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Egg-collection equipment for *Liriomyza* leafminers (Diptera: Agromyzidae)

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Abstract Egg-collection equipment for *Liriomyza* leafminers was designed using a small glass tube with a glucose solution supported between two thin films. Adult female *L. trifolii* (Burgess), which were previously reared on kidney bean for 2 to 4 days, laid abundant eggs in 5 or 10% glucose solutions. Hatchability of the eggs in the solutions was greater than 80%. The equipment was used successfully to collect eggs from another leafminer species, *L. sativae* Blanchard.

Keywords Egg-collection equipment · Leafminer · *Liriomyza trifolii* · *Liriomyza sativae* · pre-rearing

Introduction

Adult female Agromyzid leafminers lay eggs in the leaf tissues of host plants (Spencer 1990). Consequently, harvesting leafminer eggs in sufficient numbers without damaging them is difficult. Here we report the development of an egg-collection method for *Liriomyza* leafminers using a modification of the equipment used for collecting the eggs of thrips (Murai and Loomans 2001). In addition, we describe several potential applications of the equipment and the eggs collected therewith.

Materials and methods

Laboratory cultures of *L. trifolii* and *L. sativae* were established from populations collected in Hamamatsu, Shizuoka, Japan in 1991, and Iwata, Shizuoka, Japan in 2004, respectively. The cultures were maintained in a gauze cage (50 x 50 x 50 cm) on the primary leaves of potted kidney bean plants, *Phaseolus vulgaris* L., that were maintained in a room under 16L:8D at 25 ±1°C (Saito et al. 1995). Pupae were collected and transferred to a plastic container (diam.: 10 cm, height: 5 cm) with honey streaked on the cap surface. The container was incubated under 16L:8D at 25 ±1°C and newly emerged female and male adults were collected and transferred to another gauze cage (50 x 50 x 50 cm) containing potted kidney bean plants maintained at 16L:8D and 25 ±1°C. After 0 to 6 days female adults were collected from the cage and used in subsequent egg-collection experiments.

The egg-collection equipment shown in Fig. 1a was modified from that used to harvest the eggs of thrips (Murai and Loomans 2001). The open end of a glass tube (diam.: 2 cm, height: 2 cm), which had honey streaked on the inner surface, was covered with a single sheet of stretched paraffin film (NOVIX-II, Asahi Techno Glass, Tokyo). Then, 200 µl of glucose solution (5, 10 and 30%) or water was placed in the center of the film, which was then covered with another sheet of the paraffin film. Adult females that had been pre-reared on kidney bean were then individually released into the glass tubes and the opposite ends of the tube were covered with gauze. The number of eggs laid in the solution was then counted daily over a two-week period under a stereomicroscope (MZ16, Leica, Germany). Hatchability of the eggs in the solution was determined on the last day of the experiment, which was performed in an incubator under 16L:8D at 23±1°C.

Results and discussion

In all of the experimental treatments, adults finished laying eggs within 5 days. The number

of *L. trifolii* eggs varied markedly between individuals; more than half of the adults did not lay any eggs, while some adults laid more than 40 eggs over the course of their lives (maximum: 48 eggs). Comparatively high numbers of eggs were obtained from adults offered glucose solutions of 5 and 10% at 2 to 4 days after emergence (DAE) (Fig. 2). The most eggs produced was 9.0 eggs/adult in adults offered a glucose solution of 10% at 4 DAE (14 of 32 adults laid eggs). At 2 DAE, adults offered a glucose solution of 5% produced 8.8 eggs/adult (7 of 16 adults laid eggs). Hatchability of eggs exceeded 80% when adults were offered glucose solutions of 5 and 10%, and was less than 50% when adults were offered a 30% glucose solution (data not shown). Eggs collected by the equipment and larvae hatched in a glucose solution are shown in Fig. 1b and 1c, respectively. No eggs were laid in water without glucose.

The equipment was also tested with another leafminer species, *L. sativae*, using combination of 4 DAE adults and a 10% glucose solution, which was shown to be the most favorable combination for egg collection in *L. trifolii*. The most eggs produced by *L. sativae* was 8.1 eggs/adult (6 of 14 adults laid eggs, maximum: 25 eggs, hatchability: 70.2%), which was similar to that observed in *L. trifolii*. These findings show that the method could potentially be applied to other *Liriomyza* species, such as *L. bryoniae* (Kaltenbach) and *L. huidobrensis* (Blanchard).

Our results indicate that pre-rearing adults on kidney beans for at least 2 days may be necessary to allow females to reach reproductive maturity, but that pre-rearing for more than five days could result in eggs being laid on the host plant before the females are transferred to the egg-rearing equipment. When offered the 30% glucose solution, a few adults laid a small number of eggs but the hatchability of the eggs was low, suggesting that female adults may avoid laying eggs in highly concentrated glucose solutions as the high osmotic pressures of such solutions may adversely affect hatchability.

In a previous study, the number of eggs produced by the *L. trifolii* population used in this study when reared on a variety of host plants was as follows: >500 eggs/adult on kidney bean and pak-choi, 100-300 eggs/adult on melon, gerbera, celery and chrysanthemum, 54.7 eggs/adult on tomato, and 21.1 eggs/adult on soybean (Saito et al. 1995). Compared with the host plants used in that study, the number of eggs obtained using the rearing equipment described here was relatively low. However, the number of eggs that could be harvested using this method could be increased by placing more adults into each glass tube and/or by scaling-up the size of the equipment used.

The observation that more than half of the adults used did not lay any eggs suggests that cues other than glucose concentration may be required by adults in order to lay eggs and/or that the paraffin film may lack physical and chemical properties that are required for

1
2 promoting egg laying. Consequently, the equipment described here could also be used to
3 elucidate which factors affect egg laying in adults. The ability to harvest eggs from the
4 glucose solution easily and without causing any damage means that, by embedding the
5 eggs in leaves of non-host plants such as carnations, *Dianthus caryophyllus* L. and the
6 strawberry, *Fragaria x ananassa* Duch., it may be possible to study the defense
7 mechanisms employed by plants in response to *L. trifolii* infestation in greater detail. The
8 equipment may also be useful in embryological studies of *Liriomyza* leafminers, as the
9 growth and development of the eggs can be observed through the film under a microscope.
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Figure 1

Figure 2

Figure legends

Fig.1 a Egg-collection equipment (diam.: 2 cm, height: 2 cm) for *Liriomyza* leafminers. **b** A female adult released into the equipment and its eggs in a glucose solution. **c** Eggs, egg shells and larvae in a glucose solution.

Fig. 2 Number of *L. trifolii* eggs collected using the egg-collection equipment described in this study. Adult females were reared on potted kidney bean plants for 0 to 6 days after emergence (DAE) and the number of eggs was determined using the apparatus filled with different glucose solutions (5, 10 and 30%). No eggs were detected in water without glucose. Bars indicate number of eggs/adult, fractions above bars indicate the number of adults that laid eggs/number of adults tested, and whiskers indicate standard errors.