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Protocols for the commercial production of *Orius tantillus* (Motschulsky) (Hemiptera: Anthocoridae)

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ABSTRACT: Protocols for the mass rearing of the anthocorid predator, *Orius tantillus* (Motschulsky), were standardised by utilising UV-irradiated *Sitotroga cerealella* (Olivier) eggs as prey. By maintaining 150 ovipositional containers with 50 *O. tantillus* adults in each, and by providing *S. cerealella* eggs as feeding and pieces of bean pods as ovipositional substrates, 86,250 nymphs could be produced per month. By taking into account the fixed and variable costs, the cost of 100 seven-day-old *O. tantillus* nymphs was worked out to be Rs. 34.30. The protocols developed can be adopted for commercial production.

KEY WORDS: *Orius tantillus*, *Sitotroga cerealella,* commercial production, cost of production, mass rearing

INTRODUCTION

Anthocorid bugs, particularly *Orius* spp., have received considerable attention because they have a great preference for thrips. Anthocorid predators of the genus *Orius* have been multiplied and released against various pests, for example *Orius albidipennis* (Reuter) against thrips and aphids in Iran (Shojai *et al.,* 2003), *Orius* spp. against mites in Brazil (Reis *et al*., 2005), *Orius laevigatus* (Fieber) against *Frankliniella occidentalis* (Pergande) in Russia (Carvalho *et al*., 2005), *Orius insidiosus* (Say) against *F. occidentalis* and *Tetranychus urticae* Koch in Germany (XueNong *et al*., 2006) and *Orius strigicollis* (Poppius) against *F. occidentalis* in Japan (Kakimoto *et al*., 2007). *Orius tantillus* (Motschulsky), *Orius maxidentex* Ghauri and *Orius indicus* (L.) are the most common amongst *Orius* spp. in India (Ananthakrishnan and Sureshkumar, 1985). *Orius tantillus* has been identified as a potential predator of different species of thrips and other major pests like *Helicoverpa* spp. and leafhoppers in many Asian countries (Ananthakrishnan and Sureshkumar, 1985; Sigsgaard and Esbjerg, 1997; Chinling, 1999; Hafeez *et al*. 2002).

The ability of natural populations of *O. tantillus* in maintaining thrips populations at low levels was reported by Muraleedharan and Ananthakrishnan (1978). However, natural populations of this predator are insufficient to regulate the thrips populations below economic levels on several crops. For the successful utilization of any bio-agent, it is important to develop protocols for its mass production. In India, very few attempts have been made to rear anthocorid predators. Mass rearing techniques have been standardized for *Cardiastethus exiguus* Poppius and *Blaptostethus pallescens* Poppius (Ballal *et al.,* 2003a, b), *O. maxidentex* and *Xylocoris flavipes* (Reuter) (Chandish R. Ballal, unpublished).

Thrips being a major problem in our country on ornamental and polyhouse crops, attempts were made to develop a cost-effective method to mass rear *O. tantillus* on the eggs of *Sitotroga cerealella* (Olivier) in the laboratory. The objective of this work was to standardise an inexpensive and simple mass rearing technique which could be adopted for commercial production of *O. tantillus* and to work out the economics of production.

MATERIALS AND METHODS

Mass rearing

The experiment was conducted at the Mass Production Laboratory, National Bureau of Agriculturally Important Insects (NBAII) (erstwhile Project Directorate of Biological Control), Bangalore. For mass production of *O. tantillus*, the items required were UV-irradiated *S. cerealella* eggs as feeding, *Phaseolus vulgaris* L. (french bean) pods (cut into pieces of approximately 4-5cm length) as ovipositional substrates, 500ml transparent plastic containers as ovipositional containers, a thin layer of absorbent cotton fibre to avoid cannibalism, ventilated jewel boxes (diameter 7.5 cm and height 2.5 cm) as hatching containers, black cloth pieces to cover ovipositional containers, a refrigerator for storing beans and *S. cerealella* eggs and miscellaneous items like tissue paper, rubber bands, brushes, etc. Laboratory studies were conducted at 26±2°C and 65±2% relative humidity. *S. cerealella* eggs were obtained from Biotech International, Bangalore, and the Mass Production Laboratory, NBAII.

Adults of *O. tantillus* were initially collected from maize fields. These adults were kept in ovipositional containers. The ovipositional containers were provided with a layer of tissue paper at the base, *S. cerealella* eggs and a thin layer of absorbent cotton fibre to prevent adults from encountering each other and avoid cannibalism. *S. cerealella* eggs were placed on bean pieces by using a moist brush and were also sprinkled on the cotton strands in the ovipositional container. *O. tantillus* adults feed on *S. cerealella* eggs placed on bean pieces as well as those on cotton strands. The freshly hatched nymphs preferred to initially feed on the *S. cerealella* eggs pasted on bean pieces. Each container was covered with a piece of black cloth and fastened with a rubber band.

After every 24 h, bean pieces with *O. tantillus* eggs were removed from the ovipositional containers and kept in hatching containers. The ovipositional containers were replenished with fresh bean pieces and *S. cereallela* eggs every 24 hrs. The hatching containers were provided with cotton strands and *S. cerealella* eggs. Dry bean pieces from the hatching containers were removed and replaced with fresh bean pieces to provide adequate moisture. Mature nymphs were transferred to new ovipositional containers after 10-12 days from the date of hatching. When adults were formed, the rearing cycle was repeated for further production.

Cost of Production

Cost of production was calculated on the basis of the feeding potential of *O. tantillus*, the facilities required (non-recurring and recurring) for production and manpower requirement. The requirement of *S. cerealella* eggs for adults was calculated based on the average number of eggs fed by the adult throughout its lifetime and for nymphs it was calculated based on the feeding potential for the first seven days of the nymphal period because seven-day-old nymphs are ideal for field release. Fixed costs included non-recurring equipments like incubator, fan, air conditioner, metal racks, furniture, etc. and minor items like plastic containers, plastic trays and other miscellaneous items like brushes, forceps, lamp, aspirator, etc. Variable costs included the cost of recurring items like manpower, building rent, electricity and water charges and consumables like beans, cotton and *S. cerealella* eggs.

Cost of non-recurring equipments was calculated @ 20 per cent depreciation per annum for 5 years and the interest on capital @ 9 per cent per annum. Interest on working capital for recurring items was also calculated at the rate of 9% per annum. Total cost was then converted for a period of one month as the mass production and supply of *O. tantillus* would be seasonal and need-based.

RESULTS AND DISCUSSION

Mass rearing

For the first time, a technique for the mass production of *O. tantillus* was developed. Environmental conditions, host eggs, ovipositional substrate, etc. play an important role for large-scale production of any predator. In our studies, *O. tantillus* could be successfully mass reared on *S. cerealella* eggs and bean pods were utilized as ovipositional substrates. It is difficult to rear thrips, which is a natural prey of *O. tantillus*, in the laboratory. So, *S. cerealella* was used as an alternative laboratory host to mass rear *O. tantillus*. *S. cerealella* eggs have been utilised to rear anthocorid predators like *O. minutus* (L.), *O. majusculus* (Reuter) and *O. insidiosus* by Niemczyk (1980), Hejzlar and Kabicek (1998) and Saini *et al* (2003), respectively. Bean pods were used as ovipositional substrates by Tommasini (2003) to rear *O. majusculus*, *O. laevigatus* (Fieber), *O. insidiosus* and *O. niger* Wolff. Ito (2007) used frozen eggs of *Ephestia kuehniella* Zeller as food and soyabean seedlings as ovipositional substrates for rearing *O. strigicollis* and *O. laevigatus.*

Based on the present studies, the production protocols for the mass rearing of *O. tantillus* were developed which can be adopted for commercial production (Fig. 1). Each ovipositional container can accomodate 50 adults emerging from the nymphal containers, with 25 females based on a 1:1 sex ratio. Each female during its lifetime can lay approximately 33 eggs, out of which 70% hatching can be expected. From one ovipositional container, approximately 575 nymphs could be harvested in one month. Based on this, by increasing the number of ovipositional containers and racks, the culture can be scaled up. In one month, from 50 containers (which can be accommodated in one rack), approximately 28,750 nymphs can be produced. Maintaining 150 ovipositional containers (with 50 adults in each), production can be scaled up to around 86,250 *Orius* nymphs per month.

The scale of production can also be improved by optimum utilization of the rearing facility and space. One slotted angle rack with four shelves can accommodate around 50 ovipositional containers, with one shelf (length 32½ inches, depth 14½ inches and height 15½ inches) accommodating about 12 ovipositional containers. In one shelf of a rack, 12 plastic trays with 25 hatching containers in each can be stacked, thus accommodating 1200 hatching containers in one shelf and 4800 in one rack. Three to four rearing cages of *O. strigicollis* and *O. laevigatus* were piled up for optimum space utilization and dry batteries were placed as weights on the rearing cages by Ito (2007). Ten per cent of the *O. tantillus* nymphs in the nymphal containers can be kept aside for further production and the remaining nymphs used for field releases. Seven-day-old nymphs of *O. tantillus* can be used for field release and the releases could be initiated 10-11 days from the date of egg collection.

Precautions to be followed

The major problems associated with large-scale anthocorid production are the risks associated with contamination with other anthocorid species, cannibalism, bio-deterioration of the culture and incidence of mites and diseases. To avoid these problems, the following steps could be adopted:

- Monitoring and cleaning (removal of old, unfed *S. cerealella* eggs) of ovipositional and hatching containers every week to avoid mite or disease incidence
- Regular provision of *S. cerealella* eggs in adequate quantity to avoid cannibalism.
- Proper UV-irradiation of *S. cerealella* eggs to avoid hatching of the larvae.
- Provision of fresh uninfected bean pods as ovipositional substrates and to avoid fungal infection.
- Regular monitoring of ovipositional and hatching containers to avoid mix-up of species.
- Rejuvenation of lab culture with wild culture to avoid bio-deterioration/ inbreeding depression.

In the present studies, it was observed that one *O. tantillus* could produce around 23 nymphs during its lifetime and the adult longevity was about one month. This indicates that *O. tantillus* has a low reproductive rate, which is in agreement with the observation of Yano (2003). Longevity and fecundity of anthocorids may differ from species to species and place to place. In Canada and Italy, 100,000 *O. insidiosus* could be reared per week (Schmidt, 1994; Tommasini, 2003). The scale of production depends to a large extent on the quantity and quality of the initial culture.

Cost of production

For commercialization of *O. tantillus*, it is important to work out a simple low-cost rearing method. This would also facilitate its inclusion in a Biointensive Integrated Pest Management programme for tackling thrips and other sucking pests. The details on the cost of production of seven-day-old nymphs of *O. tantillus* are presented in Table 1. The requirement of *S. cerealella* eggs for feeding was calculated based on the adult feeding potential during its lifetime and the nymphal feeding potential for seven days. One cc of *S. cerealella* eggs contains 30,000 eggs. One adult feeds on 122 *S. cerealella* eggs during its lifetime. Therefore, for feeding 7,500 adults, 31 cc of *S. cerealella* eggs are required. One nymph feeds on approximately 16 *S. cerealella* eggs in seven days and thus a total of 46 cc of *S. cerealella* eggs will be consumed by 86,250 nymphs. Therefore, for both adult and nymphal stages, a total of 77 cc of *S. cerealella* eggs would be required in a month, which would cost Rs. 2,002 at the rate of Rs. 26 per cc. A total of 924cc of *S. cerealella* eggs costing Rs. 24024 would be required in a year (Table 1).

The expenditure on non-recurring items (equipments and other rearing materials) was Rs. 28,473 per annum (considering depreciation @20% and 9% interest on capital per annum). The expenditure on recurring items like building rent, water, electricity charges, salary for contractual workers and consumables like beans, *S. cerealella* eggs, cotton, etc. was Rs. 3,26,590 (including 9% interest on capital) (Table 1). The total expenditure for producing 86,250 *O. tantillus* nymphs per month was calculated as Rs. 29,588. Thus, the cost of 100 seven-day-old *Orius* nymphs would be Rs.34.30. Based on the cost of *S. cerealella* eggs alone, the cost of one *Orius* nymph was calculated as Re. 0.82 (Gupta and Ballal, 2006). The cost of production of different *Orius* species can vary based on rearing procedures adopted. Schmidt (1994) and Mendes *et al.* (2005) calculated the cost of production of *O. insidiosus* as 0.03 Canadian dollar/ bug and 0.069 US dollar/adult, respectively. The latter has included the fixed and variable costs for calculating the cost of production as in the present studies. A unit which has extra space to accommodate five racks for the production of *O. tantillus* can save the rental charges and then the cost of 100 nymphs would be Rs. 26.70. If a production facility for the host (*S. cerealella*) is also available in the same unit, the cost of production of *O. tantillus* can be further reduced. For instance, the commercial cost of 1cc of *S. cerealella* eggs is Rs. 26, whereas the actual production cost is only Rs. 7 (Biotech International Pvt. Ltd., Bangalore, personal communication). In-depth studies are required to improvise the present protocols leading to improvement in fecundity of *O. tantillus* and further scaling-up of the production for commercial uptake.

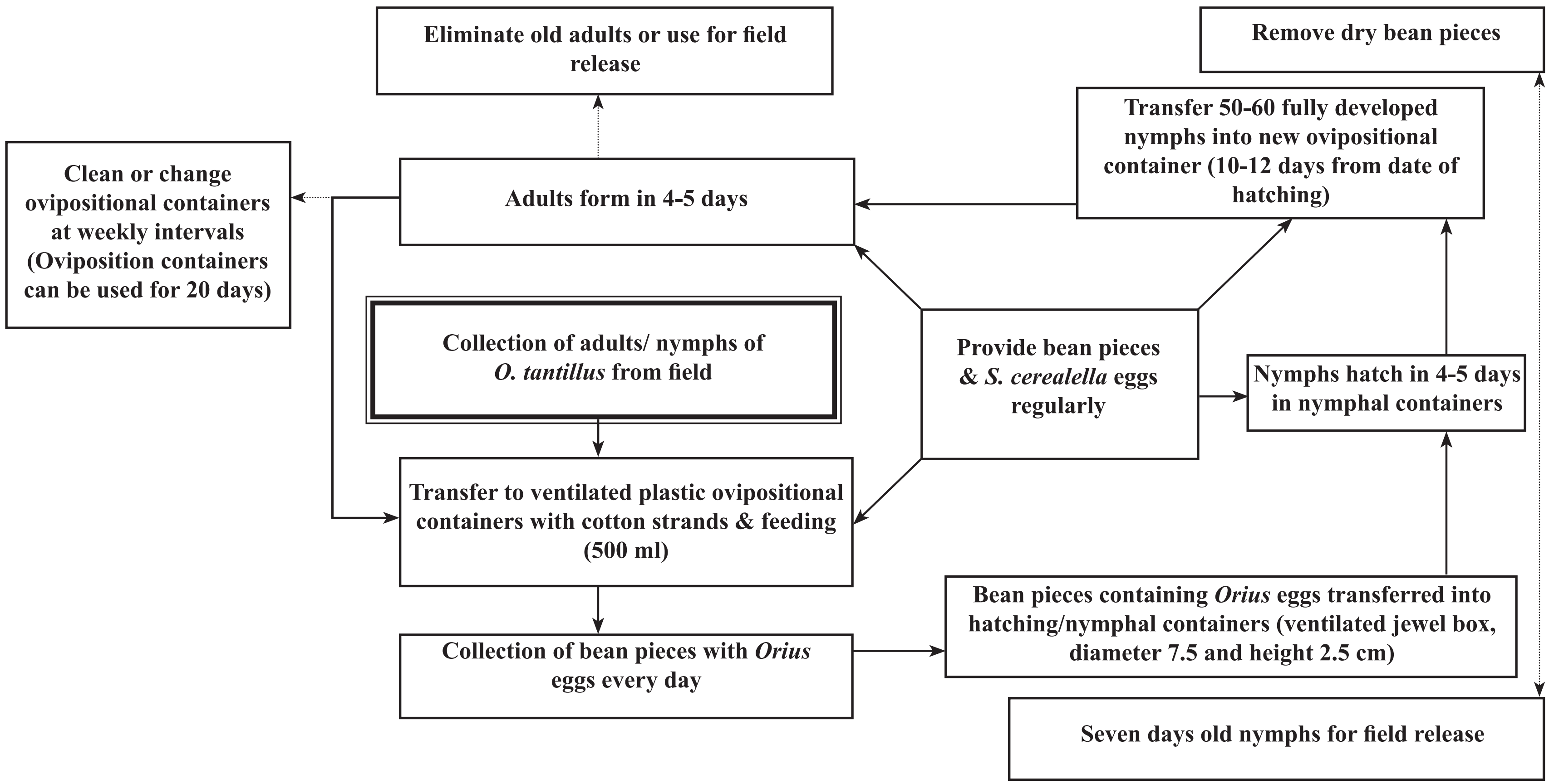

Fig. 1. Schematic diagram for mass production of *O. tantillus*; ⟶ regular and essential steps; ⇢ steps to be followed based on need

Table 1. Cost of production of *Orius tantillus*

A	Non-recurring equipment and materials			
	Particulars	Period (Years)	Quantity Required (Nos.)	Total cost (Rs.)
a)	BOD*	5	1	30,000
b)	Fan	5	2	2000
c)	AC*	5	1	35,000
d)	Racks (4 partitions)	5	5	12,000
e)	Furniture (Chair, table & stool)	5	2 each	11,000
f)	Plastic containers (ovipositional)	5	500	12,500
g)	Hatching containers	5	1000	5000
h)	Plastic trays	5	60	21,00
i)	Miscellaneous (Brushes, forceps, lamps, aspirator, etc.)	5		
	Total		112100	
Depreciation calculated @ 20% per annum for 5 years and the interest on capital @ 9% per annum for 5 years were calculated, the value per year is Rs. 28473, value per month is Rs. 2372 (i)				
B	Recurring items			
a)	Building rent	1		72000
b)	Electricity & water	1		48000
c)	Beans	1	360 kg	9000 (Rs. 25/Kg)
	Cotton	1	20 rolls (500gm)	2600
	S. cerealella eggs	1	924cc	24024 (Rs. 26/cc)
For adults 31 cc eggs and for nymphs 46 cc eggs (up to seven days) required for one month. Therefore in a year 924cc *S. cerealella* eggs are required for feeding				
d)	Contractual workers	1	4	144,000
			Total cost per annum	299624
			Total cost per month	24969 (ii)
The interest on capital @ 9% per annum for recurring items is Rs. 26966, Value per month is Rs. 2247 (iii)				
Total cost per month (i + ii + iii)				2372+ 24969+ 2247= Rs. 29588
Cost of production of 86,250 *O. tantillus* nymphs (7-day-old) per month from 150 containers holding 50 adults in each				Rs. 29588
Cost of 100 *O. tantillus* nymphs (7-day-old)				Rs. 34.30

* Not essential, but can improve scale of production during adverse conditions

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