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Authors: Rondon, Silvia I., Price, James F., and Cantliffe, Daniel J.

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## DEVELOPMENTAL TIME, REPRODUCTION, AND FEEDING OF TWO SUBSPECIES OF *COLEOMEGILLA MACULATA* (COLEOPTERA: COCCINELLIDAE) IN THE LABORATORY

SILVIA I. RONDON<sup>1</sup>, JAMES F. PRICE<sup>2</sup> AND DANIEL J. CANTLIFFE<sup>3</sup>

<sup>1</sup>Oregon State University, Crop and Soil Sciences, Hermiston Agricultural Research and Extension Center  
P.O. Box 105, Hermiston, OR 97838

<sup>2</sup>University of Florida, Institute of Food and Agriculture, Gulf Coast Research and Education Center  
14765 CR 672, Wimauma, FL 33598

<sup>3</sup>University of Florida, Institute of Food and Agriculture, Horticultural Sciences Department  
P.O. Box 110690, Gainesville, FL 32611

Coccinellids (lady beetles, lady bugs or ladybird beetles) have been used in biological control programs because of their ability to prey on economically important pests such as aphids (Hagen & Van den Bosch 1968; Hagen 1974; Frazer 1988; Rondon et al. 2004), whiteflies (Hoelmer et al. 1994) and mites (Chazeau 1985; Rondon et al. 2004) on maize, *Zea mays* L. (Kieckhefer & Elliot 1990), alfalfa, *Medicago sativa* L. (Giles et al. 1994), and potato, *Solanum tuberosum* L. (Grodén et al. 1990; Hilbeck & Kennedy 1996). Lady beetles are probably the most visible and well known beneficial predatory insects with over 450 species found in North America (Gordon 1985). *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), the pink spotted lady beetle, is a new world species distributed from southern Canada, U.S. (east of the Rocky Mountains), and Central and South America (Timberlake 1943; Wright & Laing 1982; Gordon 1985; Munyaneza & Obrycki 1998). Three subspecies of *C. maculata* have been described based on morphological characters such as spot patterns, color, body size, genitalia, and geographical distribution (Gordon 1985). According to Gordon (1985) these subspecies are *C. m. fuscilabris* (Mulsant), *C. m. lengi* Timberlake and *C. m. strenua* (Casey). *Coleomegilla m. fuscilabris* is found in the southeastern U.S., including Florida, while *C. m. lengi* is found from Ontario, Canada, through northwestern Georgia. *Coleomegilla m. lengi* has not been reported in Florida (Peck & Thomas 1998). The criteria to determine subspecies based only on morphological and geographical distribution has been challenged several times and even by Darwin (1964). The assertion that “biology should overrule taxonomy and that the term subspecies should be referred to as species rather than subspecies” (anonymous) is open to discussion. Nevertheless, geographic distribution has been defining in allocation of species (Odum 1950); however, the final determination of genotypic characteristics should be considered as definitive in insect identification. There has not been a determination of the actual distribution of subspecies since Gordon (1985). To our knowledge, no further surveys have been made to update the records.

In Florida, there has been an increasing interest from the biological control industry to introduce *C. m. lengi* (non-native), which is thought to have a greater reproductive capability, a highly attractive biological characteristic desired by producers of beneficials, than *C. m. fuscilabris* (native) (Griffin & Yeargan 2002). However, concerns regarding the possibility of cross genetic contamination between *C. m. fuscilabris* and *C. m. lengi* prevented the introduction (Peres 2000). Studies by Peres & Hoy (2002) indicated that there was a reproductive near incompatibility between the subspecies during the first and second generations (F1, F2); conversely, Krafus & Obrycki (2000) indicated that high levels of gene flow among subspecies might be possible. Due to this contradiction, more basic information to create a strong argument regarding the possibility to introduce *C. m. lengi* into Florida was needed. Thus, the objective of this research was to compare the development, oviposition, and feeding behavior of *C. m. fuscilabris* and *C. m. lengi*, on the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) as prey in the feeding behavior study, and strawberry, *Fragaria* × *ananassa* Duchesne, as a substrate. Strawberry plants were maintained following Paranjpe (2003) protocols. Experiments were conducted at the biological control laboratory, Protected Agricultural Project Research Station, University of Florida, Horticultural Sciences Department in Gainesville, FL. Both subspecies of *C. maculata* were provided by Entomos (Gainesville, FL) (voucher specimens can be found at DPI) where they were reared on undisclosed artificial diet.

From an initial colony (40-50 females per cage) from Entomos, 20 egg masses of *C. m. fuscilabris* and 20 of *C. m. lengi* were randomly selected and isolated in individually labeled 10-cm diameter Petri dishes and maintained at 26 ± 1°C, 80 ± 5% R.H., and 16:8 h (L:D) photoperiod. Eggs were checked for larval eclosion every 12 h. After eclosion, 20 larvae were collected randomly and isolated in 30 ml plastic cups. Larvae were fed every second day with an undisclosed proprietary artificial diet

(1.8 g), to which were added bee pollen (0.05 g) and shrimp eggs (0.01 g). Water was provided through wet cotton balls. Larvae were transferred to clean plastic cups twice a week. Daily observations were made and the number of days from instar to instar was recorded. Instars were distinguished by the presence of cast exuvia. After adults emerged, one female and one male were paired ( $n = 20$ ) in 30-ml plastic cups for 48 h to facilitate mating. Gender was determined by examining the last abdominal sclerite under a dissecting microscope. After 48 h, females were isolated in plastic cups ( $15 \times 15 \times 10$  cm) to determine viability of eggs (% eclosion), survival (larva to adult), number of egg masses, and number of eggs per mass produced by each female. A small piece of gray, thick fur served as an oviposition substrate. Longevity of adults also was measured. The experiment was repeated three times with 20 replications per treatment. The data are presented as average ( $\pm$ SE) over the three experiments ( $P \leq 0.05$ ). The measure of the developmental time was analyzed by *t*-test for independent samples. In general, there were no significant differences between the developmental periods (egg to adult) of *C. m. fuscilabris* ( $23 \pm 4$  days) and *C. m. lengi* ( $22 \pm 3$ ). There were no significant differences between *C. m. fuscilabris* and *C. m. lengi* in development periods of their eggs ( $3 \pm 3$ ;  $2 \pm 1$ , respectively), 1st ( $3 \pm 2$ ;  $3 \pm 1$ ) 2nd ( $4 \pm 1$ ;  $3 \pm 1$ ), 3rd ( $4 \pm 1$ ;  $5 \pm 1$ ) and 4th instar ( $3 \pm 1$ ;  $3 \pm 1$ ) larval; pre-pupal ( $3 \pm 2$ ;  $3 \pm 1$ ) and pupal stages ( $3 \pm 2$ ;  $3 \pm 1$ ). Female adult longevity is significantly greater in *C. m. lengi* ( $43 \pm 6$  days) as compared with *C. m. fuscilabris* ( $38 \pm 7$ ). However, there was no significant difference between male adult longevity in the two subspecies (*C. m. fuscilabris*,  $31 \pm 5$  days; *C. m. lengi*  $36 \pm 4$ ). The percentage of eclosion of *C. m. fuscilabris* eggs to larvae ( $95 \pm 3$ ) was greater than of *C. m. lengi* ( $86 \pm 4$ ); in contrast, the percentage of survivorship (larva to adult) was significantly greater among *C. m. lengi* ( $73 \pm 5$ ) than among *C. m. fuscilabris* ( $65 \pm 3$ ). The number of egg masses produced by *C. m.*

*lengi* per female per day ( $3 \pm 1$ ) was not significantly different from those produced by *C. m. fuscilabris* ( $4 \pm 1$ ). However, there were significantly more eggs oviposited in each *C. m. fuscilabris* mass ( $11 \pm 3$ ) than in each *C. m. lengi* egg mass ( $8 \pm 3$ ). Also, there was significantly more estimated number of eggs produced by *C. m. fuscilabris* (1,672 eggs) than *C. m. lengi* (1,032) over the female's lifetime.

An experiment was conducted to determine the consumption of *A. gossypii* by *C. m. fuscilabris* and *C. m. lengi*. Each experimental unit consisted of a 10-cm diameter Petri dish, where one strawberry leaflet, one individual of a single predator subspecies, and ten prey were placed. All instars and the adults of each subspecies were evaluated. The predators tested were starved for 8 h prior to providing them with *A. gossypii*. Ten individual prey, without a predator, per Petri dish served as a control for the experiment. Aphids were removed from infested leaves in the colony by using a wet, fine, camel hair brush. The strawberry leaflets were isolated with lanolin to confine the prey on the upper side of the leaf relative to the Petri dish. Petri dishes were sealed with Parafilm® and labeled. Each experiment was maintained at  $21 \pm 2^\circ\text{C}$ ,  $65 \pm 5\%$  R.H., and 16L: 8D photoperiod. Samples were examined under a stereo microscope and the number of prey consumed at 24 h was recorded. Each experiment was repeated three times with five replications per treatment in a block design. The feeding data are presented as average number ( $\pm$ SE) of prey consumed by a predator at 24 h. All data were analyzed with SAS (SAS Institute 2000). The general linear model (GLM) procedure was used to construct analysis of variance (ANOVA). Averaged over all five feeding stages, *C. m. fuscilabris* consumed more aphids ( $8.4 \pm 1.1$ ) than did *C. m. lengi* ( $6.5 \pm 1.5$ ) (LSD,  $0.05 = 1.96$ ;  $F = 1.19$ ;  $df = 2, 20$ ;  $P > 0.09$ ) in 24 h (Table 1). Aphid consumption by *C. m. fuscilabris* 1st instar was significantly greater as compared with *C. m. lengi* ( $F = 1.84$ ;  $df = 4, 20$ ;  $P >$

TABLE 1. CUMULATIVE AVERAGE CONSUMPTION (MEAN  $\pm$  SE) BY TWO SUBSPECIES OF *COLEOMEGILLA MACULATA* DE-GEER (COLEOPTERA: COCCINELLIDAE) PREYING ON THE COTTON APHID, *APHIS GOSSYPYII* GLOVER (HOMOPTERA: APHIDIDAE), DURING 24 H ( $N = 10$ ).

Life Stage	Number of Aphids Consumed		
	<i>C. m. fuscilabris</i>	<i>C. m. lengi</i>	Significance
1st instar	7 $\pm$ 1	4 $\pm$ 1	n.s.
2nd instar	9 $\pm$ 1	7 $\pm$ 2	n.s.
3rd instar	9 $\pm$ 1	8 $\pm$ 1	*
4th instar	9 $\pm$ 1	7 $\pm$ 1	*
adults	9 $\pm$ 1	7 $\pm$ 1	*
Total	9 $\pm$ 1	7 $\pm$ 1	*

Mean ( $\pm$  SE) within subspecies. Each treatment was repeated three times with five replications in each treatment. n.s. = no significant different; \* = significant different ( $P < 0.05$ )

0.06). Also, *C. m. fuscilabris* 4th instar consumption of aphids was greater than that of the 4th instar of *C. m. lengi* ( $F = 3.84$ ;  $df = 4, 20$ ;  $P > 0.05$ ) and adult *C. m. fuscilabris* consumed more aphids than did adult *C. m. lengi* ( $F = 3.84$ ;  $df = 4, 20$ ;  $P > 0.05$ ). *C. m. fuscilabris* 2nd and 3rd instar consumption of aphids was not significantly different from that of 2nd and 3rd instar of *C. m. lengi* ( $F = 3.04$ ;  $df = 4, 20$ ;  $P > 0.126$  and  $F = 2.02$ ;  $df = 4, 20$ ;  $P > 0.15$ , respectively).

Although immature and adult *C. m. lengi* are larger than those of *C. m. fuscilabris* (Peres 2000), this morphological advantage does not provide any significant benefit to *C. m. fuscilabris* as compared with *C. m. lengi*. For instance, considering total egg production as a measure of a successful candidate for mass rearing for commercial purposes, our data indicated the advantage of *C. m. fuscilabris* as a mass reared subject. The 38-day life of *C. m. fuscilabris* and 43-day life of *C. m. lengi* adults were lower as compared with the 3 months reported by Wright & Laing (1978). We also observed a 3-day pre-ovipositional period in contrast to the 5 to 15 days reported by Hodek (1973). This situation may have occurred because our insects came from a commercial colony fed on an artificial diet for many generations. In nature, *C. maculata* spends time selecting ovipositional sites based on availability of food such as aphids and eggs of various species (Nault & Kennedy 2000). Our observations indicated that *C. m. fuscilabris* seems to be more aggressive than *C. m. lengi* (unpublished data). *C. m. fuscilabris* take only few second before starting to manipulate and consume (handling time) the prey (Rondon et al. 2004) as compared with *C. m. lengi*. Although no striking advantages emerged for one subspecies over the other, further studies are still needed. Results from our laboratory experiments provide the basis to further evaluate the possible introduction of *C. m. lengi*.

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#### SUMMARY

After measuring the developmental time, reproduction, and feeding of both subspecies of *C. maculata*, we conclude that there were no significant differences between subspecies in developmental periods but there were different levels of female longevity, eclosion, survival, and number of eggs per mass. In general, *C. m. fuscilabris* con-

sumed more *A. gossypii* than *C. m. lengi* in 24 h and produced more eggs per female. Further studies are needed to conclude if the introduction of *C. m. lengi* into the ecosystem of Florida would bring additional benefits to the present predator complex.

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