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## MASS REARING OF A TROPICAL MINUTE PIRATE BUG, *ORIOUS PUMILIO* (HEMIPTERA: ANTHOCORIDAE)

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Minute pirate bugs are considered important beneficial insect predators that can be useful in the biocontrol of flower thrips and some other insects which infest peppers and other vegetable crops (Funderburk et al. 2000; Ramachandran et al. 2001; Reitz et al. 2003; Funderburk 2009). *Orius insidiosus* (Say) and *O. pumilio* (Champion) (Hemiptera: Anthocoridae) are 2 naturally occurring species of *Orius* in Florida (Herring 1966) while a third, *O. tristicolor* (White), has become established (Halbert 2009). Both *O. insidiosus* and *O. pumilio* have been identified as major predators of *F. bispinosa* infesting Queen Anne's lace (*Daucus carota* L.; Apiaceae) and false Queen Anne's lace, (*Ammi majus* L.; Apiaceae), on an organic farm in Alachua County, Florida (Shapiro et al., 2009; Shirk et al., unpublished data 2011). *O. insidiosus* is commercially available as a biocontrol agent for use in greenhouse and field conditions. *O. pumilio* shows promise as a biological control agent because of its tropical and subtropical distribution (Herring 1966) which may limit concerns for its introduction into more temperate regions. Rearing conditions and life table data for *O. pumilio* are described here to facilitate its production as a biocontrol agent.

The *O. pumilio* colony was acquired as a gift from a commercial supplier (Shapiro & Ferkovich 2009). The colonies were maintained in 34 × 21 × 9 cm containers (Rubbermaid, Huntersville, North Carolina) with buckwheat hulls, fed on *Ephesthia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and Hydrocapsules® containing water (Hydrocapsule Inc., Jasper, Georgia) at 25 °C, 75-80% RH, 14:10 h L:D as previously described (Shapiro et al. 2009). Fresh green beans (*Phaseolus vulgaris* L.; Fabales: Fabaceae) were used as an ovipositional substrate and added along with new food to the colonies on every second or third day after origination. Eggs oviposited into the green beans were counted under a stereomicroscope. New colonies were started weekly from approximately 18,000 eggs oviposited into green beans over a 7-d period, i.e., embryos were 0-7 d old at colony start date.

To establish the optimum period of egg production by mass-reared colonies, the peak oviposition rate was established as the intersection of increasing and decreasing ovipositional rates determined by linear regression (Fig. 1). The rate of increase in oviposition was computed as the slope (0.028 eggs/female/d) between d-14 after

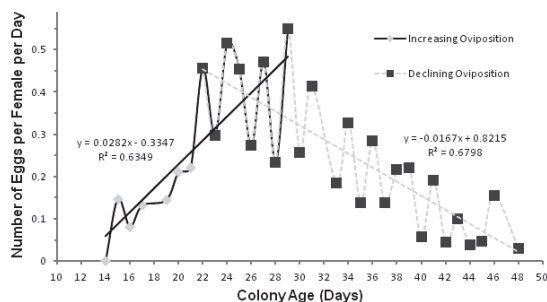


Fig. 1. Biphasic age-dependent egg production by *Orius pumilio* colonies. The number of eggs produced per female per d was computed by dividing the number of eggs oviposited into a bean by the estimated number of females originally placed in culture by the number of d that the bean was exposed. The number of females in each colony was computed on the basis of the number of eggs originally placed in culture assuming a 1:1 female:male ratio (van den Meiracker 1994) and a 50% hatch rate as determined here. The ovipositional data are the means of data compiled over 15 mo.

colony origination (the first d eggs were observed) and d-30 (the d the highest egg oviposition was observed). The rate of decline in oviposition was computed as the slope of oviposition (-0.017 eggs/female/d) between d-22 (the first d of the major oviposition activity) and d-48 (the last d eggs were deposited). Maximum mean daily egg production occurred on the 26th d after colony origination. Within 5 d after the maximum, a decline in oviposition was observed, establishing the limits of maintenance of a productive culture.

To establish timing of major developmental events, green beans were provided to sub-colonies and groups of 0-4 h old eggs and the nymphs were monitored daily for development. The time to maximum number hatched was 104 h after oviposition but the maximum number hatched never exceeded 50% of the eggs oviposited into green beans. Nymphs from 0-4 h synchronized eggs were isolated on d-12 by narcotizing with CO<sub>2</sub> and sieving from the buckwheat hulls. The nymphs were transferred individually into 0.5 mL micro-centrifuge tubes containing *E. kuehniella* eggs and Hydrocapsules and then monitored every 4 h as they completed development. Adult eclosion occurred 16.5 d ( $s = 0.3$ ) after oviposition, and the time to first egg deposition was 5.3 d ( $s = 1.6$ ) later.

As a measure of reproductive capacity, the amount of yolk protein accumulated in adult females following forced egg retention by removal of the oviposition substrate was determined using a double monoclonal antibody ELISA performed essentially as described by Shapiro and Ferkovich (2002). Sub-colonies of *O. pumilio* were housed in containers (16 × 16 × 5 cm) that were started with approximately 1000 eggs oviposited within a 24-h period and maintained under similar conditions as the large colonies. One set of sub-colonies had green beans as an ovipositional substrate presented continuously throughout the sampling period from 17 d to 44 d of the colony. A second set was provided green beans until 72 h prior to collection of the females which prevented oviposition of eggs (Shapiro & Shirk, 2010). The amount of yolk protein accumulated in the females that had green beans present did not change significantly over the ovipositional period (Fig. 2). When the ovipositional substrate was withheld for 3 d prior to collection, 24-d ovipositing females contained twice as much yolk protein per female (20.0 µg/♀;  $s = 3.5$ ), which was significantly more than corresponding 24-d females that had continuous oviposition substrate (9.7 µg/♀;  $s = 0.95$ ) (Fig 2). Although the 31-d females without substrate contained 16.9 µg/♀ ( $s = 5.5$ ), the amount was significantly different from the 24 d females but not from older cultures (Fig 2). This suggests that the capacity to rapidly accumulate eggs slowly declined as the females aged, resulting in reduced ovipositional activity. Taken together with the ovipositional rate, the maximum period to maintain a colony for egg production would end on d 31.

Many species of *Orius* occur throughout the world and are suitable as augmentative biocontrol agents of pest insects of field and greenhouse produced crops (Bonte & De Clercq 2008; Funderburk 2009; Bonte & De Clercq 2010). The data presented here demonstrate that *O. pumilio* can be reared using a mass-colony protocol for use as a biocontrol agent of thrips.

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#### SUMMARY

Mass-reared colonies of *Orius pumilio* were monitored to establish growth and development parameters. Mass colonies had maximal oviposition from 16 d to 31 d after establishment with eggs 0-7 d old, peaking at 26 d. The difference in

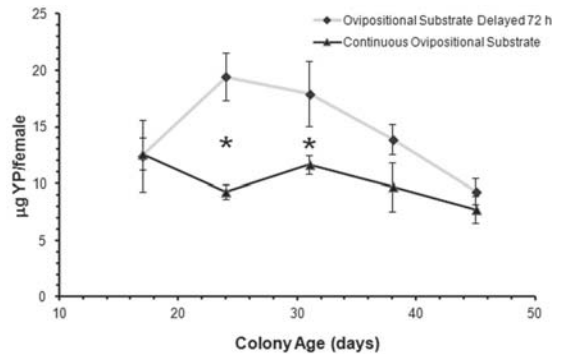


Fig. 2. Yolk protein accumulation in adult female *Orius pumilio*. Data for yolk protein (YP) accumulation in adult female *Orius pumilio* were acquired using KCJr software ver. 1.41.2 and a µQuant plate reader (Bio-Tek, Winooski, Vermont) and analyzed using Excel (Microsoft, Redmond, Washington). Lyophilized protein from 50-egg *O. insidiosus* samples was used as a quantitative standard for yolk protein (Shapiro & Ferkovich 2009). For each ELISA, 12 samples of 5 adult female *O. pumilio* from each sub-colony age, either with continuous green bean oviposition substrate or without substrate for 3 d prior to collection were assessed and each assay was replicated in triplicate. A Newman-Kuehls test for the effect of age and withholding ovipositional substrate was conducted for the amount of YP accumulated; Variable YP (µg/female) Approximate Probabilities for Post Hoc Tests Error: Between MS = 8.6713, df = 20,000. Significant differences between mean YP values for 24 d and 31 d are noted with a \* between the error bars.

accumulation of yolk protein in females denied an oviposition substrate vs. those provided substrate showed that females from 24-d to 31-d colonies had the capacity to produce the largest amounts of egg material. Time to 50% egg hatch was 104 h. Adult eclosion occurred at 16.5 d ( $s = 0.3$ ) after oviposition. Time to first egg deposition was 5.3 d ( $s = 1.6$ ) after adult eclosion. These parameters show that *O. pumilio* can be efficiently reared in mass quantities as an augmentative biocontrol agent for flower thrips.

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