NOTE

Age-Grading Eggs of the Tarnished Plant Bug (Heteroptera: Miridae)[1]

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The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), feeds and oviposits on many plants, including economically-important hosts in the southeastern U. S. (Young, Ann. Entomol. Soc. Amer. 79: 747-762, 1986; Fleischer and Gaylor, Environ. Entomol. 16: 379-389, 1987). Determining the age structure of a population on various hosts is important for studies of population dynamics, colonization, or the impact of natural enemies.

A method of age-grading adult TPB is available (Stewart and Gaylor, J. Entomol. Sci. 25: 216-218, 1990). The age of nymphs can be estimated by morphological differences between instars. However, there is no comprehensive method of age-grading TPB eggs. The green pigment of developing nymphs can be observed in 7 to 10 d-old TPB eggs (Snodgrass and McWilliams, J. Econ. Entomol. 85: 1162-1166, 1992). Sorenson (Utah Agric. Exper. Stn. Bull. 284, 1939) observed changes in the "cap-end" or operculum of the egg and the appearance of an eye and dorsal scent gland as eggs of *Lygus* spp. aged. Our objective was to develop a method by which the age of TPB eggs could be accurately estimated.

TPB eggs of a known age were obtained by caging adult females on terminals of fleabane, *Erigeron strigosus* (Muhlenberg ex Willdenow), at 27°C in an environmentally-controlled room. The females were removed after 12 h. We chose fleabane because TPB eggs can be easily retrieved from oviposition sites within the flower buds.

Cohorts of eggs (range = 6 to 18 h old) were dissected from the flowers, placed on filter paper inside a Petri dish and maintained at 27°C. The filter paper was kept moist by adding a few drops of water twice daily until the eggs hatched. Photographs and observations of eggs were taken at 24-h intervals using a dissecting microscope at 250x and 500x, respectively. Total egg length, width (at widest point), length of the serosal cuticle, and eye diameter were recorded for 12 eggs as they aged. Linear regressions were performed using the mean values of these variables versus age (SAS Institute, SAS/STAT user's guide. Cary, NC, 1988).

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Eggs are elongate, widened posteriorly, and slightly incurved along their ventral length. Newly-laid eggs (range = 6 to 18 h old) are pale yellow, moderately flaccid, 949 ± 4 μm long (mean ± SE) and 270 ± 4 μm wide. Eggs are similar in appearance, but relatively turgid, when 36 h old (Fig. 1a). The operculum is slightly invaginated at 12 and 36 h after oviposition.

All 12 of the eggs hatched between 174 and 186 h of age. Sorenson (1939) reported that evagination of the egg cap occurs in *Lygus* spp. just before hatch. However, we found that the operculum was partially evaginated by 60 h (Table 1, Fig. 1b). Evagination of the operculum and egg elongation results from the stretching of the serosal cuticle (Cobben, Centre for Agric. Publ. and Documentation, Wageningen, 1968). The serosal cuticle increases linearly in length throughout egg maturation (Table 2). Consequently, a linear increase in total egg length also occurred. The width of eggs did not linearly increase during maturation. Generally entire, filamentous strands were evident on the surface of the serosal cuticle at 84 h (Fig. 1c). These strands were parallel to the longitudinal axis of the egg but, in 10 of 12 eggs, most of the strands had torn by 108 h (Fig. 1d).

Table 1. Total length, serosal cuticle length, and width of TPB eggs, and embryonic eye diameter versus age (± 6 h). Means ± SEM (μm) are reported.

Median age, h	No. eggs	Total length	Serosal cuticle length	Width	Eye diameter
12	12	949 ± 4	–	270 ± 4	–
36	12	948 ± 4	–	258 ± 2	–
60	12	1022 ± 8	38 ± 3	298 ± 4	–
84	12	1160 ± 8	142 ± 7	328 ± 5	–
108	12*	1194 ± 11	170 ± 9	329 ± 4	34 ± 4
132	12	1213 ± 12	188 ± 9	321 ± 4	72 ± 2
156	12	1227 ± 10	225 ± 10	324 ± 3	97 ± 1
180	3	1260 ± 53	243 ± 49	320 ± 0	107 ± 7

* Eyes were visible in 5 of 12 eggs at 108 h.

Faint, red eyes were visible by 108 h in 5 of 12 specimens (Fig. 1d). In all eggs, the eyes were considerably larger and more distinct by 132 h (Fig. 1e, Table 1) and grew linearly in diameter until hatch (Fig. 1f, Table 2).

At 132 h, the orange-colored dorsal abdominal scent gland (≈ 60 μm diameter) was faintly visible on the embryo ≈ 380 μm from the posterior end of the egg. This spot was clearly visible by 156 h. Also at 132 h, the distal segment of the developing nymphal antennae appeared as a pair of orange stripes located posteriorly on the venter of eggs. Both the dorsal abdominal scent gland and the

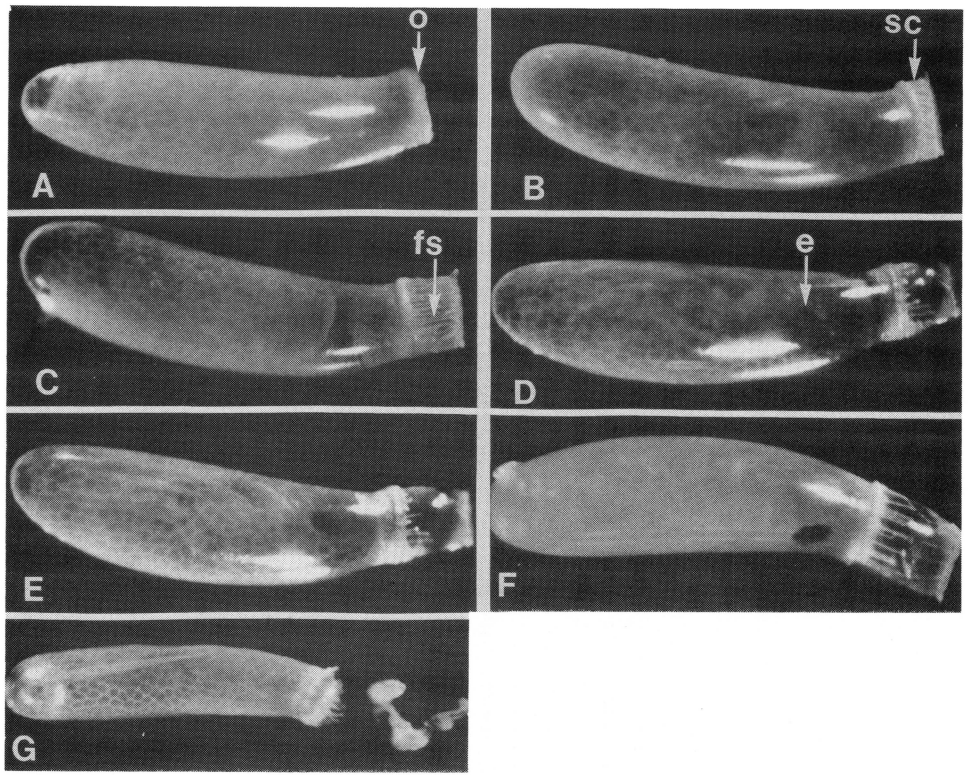

Fig. 1. Lateral view of tarnished plant bug eggs taken at A) 36, B) 60, C) 84, D) 108, E) 132, F) 156 h after oviposition and G) after hatching. Photomicrographs were taken at 250X. Letters indicate the following characters: e, eye; fs, filamentous strand; o, operculum; sc, serosal cuticle.

Table 2. Parameter estimates (± SEM, μm) for linear regression equations of total length, serosal cuticle length, width of TPB eggs, and embryonic eye diameter versus age in hours.

Dependent variable	Parameter estimates		r^2	F	P
	Intercept	Slope			
Total length	1096.3 ± 17.6	0.89 ± 0.12	0.96	54	0.018
Serosal cuticle length	52.8 ± 15.3	1.07 ± 0.10	0.98	105	0.009
Egg width	338.0 ± 9.20	-0.10 ± 0.06	0.56	2.6	0.250
Eye diameter	-68.7 ± 27.1	1.01 ± 0.19	0.94	30	0.032

antennae became more obvious as the eggs matured, but they are not visible in our black and white photomicrographs.

A green tint, first observed in 156-h-old eggs, was even more apparent at 180 h. The heads of nymphs began to move anteriorly within ≈ 2 h of hatch and eventually ruptured the serosal cuticle. A delicate, mesh-like network on the chorion of TPB eggs was most evident in hatched eggs (Fig. 1 g), but it was not hexagonal as previously reported (Sorenson 1939). Hatched eggs were deflated and somewhat translucent.

Because the appearance of TPB eggs change in a predictable pattern with development, age can be estimated by non-destructive, visual examination. The length and appearance of the serosal cuticle is a useful criteria for determining the age of eggs. The evaginating serosal cuticle may be the only visible portion of a TPB egg embedded into plant tissue. The eye spot, dorsal scent gland, and other characters are observable on some important hosts (e. g., *Erigeron* and *Conyza* species) that serve important roles as harborage for TPB populations. Of course, the rate of egg development depends on temperature, but this does not affect our ability to group eggs into cohorts. Grading egg development has potential for use in field and laboratory studies when timing insecticidal applications or parasitic releases are critical.

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