A simple mass-rearing method for predaceous Orius bugs in the laboratory

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Abstract
Orius strigicollis (Poppius) and O. laevigatus (Fieber) (Heteroptera: Anthocoridae) can be reared successfully using a plastic Petri dish (90 mm in diameter, 20 mm in depth) as a rearing cage with wheat grains to prevent cannibalism and excessive moisture. Frozen eggs of Ephestia kuehniella were supplied as food and a soybean seedling was provided as an oviposition substrate. The rate of individuals reaching the adult stage averaged 74–87% when the initial nymphal density was 100–400. In addition to the high breeding capacity, these insects could be separated without difficulty from wheat grains by using a suitable sieve, and wheat grains were reusable after sterilization in an oven.

Key words: Cannibalism; Orius laevigatus; Orius strigicollis; predator; rearing method

INTRODUCTION
Anthocorid bugs, particularly Orius species, are important natural enemies of agricultural pests such as thrips, aphids and spider mites (e.g. Nakata, 1995; Wang, 1998; Lattin, 1999). Several species of Orius bugs are now marketed as biocontrol agents against thrips that attack crops in greenhouses (Jacobson, 1993; Van den Meiracker and Sabelis, 1993; Van Lenteren et al., 1997; Shipp and Wang, 2003; Yano, 2004). It is important to study the appropriate use of these biocontrol agents in the greenhouse, as well as the efficacy of indigenous populations of Orius species as native predators against pests in the open field. Studies on the susceptibility to insecticides or acaricides in these predators are also needed. To assist with the above endeavors, a simple mass-rearing technique in the laboratory needs to be developed.

Although Orius bugs are successfully bred on eggs of the Mediterranean flour moth, Ephestia kuehniella Zeller, as an alternative food (Schmidt et al., 1995; Cocuzza et al., 1997; Montserrat et al., 2000; Yano et al., 2002), cannibalism in rearing cages under crowded conditions may be a problem (Chambers et al., 1993; Tommasini et al., 2002; Baniameri et al., 2005). To prevent such cannibalism, researchers have utilized folded paper tissue (Chambers et al., 1993), shredded paper (Arijs and DeClercq, 2001), a mesh sheet (Shimizu and Kawasaki, 2001) or unhulled rice grains (Yano, personal communication; Ito and Nakata, 1998) to expand the surface area in the rearing cage and as shelter materials for several Orius species. For mass-rearing, however, very few studies have been done on the breeding capacity of rearing cages: therefore, this study focused on evaluating the plastic Petri dish as a mass-rearing cage using two Orius species, Orius strigicollis (Poppius) and O. laevigatus (Fieber) (Heteroptera: Anthocoridae). The former is a common species in the southwestern part of Japan, Taiwan, and southern China (Yasunaga, 1997). It is registered as a biotic pesticide and is commercially available in Japan. The latter is widespread throughout the Mediterranean region and is also registered and sold in Europe (Copping, 1998).

MATERIALS AND METHODS
Adults of O. strigicollis were collected in August 2001 in Miyazaki, Japan, and those of O. laevigatus were collected in October 1999 in Montpellier, France. Insects used in this study were the progeny of these populations.

A plastic Petri dish (90 mm in diameter, 20 mm
in depth: Nipro Co., Osaka) was employed as a rearing cage and 30 g of wheat grains (dried and sterilized in an oven at 90°C for 5 h) were put into the cage (about 7–8 mm in depth) to prevent potential cannibalism and excessive moisture. Ample amount of frozen eggs of *E. kuehniella* (0.2–0.3 g) were supplied as food, and a fresh soybean, *Glycine max* Merril, seedling (wrapped around the snipped stem with a piece of water-soaked cotton to maintain moisture) on a piece of Parafilm® M was provided as an oviposition substrate and water supply. Since 1st instar nymphs of *Orius* can readily escape through gaps between the lid and the body of a Petri dish through the small projections of the lid surface for ventilation, the lid was used in an inverted position. Three or four Petri dishes were piled up and dry batteries were placed as weights on the dishes (Fig. 1A, B). Food and water were supplied every other day, but the cage was not changed, and the wheat grains and soybean seedling were not changed during the experiment. The rearing cages were kept under a photoperiod of L16:D8 at 22°C and 45% RH in an incubator.

To examine the relationship between nymphal density and the rate of successful rearing to the adult stage, 100–400 nymphs (1st–2nd instar) of both species were confined to each cage using an aspirator and reared until they developed to the adult stage. Adults were counted and removed from the cage every other day until development was completed. Since newly hatched nymphs are very small and susceptible to damage when they are transferred into the cage, older 1st and 2nd instar nymphs were used in this experiment. The experiment was replicated 6–11 times for each density.

To examine the rearing capacity of the cage, one or two soybean seedlings bearing various densities of *Orius* eggs (Fig. 1C) were placed in each cage. Nymphs were reared and adults were counted as described above. Nymphs were not counted to avoid damage from manipulation.

A sieve (1.25 mm mesh, 150 mm in diameter and 50 mm in depth) was used to separate insects from wheat grains. Insects through the sieve were col-

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**Fig. 1.** An overview of the rearing cages of *Orius* bugs using a plastic Petri dish with wheat grains and a soybean seedling on a piece of Parafilm® M (A), a close-up of the cage (B), a soybean seedling bearing *Orius* eggs (C), and the separation of insects from wheat grains using a sieve (1.25 mm mesh) (D).
lected into a large glass Petri dish (150 mm in diameter) (Fig. 1D).

RESULTS AND DISCUSSION

Although the lid of the Petri dish was used in an upturned position, air supply for the bugs was apparently sufficient, because many insects successfully developed to the adult stage. The numbers of adults that developed in *O. strigicollis* and *O. laevigatus* cages increased linearly with nymph density within the range examined (100–400 nymphs) (Fig. 2), and there were no significant differences in the proportion of individuals that reached the adult stage in either species (*p* > 0.05, Tukey’s multiple comparison test). The mean percentages reaching adulthood in *O. strigicollis* were 76.4, 74.2, 75.9 and 74.1% at densities of 100, 200, 300 and 400, respectively. They were 86.7, 86.8, 83.1 and 77.7% at the same densities in *O. laevigatus*. Although *O. laevigatus* showed a higher adult rate than *O. strigicollis* at each density, significant differences were found only at densities of 100 and 200 (*p* < 0.05, Mann-Whitney’s *U* test). About 300 and 310 adults developed on average at the highest nymphal density of 400 in *O. strigicollis* and *O. laevigatus*, respectively.

The developmental period (egg + nymph) at each density was not significantly different (*p* > 0.05, Tukey’s multiple comparison test), namely, it was 22.4 ± 0.5, 22.6 ± 0.5, 22.5 ± 0.5 and 22.6 ± 0.5 days at a density of 100, 200, 300 and 400, respectively in *O. strigicollis*. It was 26.2 ± 1.2, 25.6 ± 0.8, 25.8 ± 0.8 and 26.3 ± 0.8 days at a density of 100, 200, 300 and 400, respectively in *O. laevigatus*. These developmental periods were comparable to the results obtained under similar conditions reported by Kakimoto et al. (2003) on *O. strigicollis* (30.2 days at 20°C and 20.8 days at 25°C) and by Alauzet et al. (1994) on *O. laevigatus* (26.8 days at 20°C and 17.8 days at 25°C).

The sex ratio of adults produced in some density...
replicates was examined in *O. strigicollis*. The number of females and males emerging was almost the same at a density of 100, 200 and 300 (\( p \geq 0.05 \), \( \chi^2 \)-test) (Table 1); therefore, it seems that the sex ratio does not change depending on nymphal density. Adult body size also seems not to differ among nymphal densities.

Another experiment of rearing capacity showed that a maximum of 521 adults emerged from the *O. strigicollis* cage and 735 from that of *O. laevigatus*, although the initial number of nymphs was not counted (Fig. 3). *O. laevigatus* is likely to be tolerant against such crowded conditions, but the actual reason for the higher production rate in *O. laevigatus* is unknown. These results indicate that several hundred adults of both species can be obtained from a small cage such as a Petri dish. This is much better than the results of Chambers et al. (1993) and potentially better than Schmidt et al. (1995) on a per cage volume basis. The former reported that *O. laevigatus* was able to produce approximately 100–200 nymphs per week from a culture of up to 10 containers (15 cm diameter, 20 cm deep) feeding on *Aphis gossypii* Glover. The latter reported that 200–600 individuals of *O. insidiosus* (Say) were reared in a “zip-lock” plastic bag (4.5 l) feeding on *E. kuehniella* eggs. In the present method, several hundred adults from a Petri dish will be satisfactory for mass rearing of *Orius* in the laboratory.

In addition to high breeding capacity, this method has an advantage of easy separation of insects from the shelter material. Insects can be separated without difficulty from the wheat grains using a suitable sieve (see Fig. 1D). Moreover, wheat grains are reusable after sterilization in an oven at 90°C. Sieving and recycling of wheat grains may be also applicable if other hygroscopic granules such as unhulled rice grains are employed for the shelter material.

These mass-reared *Orius* bugs could be used for biological and physiological studies in the laboratory.

### REFERENCES


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