

## Evaluation of a rearing-method for the predator *Orius insidiosus*

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### Abstract

The development of mass-rearing techniques is aimed to produce large numbers of natural enemies of a good quality at a competitive price. The objective of this study was to investigate a rearing-system for *Orius insidiosus* (Say) (Rhynchota Anthocoridae). Three types of containers and different densities of eggs of the predator per container were tested in a climate room at  $26 \pm 2$  °C,  $70 \pm 10\%$  RH and photophase of 12 h. Also, one type of container and different densities of adults of the predator were tested. The immature stages were reared in plastic bags, Petri dishes, and glass jars at densities of 100, 250, and 400 predator eggs. Glass jars were used to rear adults, at densities of 250, 400, and 550 adults. Both Petri dishes and glass jars were suitable for rearing *O. insidiosus* at a density of 100 eggs per container, with a mass-production of newly-emerged adults of 55.2% and 50.5%, respectively. At the density of 250 eggs per container, only glass jars were suitable for nymph rearing with 53.6% production of adults. For adult rearing, the density of 400 adults per container was most suitable, with the highest number of eggs laid per female. The results showed that glass jars were best compared to others containers tested for the *O. insidiosus* rearing. Corrugated cardboard strips as hiding places for nymphs and adults, eggs of *Ephestia kuehniella* Zeller as food, an inflorescence of *Bidens pilosa* L. as oviposition site and a source of moisture should also be included to maximize the mass-rearing efficiency of *O. insidiosus*. This rearing-method could be a starting mass-rearing system for *O. insidiosus* in our conditions and that would be improved in co-operation with a Brazilian producer of natural enemies.

**Key words:** biological control, mass-rearing methods, *Orius* spp., predator, food, oviposition site.

### Introduction

Interest in biological control has increased considerably as a response to the various effects of pesticides on the environment and as a result of new international trends, which favors conservation and the sustainable use of biological resources. International food production policies increasingly demand alternatives to the use of chemical control, and biological control resurfaces with new energy in this scenario by means of techniques that make it viable to be used economically. According to van Lenteren (2000) often populations of predators and parasitoids that are naturally present in the agroecosystems are insufficient to maintain the density of a pest organism below the economic injury level. Thus, mass-production and release of natural enemies is needed to obtain sufficiently low pest populations.

One of the most serious pests worldwide is thrips. The use of predators of the genus *Orius* for the control of thrips has been especially successful in several countries in Europe (Van den Meiracker and Ramakers, 1991; Chambers *et al.*, 1993; Riudavets and Castañe, 1998; Tommasini, 2003). This success can be attributed mostly to: 1) the difficulty in controlling this pest with conventional insecticides, because thrips live concealed in flowers and in meristematic parts of the plant; 2) the rate with which thrips acquire resistance to insecticides; and 3) the good performance of *Orius* predators, because they also live concealed in the same habitat as thrips (Immaraju *et al.*, 1992; Van den Meiracker 1999; Linus *et al.*, 2002; Silveira *et al.*, 2004).

*Orius insidiosus* (Say) is the most common *Orius* species in Brazil, and several studies on its behavioral and biological features have been conducted under Brazilian

greenhouse conditions (Bueno *et al.*, 2003; Bueno, 2005; Carvalho *et al.*, 2005; Mendes *et al.*, 2005).

Many insects have been successfully reared in small numbers in the laboratory, but a large-scale rearing requires specific procedures and adaptations compared to small-scale laboratory rearing (Nordlund and Greensberg, 1994). Often the success of biological control by an increase of released natural enemies depends on an economically efficient mass-rearing. For *O. insidiosus*, which is already sold commercially, different rearing-systems have been proposed and used (Isenhour and Yeargan, 1981; Schmidt *et al.*, 1995; Blumel, 1996; Bueno, 2000; Tommasini *et al.*, 2004; Mendes *et al.*, 2005a). Thus, based on the knowledge presented in these papers, we attempted to develop cheaper and more effective mass-rearing systems, considering the resources available in Brazil. The development of new techniques for the mass-production of natural enemies is expected to lead to reduce costs and to make the use of biological control agents a feasible undertaking in our country. This work had as objective to investigate a rearing system for the predator *O. insidiosus*.

### Materials and methods

Experiments were conducted in a climate room at  $26 \pm 2$  °C,  $70 \pm 10\%$  RH and a 12-hour photophase, in the Laboratory of Biological Control of the Department of Entomology at the Federal University of Lavras, MG, Brazil. The insects used in the experiments came from a research stock-rearing of *O. insidiosus* started according to the methodology proposed by Bueno (2000) and Mendes *et al.* (2005a) with adults originally collected in

the municipality of Lavras, MG, in farmer's friend (*Bidens pilosa* L.) inflorescences. This rearing is periodically renewed with adult insects collected in the field.

The mass-rearing methods employed by commercial producers in other countries are usually not in public domain. We developed a mass-rearing based on experience obtained with the research on rearing of *O. insidiosus* maintained at our laboratory and data from the references mentioned in the introduction of this paper. According to Parra (2002), a research rearing consists of insect colonies reared on a small-scale basis, which can be increased in order to be used in applied researches especially in cases of biological control that require seasonal inoculative releases of the natural enemy.

### Rearing containers

Three types of containers were evaluated in the laboratory to optimize the mass-rearing of *O. insidiosus* (figure 1).

#### 1. Plastic bag

(Adapted from a model used by Schmidt *et al.*, 1995). Transparent plastic bags (27 × 31 cm), with a 4.0-liter capacity, equipped with a hermetic seal in their upper part were used (figure 1A). Adaptations and adjustments: (1) an EVA (Ethylene Vinyl Acetate rubber-like material) strip (5 cm width × 45 cm length) was attached to the side ends of the container with transparent adhesive tape (5 cm wide); (2) to promote aeration inside the plastic bag, an opening (5 × 6 cm) was made in one of the walls, closed with a voile-type fabric, using transparent adhesive tape (5 cm wide); (3) two cotton discs (5 cm in diameter) moistened with distilled water, and eight corrugated cardboard strips (2 × 45 cm) were attached to the inside walls of the plastic bag as hiding places for nymphs and adults.

#### 2. Petri dish

(Adapted from a model used by Mendes and Bueno, 2001). Petri dishes (20 cm in diameter) with 800 ml capacity, sealed with PVC film were used (figure 1B). Six corrugated cardboard strips (2 × 45 cm) were placed inside, in addition to a piece of cotton (approximately 1 g) moistened with distilled water.

#### 3. Glass jar

(Adapted from models used by Isenhour and Yeorgan, 1981; Tommasini and Nicoli, 1993; Blumel, 1996). Glass jars (11 cm in diameter × 16 cm in height) with 1.7-liter capacity and plastic screw-on lids were used (figure 1C). Adaptations of this container: (1) ventilation opening: an opening (9 cm diameter) was made on the plastic lid, leaving only the screwing ring intact and a voile-type fabric was added (14 cm diameter); (2) in order to maintain humidity within the glass jar, three acrylic vials (5 ml) filled with cotton, moistened with distilled water, were placed inside; (3) eight corrugated cardboard strips (2 × 45 cm) were placed inside the jar as shelters for the insects.

### Immature stage rearing

Densities of 100, 250, and 400 *O. insidiosus* eggs were evaluated in each type of container. The eggs (laid

in farmer's friend inflorescences) were obtained from the research rearing. Next, the inflorescences containing eggs were grouped into a small bouquet, held together at the bottom with cotton and maintained in 10 ml glass vial containing water.

*Ephestia kuehniella* Zeller eggs glued on filter paper (2 × 8 cm) at amounts varying according to each density were used as a source of food for the nymphs. According to Yano *et al.* (2002), the ideal number of eggs for the development of the immature stage of *Orius sauteri* (Poppius) is approximately 7.5 eggs per individual/day and, according to Parra (1997), one gram of *E. kuehniella* eggs has, on average, 36,000 eggs. Thus, based on these data, 0.06 gram of *E. kuehniella* eggs were daily placed in the containers for the density of 100 eggs of the predator; 0.12 gram for the density of 250 eggs and 0.18 gram of eggs for the density of 400 eggs. A higher amount of eggs of the prey than that proposed by Yano *et al.* (2002) was used, because: (1) the species in the present study was *O. insidiosus* which is larger than the *Orius* species used by these authors and (2) the study was conducted in a gregarious rearing, taking into account cannibalism among nymphs and adults.

The percentage of emerged adults obtained for each density of eggs of the predator per container and at each type of container was determined. This measurement was performed four days after observing the first adult in the container (on average 20 days after placing the eggs in the container). The individuals were removed from the container with a manual aspirator, and were counted and separated by sex. The experimental design was comprised of completely randomized blocks, with 10 blocks, in a factorial layout; and consisted of three types of containers and three densities of eggs of the predator within each block.

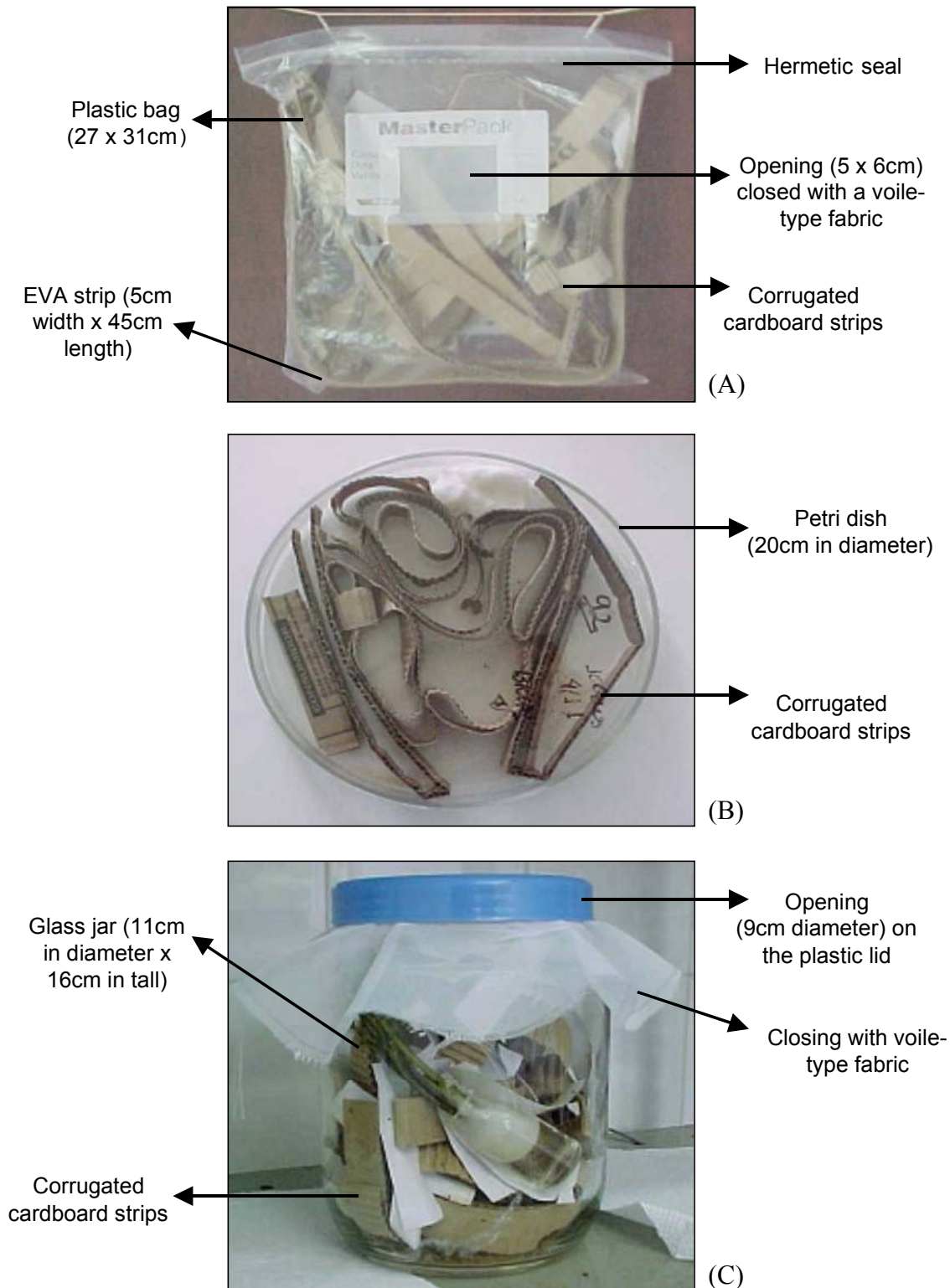
### Adult stage rearing

For the adult only the glass jar container, which proved to be the most suitable for rearing nymphs, was used. Densities of 250, 400, and 500 adults per container were tested. Eight corrugated cardboard strips (2 × 45 cm) (shelter), and three acrylic vials (10 ml) containing moistened cotton (as a source of water) were placed inside the glass container. Adult individuals were placed in the container at the proportion of approximately 2/3 females, 1/3 males. *E. kuehniella* eggs were added three times a week, according to the adult density under study.

Yano *et al.* (2002) reported that the intake of five eggs per adult would maximize the reproductive characteristics of *O. sauteri* adults. Thus, taking into consideration the data by these authors for the density of 250 adults per container, 0.08 gram of *E. kuehniella* eggs was supplied; 0.13 gram of eggs of the prey were provided for the 400 adults per container density, and 0.18 gram of *E. kuehniella* eggs were provided for the 550 adults per container density. A bouquet containing 10 farmer's friend inflorescences was also placed in the container. The food and farmer's friend inflorescences were replaced three times a week. The number of eggs laid by the females at each density of adults of the predator was recorded, using a stereoscopic microscope.

To evaluate the effect of the density of adults of the predator on oviposition, the number of eggs laid at every two days was divided by the initial number of females present in each replicate. This was done to obtain the mean number of eggs/female and to allow comparisons between treatments. The design was organized as completely randomized blocks, and the three different densi-

ties of adults of the predator were present within each block (10 blocks). The data were submitted to analysis of variance and the means were compared by Scott-Knott test at 1% probability (Scott and Knott, 1974). The oviposition curve made for *O. insidiosus* was based on the mean daily number of eggs/female, according to methodology used by Castañe and Zalom, (1994).



**Figure 1.** Rearing containers used to rear *O. insidiosus*: Plastic bag (A), Petri dish (B), Glass jar (C).

## Results and discussion

### Rearing of the immature stage of *O. insidiosus*

There was no effect of container type and egg density interaction on the rearing of the immature stage of *O. insidiosus*. However, when these factors were evaluated separately, a significant effect was found indicating the presence of an adequate egg density of *O. insidiosus* for each type of container in the rearing of nymphs of the predator (table 1). Emergence of adults from *O. insidiosus* nymphs reared in the plastic bags was the lowest (15%), in relation to those in the other containers (table 1). This container was, therefore, considered unsuitable for rearing nymphs of the predator. The low survival was probably due to the difficulty to maintain a sufficiently low humidity in this type of container. Plastic bags do not allow water vapor to exit, leading to condensation of the inside of the container's walls, and wetting the interior of the bag, and this, on its turn, resulting in nymphal mortality. In addition, the internal temperature in the plastic bag is probably higher than outside the bag. With respect to the different densities of eggs of the predator inside this plastic bag we found that at the density of 100 eggs/container, adult emergence was 28.6%, being higher than at the other densities tested (19.7% and 15% for 250 and 400 eggs/container, respectively) (table 1). This indicates that, at a smaller egg density, the nymphs, after hatching, find better conditions for survival due to more available space in the plastic bag, mainly because of lower cannibalism. Schmidt *et al.* (1995) reared the predator *O. insidiosus* in plastic bags but did not show results of emergence of adults. According to these authors, the container was suitable for rearing *O. insidiosus* and had good attributes such as being transparent, easy to handle, and having a shape that makes it difficult for the nymphs to escape. However, some handling and shape were not considered easy characteristics when plastic bags were used in our study on mass-rearing *O. insidiosus*.

The emergence of adults of *O. insidiosus* from nymphs reared in Petri dishes was similar to that in glass jars only at the densities of 100 and 400 eggs of the predator per container (Table 1). Of all densities of eggs of the predator tested in Petri dishes, the density of 100 eggs /container showed the best result (adult emergence of 55.2%). At densities of 250 and 400 eggs of the predator per container there was no significant difference in the emergence of *O. insidiosus* adults (44.7% and 45.6%, respectively).

The small volume of the Petri dishes (800 ml) can ex-

plain the limited number of nymphs that could be reared inside them. Alauzet *et al.* (1992) reared *Orius majusculus* (Reuter) in acrylic boxes of 600 ml at density of 100 predators, observing an emergence of 41%. Thus, our results were slightly better than those found by these authors. Since results were satisfactory at the density of 100 predator eggs per container, Petri dishes can be used for small-rearing of this predator in the laboratory.

Tommasini *et al.*, (2004) proposed as rearing unit for *O. laevigatus* transparent plastic boxes (3.6 dm<sup>3</sup>) starting from ca 1,500 eggs of the predator, then mass-rearing of the predator has been improved in co-operation with the Italian mass producer of natural enemies.

The container presenting the best results for rearing *O. insidiosus* nymphs was the glass jar (table 1) both at a density of 100 predator eggs per container (adults emergence of 50.5%), and at 250 predator eggs (adults emergence of 53.6%), indicating that space could be limiting at densities above 250 eggs/container. Of the various containers tested, the glass jar has the advantage of allowing greater visibility of its interior, facilitating monitoring on the development of insect, in addition to being easy to clean. In the glass jar also moisture control was better inside. Altogether, we concluded that glass jar is the best for rearing *O. insidiosus* nymphs at densities of up to 250 eggs per container. In addition, according to Parra (1999), suitable containers for use in rearings should be cheap, transparent, and easily available, made of a non-toxic material, and should give possibilities of sufficient management of humidity. In our experiments the glass jar met all these prerequisites.

Blumel (1996) found percentage of emergence of 52.8% when rearing *Orius laevigatus* (Fieber) in 2.7-liter jars containing up to 500 individuals. Tommasini and Nicoli (1993) reported a nymphal mortality of 37.3% in *O. insidiosus* for insects reared individually, indicating that part of the mortality that occurs during the nymphal stage is typical of the species and does not depend on the type of rearing container to which they are submitted. Van den Meiracker (1999) found survival rates of 81% and 73% for *O. insidiosus* females and males, respectively, when this predator was reared at a density of one pair per 1,000 ml container, and 59% and 63% for females and males, respectively, when the same species was reared at a density of 8 pairs per 1,000 ml container. The author also observed a reduction in the order of 19% in female fecundity when they were reared at a density of 8 pairs, compared to one pair per 1,000 ml container.

We concluded that in this study cannibalism and other factors inherent to the collective rearing of *O. insidiosus*

**Table 1.** Adult emergence (%) after rearing the immature stages of *O. insidiosus* in different containers and densities. 26 ± 2 °C, 70 ± 10% RH and a 12h photophase. Means followed by different upper-case letters in the columns and lower-case letters in the rows were different among themselves by Scott & Knott test (P ≤ 0.01).

Densities	Adult Emergence (%)		
	Plastic bag	Petri dish	Glass jar
100 eggs	28.6 ± 5.85 A b	55.2 ± 4.45 A a	50.5 ± 5.13 A a
250 eggs	19.7 ± 4.70 B c	44.7 ± 5.59 B b	53.6 ± 9.80 A a
400 eggs	15.0 ± 4.32 B b	45.6 ± 15.78 B a	41.2 ± 18.02 B a

accounted for about 15% mortality. According to Van den Meiracker (1999), a high mortality in a rearing of this predator is not necessarily the result of cannibalism, but also the consequence of ingestion of less food or of increased frequency of escape reactions. However, according to this author, it is essential to synchronize the age of predators in the rearing containers to reduce cannibalism.

Thus, in this experiment, the density of 100 eggs of the predator per container maximized emergence of the adults of *O. insidiosus* in the three types of containers. In the glass jar, the density of 250 eggs of the predator per container showed optimal rearing conditions of *O. insidiosus* also.

### Rearing *O. insidiosus* adults

The highest number of eggs/female was obtained at a density of 400 adults per container (22.7 eggs), indicating that this density is the most adequate for maintaining adults in rearing containers compared to the others studied (19.2 and 20.3 eggs were obtained for densities of 550 and 250 adults per container, respectively) (figure 2). Peters and Barbosa (1977) reported that the fecundity of insects frequently decreases as the population density increases, and according to Van den Meiracker (1999), the rate of oviposition of *O. insidiosus* was 12% lower when they were arranged in groups rather than in pairs.

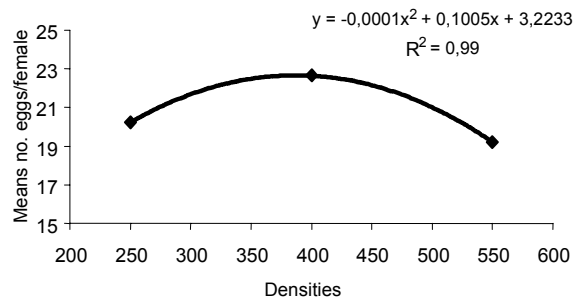
Considering the different densities of adults/container, it was observed that the peak of oviposition of *O. insidiosus* occurred approximately on the 10<sup>th</sup> day when 250 adults were maintained collectively; at 400 adults per container, the peak occurred on the 6<sup>th</sup> day, while at the density of 550 adults/container, it occurred on the 8<sup>th</sup> day after adult emergence (figure 3). *O. insidiosus* females laid eggs until approximately their 33<sup>rd</sup> day of adult life, at all the densities studied. Castañe and Zalom (1994) found an oviposition peak on the 4<sup>th</sup> day of oviposition (on green bean pods at 25 °C), and egg laying until the 27<sup>th</sup> day after oviposition had started.

Most *O. insidiosus* eggs were laid during the first two weeks after the beginning of oviposition at all densities (figure 3). For *O. majusculus* and *O. laevigatus* Blumel (1996) observed that most eggs were laid in the first week.

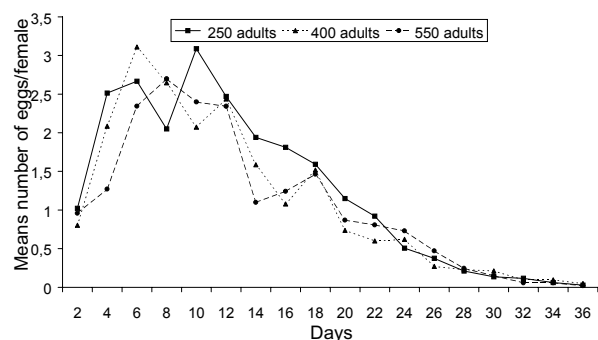
Rearing the predators in groups instead of individually leads to a reduction in oviposition activity as a response to a larger number of predators in the container (Van den Meiracker (1999). Thus, the female may “interpret” this situation that will likely lead to shortage of feeding resources later on, or it could even be caused by the reduction in physical space for oviposition. Also, according to Tawfik and Ata (1973) individual *Orius* spp. females lay their eggs rarely in clusters, but this, by necessity, occurs in collective rearings.

We observed that at the density of 400 adults only 3.5 % of the eggs were laid by the females during the fourth week of adults’ life of the predator, so this might be the right time to dispose of these females in a mass-rearing and start a new generation.

One aspect emphasized by Schmidt *et al.* (1995) is that, despite existing results recommending the addition



**Figure 2.** Effect of different densities of adults on *O. insidiosus* oviposition.



**Figure 3.** Oviposition trend of *O. insidiosus* at different densities (n=10), at 26 ± 2 °C, 70 ± 10% RH and 12-hour photophase.

of pollen to the diet of adults, which would increase fecundity and longevity, this component has not been incorporated into *Orius* rearing-methods (Isenhour and Yeargan, 1981; Tommasini and Nicoli, 1993; Schmidt *et al.*, 1995; Blumel, 1996; Tommasini *et al.*, 2004). However in the rearing-system we propose here, pollen was included in the form of farmer’s friend inflorescences. So these inflorescences provide alternative food, but present also the most suitable oviposition substrate for the predator (Mendes *et al.*, 2005b), in which the females showed better reproductive performance.

### Conclusion

The glass jar is the most suitable container, both for nymphal (at a density of 250 eggs of the predator/container) and for adult rearing (at a density of 400 adults/container) of *O. insidiosus*, both in terms of adult production, egg production, maintenance, cleaning and lowest amount of labor. However, further studies are needed in order to obtain higher yields of *O. insidiosus* 5<sup>th</sup> instars nymphs and adults, because these are the stages used for releases in protected cropping systems.

This rearing-method could be a starting mass-rearing system for *O. insidiosus* in our conditions. Improvements could be done in co-operation with a Brazilian producer of natural enemies.

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