearing method to obtain large quantities of *L. chinensis* eggs.

Keywords Stone leek leafminer · *Liriomyza chinensis* (kato) · *Allium fistulosum* · Mass-rearing · Welsh onion

Introduction

The stone leek leafminer, *Liriomyza chinensis* (Kato) (Diptera: Agromyzidae), is a destructive pest of *Allium* spp. in Japan, Korea, China, and other Asian countries (Tran and Takagi 2005). This species was recorded nearly 40 years ago in France (Martinez 1982) and now is expanding its range in Eastern Europe (Martinov et al. 2016; Papp and Cerny 2017). Leaf punctures and mines made by female adults and larvae, respectively, reduce leaf photosynthetic rate (Choi et al. 2003) and often cause unacceptable damage to marketable parts of the green onion (Ueno and Tran 2015). Recently, a new strain (strain B) of *L. chinensis* was detected throughout Japan and the larval mining damage of this strain is far greater than that of the native strain (strain A) (Tokumaru and Uesugi 2019).

Insect pest control is indispensable for field-grown Welsh onion plants, *Allium fistulosum* L. Although one *A. fistulosum* parental line, 'Negi Chuukanbohon Nou 1', with high resistance to the rust *Puccinia allii* (de Candolle) Rudolphi, has been developed (Wako et al. 2012), no *A. fistulosum* line with insect resistance has been developed. Thus, developing a line of *A. fistulosum* with insect resistance is of great interest.

One *A. fistulosum* variety, 'Beicong', has shown high resistance to *L. chinensis* (Sueyoshi et al. 2006), as evidenced by fewer leaf punctures and reduced mining. Therefore, this variety holds promise as a resistant breeding stock. However, it is difficult to select highly resistant individuals displaying antibiosis using a damage index in the field because the number of laid eggs or settled adults per leaf is variable. For this reason, the resistance of individual plants is tested by conducting laboratory experiments, which require the production of large quantities of *L. chinensis* eggs.

Allium fistulosum plants have been used for rearing *L. chinensis* in previous studies (Tokumaru and Okadome 2004; Tran and Takagi 2005). However, the number of adults introduced into the rearing cage was small (20–50 mixed sexes of *L. chinensis* adults) and, for this reason, it was not possible to obtain large quantities of eggs with these rearing methods.

Here we report a new mass-rearing method that allows *L. chinensis* to produce eggs year-round. In a separate publication, we report a new method for evaluating the antibiosis

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of *A. fistulosum* that have varietal resistance to *L. chinensis* via the artificial inoculation of plants with *L. chinensis* eggs (Takeda et al. 2020).

Materials and methods

Insect rearing

An adult colony of *L. chinensis* (strain A) was collected in October 2011 at the NARO Institute of Vegetable and Tea Science (north lat. 34° 61′, east long. 136° 25′ alt. 61 m), Tsu City. *Liriomyza chinensis* adults from this site were added regularly between 2011 and 2015 to the laboratory population to reduce inbreeding effects. Adults were reared on *A. fistulosum* plants (cv 'Kujou futo'; Takii and Co., Ltd.) grown from seeds. *Allium fistulosum* seeds were sown in two cell trays (128 cells per tray, 2 seeds per cell) with horticulture soil (Kumiai Nippi; Nippon Hiryou Co., Ltd.) every 2 weeks in a glass house year-round. In winter, the glass house was heated such that the temperature was maintained at or above 15 ℃. Approximately three months after germination, seedlings with three leaves were transplanted into six plastic pots with six plants per pot (top diameter: 45 mm, height: 60 mm) in a rearing cage (Cage A: 300 × 150 × 200-mm high, both sides had nylon mesh for ventilation). Two bottles (φ30 mm, 50-mm high) containing a solution of approximately 20% hydromel were placed in Cage A. The hydromel was served as a food source for adult flies.

Adult flies were introduced into Cage A using one of the three mass-rearing methods, as shown in Table 1. For Method I, live adults from the previous seven-day period and newly emerged adults were introduced into Cage A to maintain 300 rearing adults on the first day, and 100 emerged adults were introduced on each of the third day and the fifth day in a 7-day period. For Method II, live adults from the previous seven-day period and newly emerged adults were introduced into Cage A to maintain 300 rearing adults on the first day, and no other adults were introduced on the third and fifth days. For Method III, live adults from the previous 7-day period and newly emerged adults were introduced into Cage A to maintain 300 rearing adults on the first day, no adults were introduced on the third day, and 100 emerged adults were introduced on the fifth day. Ages of added adults were 0—3 days old. Numbers of live adults were recorded beginning in June 2015.

Table 1 Number of *L. chinensis* adults introduced into Cage A weekly for each of three mass-rearing methods

Day of introduction in a seven-day period	Number of *L. chinensis* adults introduced into Cage A		
	Method I (2015/6/5–2016/1/3)	Method II (2016/1/4–2016/8/7)	Method III (2016/8/8–2019/5/31)
First day	300[a]	300[a]	300[a]
Third day	100[b]	None	None
Fifth day	100[b]	None	100[b]
Total	500	300	400

[a]On the first day, live adults from the previous seven-day period and newly emerged adults were introduced into Cage A

[b]On the third day or fifth day, newly emerged adults were added into Cage A

Cage A was placed in a growth chamber (TAM131AM-SV; Toshiba Carrier, Co., Ltd.) with temperature and photoperiod set to 22 ℃ and 14 L:10 D, respectively. Relative humidity was not controlled and fluctuated from 20 to 60% throughout the year. The six pots in Cage A were exchanged with new pots three times per week on the first, third, and fifth days in a 7-day period. The removed pots were well watered in a plastic tray and placed in the same growth chamber for a week. From these well-watered pots, 12 pots each were placed in a second cage for pupation and adult emergence (Cage B: 220 × 150 × 250-mm high, both sides had nylon mesh for ventilation). Cage B was placed in the same growth chamber. The developmental time of *L. chinensis* from egg to adult emergence on *A. fistulosum* plants at 22.5 ℃ is 26.8 ± 0.12 days (mean ± SE), or approximately four weeks (Tran et al. 2007). Thus, the next-generation adults that emerged in Cage B after approximately 4 weeks were transferred into Cage A (see Fig. 1). Any extra live emerged adults not transferred into Cage A were discarded on the first, third, and fifth days in a 7-day period so that ages of transferred adults were 0–3 days-old. The number of live emerged adults in Cage B was counted on the first, third, and fifth days in a 7-day period and recorded beginning in June 2015. At the end of each 7-day period, the weekly total number of live emerged adults was calculated. Two fresh bottles of hydromel in Cage A were exchanged on the first day and hydromel was added to the bottles on the fifth day in a 7-day period.

Recording of number of live and dead emerged adults reared with Method III

To examine the number of next generation adults precisely, both live and dead emerged adults were counted on the first, third, and fifth days every 7 days from August 12 to September 27, 2019. The adults that were counted had emerged from the pots exchanged during the period July 15 to August 23, 2019. During this period, only six pots were placed in Cage B to allow precise counts of the emerged adults. The number of female and male emerged adults reared with Method III was counted from May 1 to May 18, 2020 and the sex ratio [male/(male + female)] was calculated.

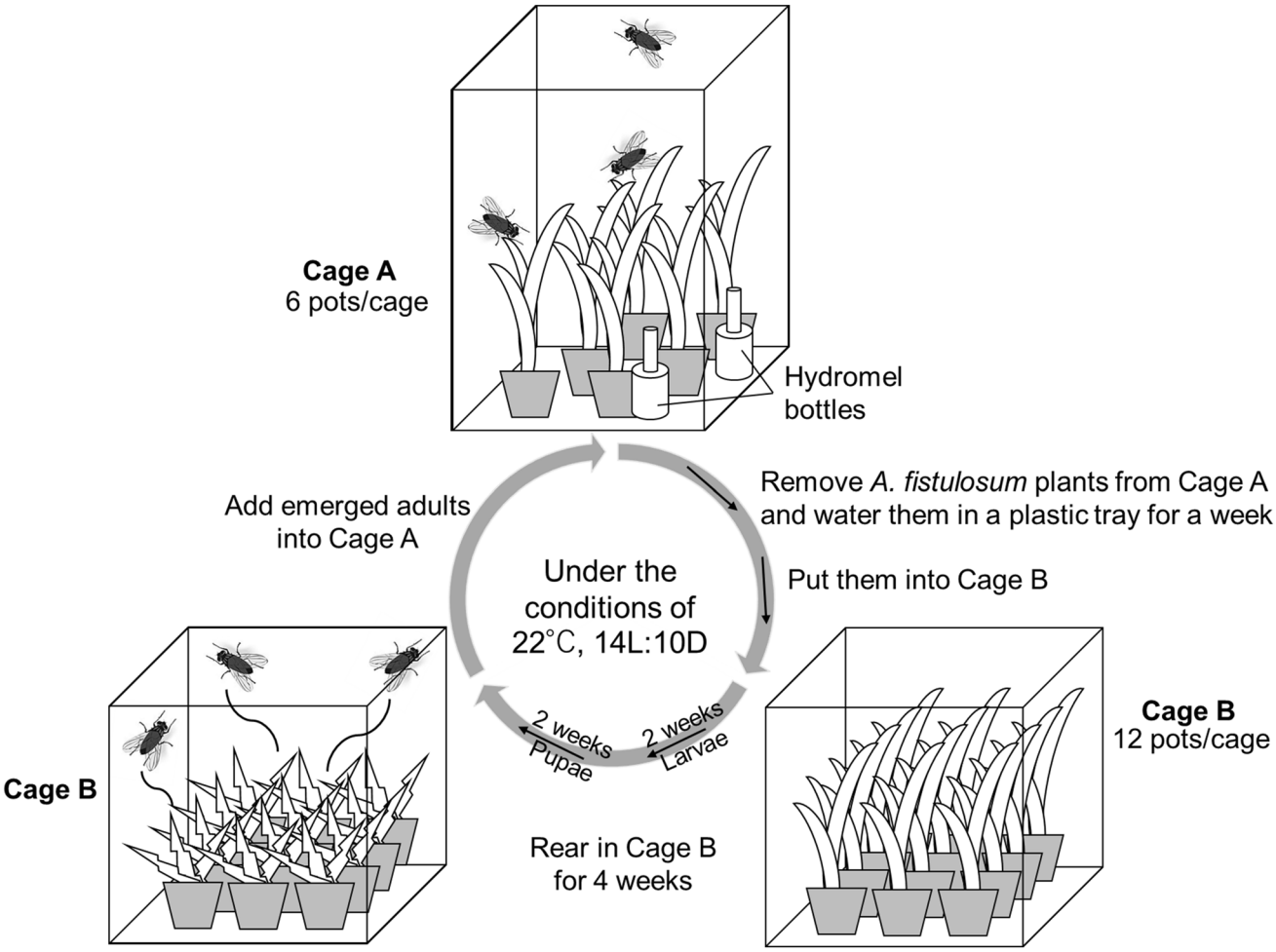

Fig. 1 A mass-rearing system for *L. chinensis*

Results and discussion

Seasonal changes in the weekly sum of live emerged adults were seen throughout the research period (Fig. 2). From 2015 to 2017, the number of live emerged adults increased around February to March and September to October and decreased around July to August and January. Beginning in 2018, using rearing Method III, the number of live emerged adults fluctuated weekly, but seasonal fluctuation remained moderate. As shown in Fig. 2, the number of live emerged adults is largest on Method III (1,963 adults, November 26, 2018), followed by Method II (1,274 adults, February 22, 2016) and Method I (930 adults, October 5, 2015). Moreover, the mean weekly total (±SD) of live emerged adults was largest on Method III (927 ± 424), followed by Method II (460 ± 268) and Method I (348 ± 195; Table 2). These results suggest that Method III is the most effective way to rear *L. chinensis* in bulk on *A. fistulosum* plants.

From June 2015 to May 2019, the number of live adults (mean ± SD) on the first day of the week after a 7-day period in Cage A was also counted (Table 3). For Method I, the number of live adults 1 week after the introduction of total 300 adults in Cage A decreased to 108.6 ± 45.0, even after 100 adults were introduced on each of the third and fifth days. For Method II, the number of live adults 1 week after the introduction of total 300 adults decreased to 87.2 ± 35.4. For Method III, the number of live adults 1 week after the introduction of total 300 adults decreased to 131.3 ± 36.5, even after 100 adults were introduced on the fifth day. The longevities of female and male adults on *A. fistulosum* plants at 25 ℃ have been reported as 12.9 ± 4.8 days and 7.1 ± 3.1 days, respectively (Tokumaru 2016). However, our data show that more than half of the adults introduced into Cage A died within a week. For all three methods, total 300 emerged adults were introduced into Cage A on the first day in a 7-day period, but the age of these adults after emergence was not known precisely (we know only that the age was 0–3 days), nor was the age of the additional 100 emerged adults known precisely. It is possible that too many adults were introduced to live longer with limited food sources.

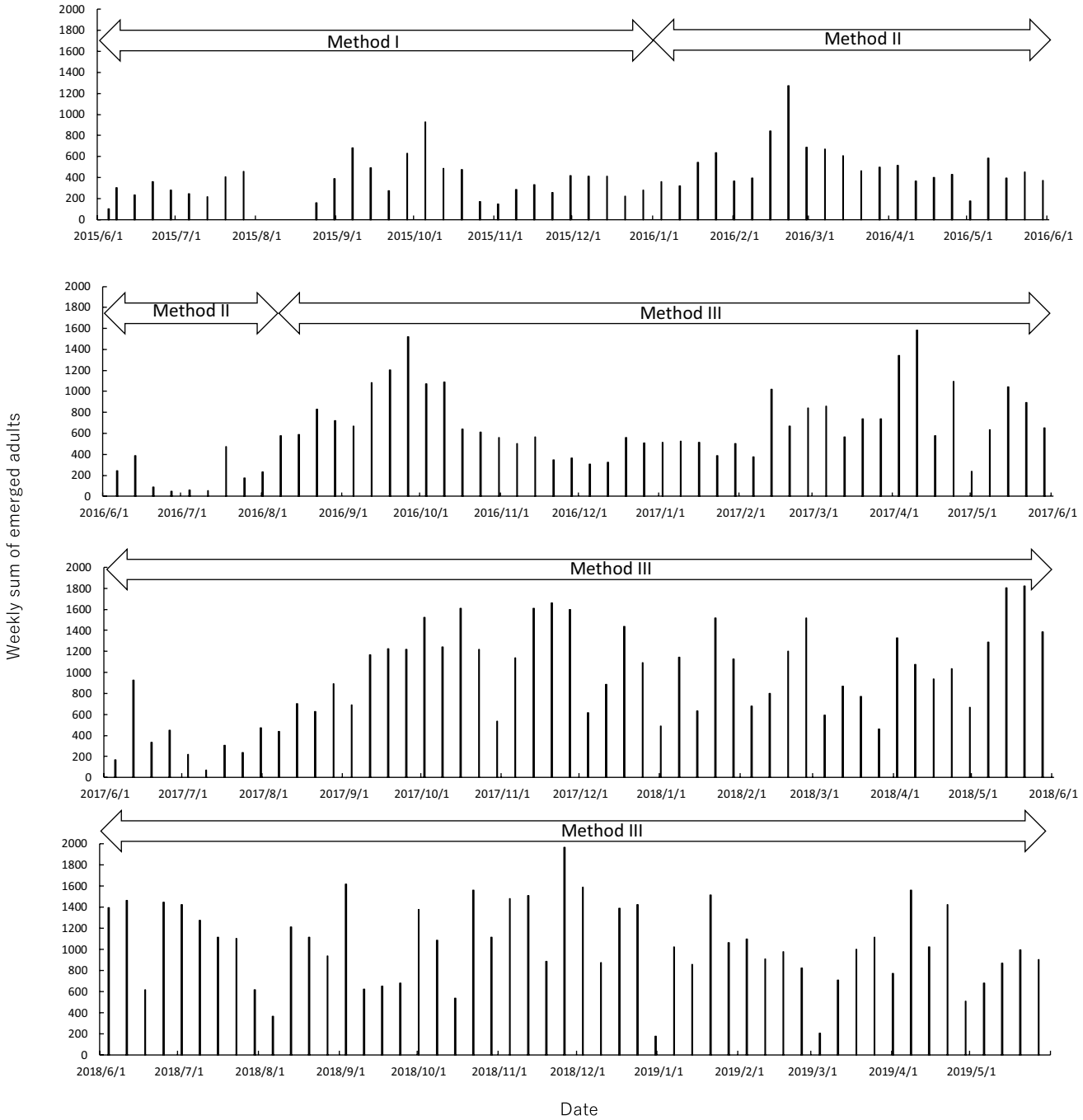

Fig. 2 Seasonal changes in weekly sum of emerged adults of *L. chinensis*. Only live adults in Cage B are included in the weekly sum. Arrows within the figure indicate the period of each rearing method (Methods I, II, and III)

Table 2 Mean (±SD) weekly total of live emerged adults in Cage B

Rearing method	Mean (±SD) weekly total	Rearing period
Method I	348±195	2015/6/5–2016/2/1
Method II	460±268	2016/2/2–2016/9/5
Method III	927±424	2016/9/6–2019/5/27

Table 3 Number of live adults (mean±SD) on the first day of the week following a seven-day period in Cage A

Rearing method	Number of live adults	Rearing period
Method I	108.6±45.0	2015/6/5–2016/1/4
Method II	87.2±35.4	2016/1/5–2016/8/8
Method III	131.3±36.5	2016/8/9–2019/5/27

In addition, the average life span of male and female adults reared with *A. fistulosum* plants and honey as food sources was reported by Tokumaru (2016) as 8.9 days and 17.8 days at 20 ℃, respectively. The survival rate would have increased with honey on the ceiling in Cage B.

Tokumaru (2016) reported that the number of next-generation *L. chinensis* adults produced by one female adult was the largest (103.6 ± 87.7) at 25 ℃, second largest (55.4 ± 55.1) at 20 ℃, and smallest (42.6 ± 40.2) at 30 ℃ (mean ± SD). Tokumaru (2016) also reported that the emergence rate of *L. chinensis* adults (No. of adult flies/No. of pupae) was highest (0.913 ± 0.090) at 20 ℃, second highest (0.682 ± 0.205) at 25 ℃, and lowest (0.440 ± 0.135) at 30 ℃ (mean ± SD). On the other hand, an optimal range of temperature for photosynthesis of *A. fistulosum* is 15–20 ℃ and high temperature affects physiological processes of *A. fistulosum*, especially photosynthesis (Levitt 1980; Yamasaki et al. 1998). In considering the emergence rate of adults and the condition of *A. fistulosum* as food sources, we set the rearing temperature to 22 ℃ and found that the measured temperature was stable at 20–25 ℃ throughout the year.

The temperature and solar radiation conditions for cultivation of *A. fistulosum* seedlings in a glass house was not stable and changed seasonally. It is reported that the nutritional value of *A. fistulosum* plants differs throughout the growing season; the sugar and ascorbic acid contents are higher in winter-grown *A. fistulosum* plants than in summer-grown plants (Ibaraki et al. 1997). Here, the temperature for cultivation of *A. fistulosum* seedlings was lowest at 15 ℃ in winter and highest at 30 ℃ in summer, and it is possible that the nutritional value changed seasonally because the *A. fistulosum* seedlings were affected by fluctuations in solar radiation and temperature. Although the temperature and photoperiod utilized in rearing *L. chinensis* were stable, the humidity varied throughout the year. These factors could have influenced seasonal fluctuations in the number of emerged adults.

The precise number of emerged adults for Method III was counted on the first, third, and fifth days in a seven-day period from August 12 to September 27, 2019 (Fig. 3). The number of both live and dead next-generation adults (mean ± SD) that emerged from *A. fistulosum* plants on which females oviposited from the first day to the third day (two days) was 380.2 ± 136.2; from the third day to the fifth day (2 days) was 393.2 ± 224.5; and from the fifth day to the first day of next period (3 days) was 467.0 ± 132.8. The weekly sum of emerged adults was 1,240.4 ± 489.5. This was approximately three times as many adults as were needed per week for oviposition in Cage A. The number of dead adults was about twice the number of live adults (Fig. 3). *Allium fistulosum* plants in Cage B had wilted by the time the next-generation adults emerged. Thus, we believe that most of the emerged adults in Cage B were not able to live

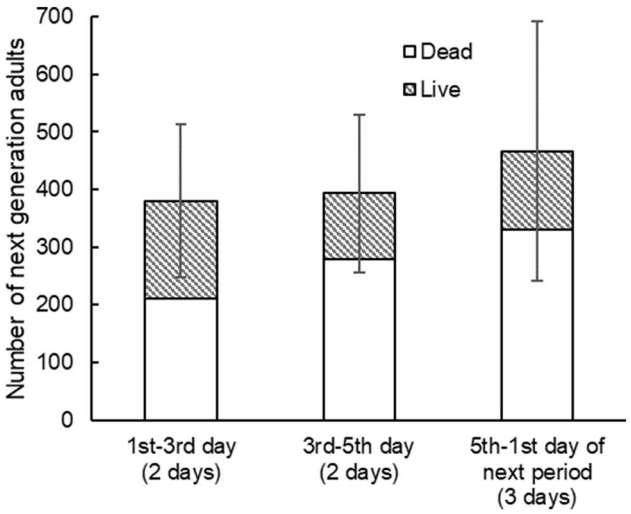

Fig. 3 The number of next-generation adults (mean ± SD) of *L. chinensis* from *A. fistulosum* plants on which females oviposited for 2 or 3 days using Method III

long without food and water and this explains the high mortality. The average life span of female adults reared with *A. fistulosum* plants and honey as food sources was reported by Tokumaru (2016) as 17.8 days at 20 ℃ and 12.9 days at 25 ℃. The average number of laid eggs per female adult was 74 at 20 ℃ and 116 at 25 ℃. Tran and Takagi (2005) reported that the average life span of female adults reared with *A. fistulosum* plants was 9 days and the average number of laid eggs was 108 at 25 ℃. In the present study, the sex ratio [male/(male + female)] was 0.47 ± 0.04 when flies were reared with Method III at 22 ℃ (mean ± SD). Assuming that (1) an average of 200 live adults are maintained in Cage A through a week when rearing with Method III, (2) approximately half of the 200 adults are female, and (3) an individual female lays between five and ten eggs per day at 22 ℃ (the oviposition rate was 3.8 at 20 ℃ and 11.7 at 25 ℃; Tokumaru 2016; Tran and Takagi 2005), we estimate that 500–1,000 eggs are laid on *A. fistulosum* leaves every day. Under these assumptions, an adequate number of eggs are laid by an adequate number of females to maintain the population from one generation to the next in Model III.

Takeda et al. (2020) established a novel method for evaluating varietal resistance of *A. fistulosum* plants against *L. chinensis* via artificial inoculation of plants with eggs. To succeed in artificially inoculating plants with eggs, 1,000 or more eggs must be produced in 24 h at 24 ℃. Assuming that an individual female lays 10 eggs per day (Tran and Takagi 2005) and the sex ratio is approximately 0.5, a population of at least 200 adults is required. Moreover, to collect eggs efficiently, it is necessary to maintain two populations, which means twice the number of eggs are required. Using Method III, the number of emerged adults (mean ± SD) per

week was 1,240.4 ± 489.5 (Fig. 3), and approximately half of them are females. Although approximately two-thirds of emerged adults died in a week (Table 3), we may be able to obtain at least 14,000 eggs per week (10 eggs/day/female × 7 days × 200 females). Therefore, we should be able to use those emerged adults not only for mass-rearing, but also for collecting eggs to use in the varietal resistance test. Using this rearing method, it is possible to collect more than 1,000 *L. chinensis* eggs in 1.5 h by one person (Takeda et al. 2020).

In summary, we report a sustainable method for mass-rearing *L. chinensis*. The most important steps in this method are as follows: (1) exchange pots planted with *A. fistulosum* for oviposition three times per week; (2) add emerged adults to maintain 300 adults in Cage A on the first day; and (3) add 100 adults to Cage A on the fifth day. Although seasonal variation in the number of emerged adults was recorded, it is possible to rear *L. chinensis* in bulk year-round with this method.

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