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**EGG PRODUCTION IN THE BOXELDER BUG *BOISEA TRIVITTATA*
(HEMIPTERA: RHOPALIDAE)**Karin A. Grimnes¹, Deborah Miller¹ and Aaron J. Wyman¹**ABSTRACT**

Boxelder bug females emerged from overwintering sites in the spring and rapidly provisioned eggs with yolk materials. Five discrete egg stages were identified based on egg size, protein content, and degree of chorion sclerotization. Females did not accumulate yolk materials into the egg until after melanization was completed, as unmelanized animals rarely possessed even stage 2 eggs. All adult females entering overwintering sites possessed only immature stage eggs (stage 1 and 2). The rate of egg vitellogenesis in the spring was rapid; a major change in numbers of more mature stage eggs (stage 3 and above) in the ovary occurred within approximately 6 days. Most mating pairs recovered in the field (92%, 12/13) possessed ovaries full of eggs in stages 3, 4 or 5. The remaining female contained only immature eggs of stage 1 and 2. This finding indicates that fully provisioned ovaries are not an absolute requirement for mating to occur. The signals that initiate vitellogenesis and control the movement of materials from fat body into eggs are unknown for the boxelder bug.

INTRODUCTION

Boisea trivittata (Say) is considered a pest species whose primary impact is one of nuisance to the homeowner when the aggregating bug swarms reach huge proportions. These insects usually do not cause economic damage, although they occasionally damage fruit such as strawberries (see Wheeler 1982).

The boxelder bug can be univoltine or bivoltine, depending on prevailing weather patterns. Even bivoltine populations can fail to mature a fall generation if the year is not warm enough for insects to enter overwintering sites as adults (Yoder and Robinson 1990). Midwestern populations appear to have predictable seasonal behavior (as summarized below from Smith and Shepherd 1937 and Tinker 1952). Boxelder bugs emerge in spring (March and April) and feed on available foliage as well as *Acer negundo* L. buds and stems. Mating occurs on warm spring days in April and May. Eggs are laid in clusters on substrates such as young leaves, bark and man-made structures in April and May and again in June and July. Nymphs occur between approximately May and Oct, with adults present in June and July and again in September until the hard frost dates in October that kill unprotected insects.

Although seasonal occurrence of eggs has been tracked for various populations, reports have been limited to observations of eggs laid on substrates (Smith and Shephard 1937, Tinker 1952, Yoder and Robinson 1990). Descriptions of ovarian anatomy (Payne 1936, Woolley 1949) mention the presence of eggs, but do not quantify them in any way. Identification of discrete stages in the egg provisioning process is needed to determine seasonal development, as is the relationship between melanization and egg provisioning during movement of resources from the fat body to the ovary.

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In this paper, we identify discrete stages of the egg provisioning process and examine the relationship between egg provisioning and melanization. A thorough understanding of these processes are necessary to characterize seasonal egg development in the boxelder bug.

MATERIALS AND METHODS

The study site was adjacent to Lang's Veterinary Clinic (T12N, R3W, Sec. 29, Pine River Township) in Gratiot County, Michigan where a large population of boxelder bugs was established. This site contains numerous pistillate boxelder trees (*Acer negundo*). The initial collections were made during the active seasons of 1990 through 1992 and supplemented during 1994 and 1999. Insects were collected and preserved in 70% ethanol. Live boxelder bugs also were collected and transported to the laboratory. These bugs were maintained at room temperature ($27 \pm 2^\circ\text{C}$) in 150 mm Petri dishes with boxelder tree leaves inserted into tubing filled with 10% maple syrup (V/V) diluted with water (Bouldrey and Grimnes 1995).

Mature preserved field-collected females were dissected in 70% ethanol, and the eggs were separated from ovarian tissue. Egg length and width were measured with an ocular micrometer and notes on coloration, opacity, and chorion formation were made. We knew that dealing with tissue in alcohol would give us slightly smaller values than fresh material, but we felt it was necessary to allow eventual analysis of previously preserved insect samples.

The protein content of eggs was determined using the Lowry Assay (Lowry et al. 1951). Individual eggs (N = 25 per stage) were homogenized in 0.5 ml water using a glass homogenizer and the Lowry Assay was performed immediately. Bovine serum albumen (BSA) was used as the protein standard.

For the melanization study, newly eclosed adults (completely bright orange) were collected daily from the field or from laboratory populations of fifth instar animals. They were maintained at $27 \pm 2^\circ\text{C}$ in the lab and observed hourly. Pictures were drawn noting the changes that occurred as the animals darkened and hardened. Approximate duration of the process was estimated by combining data from identically staged animals.

Analysis of variance was performed on the data using the JMP statistical program (SAS Institute 1998). Comparison of means was accomplished with the Tukey-Kramer's HSD statistic.

RESULTS AND DISCUSSION

Egg stage characterization. We were able to visually differentiate five stages of eggs from mature females on the basis of color, shape and the presence and degree of chorion sclerotization (Table 1). Eggs laid on substrates were labeled as stage 6 eggs. Eggs changed shape from round to oval, changed color from translucent white to milky white indicating accumulation of yolk materials, and began/completed chorion formation and tanning. Although the chorion appeared brown in the alcohol-preserved specimens, our observations of freshly laid eggs indicated the true color at laying was a light tan color that darkened progressively to red as noted in other studies (Smith and Shepherd 1937, Yoder and Robinson 1990).

An ANOVA was performed on egg length and width for all stages (Table 1). Egg length varied significantly with egg stage; however, all pair-wise comparisons with Tukey-Kramer HSD revealed that stage 3, 4 and 5 egg lengths were not significantly different from each other. A similar (but not identical) pattern was noted for width which was significant across stages overall; although stage 3 and 4 eggs overlapped in size, stage 5 eggs were significantly narrower than either stage 3 or 4 eggs. The correlation between egg length and

Table 1. Characteristics of Egg Stages in *Boisea trivittata* using JMP analysis (Mean \pm SE)

Egg Stage ¹	Egg Length (μm)	Egg Width (μm)	Protein content ($\mu\text{g}/\text{egg}$)	Description of egg stage
1	308 \pm 64a	284 \pm 62a	Not Determined	small, round, translucent, non-vitellogenic
2	806 \pm 179b	560 \pm 112b	8.4 \pm 1.1a	square-shaped, white, non-vitellogenic
3	1632 \pm 130c	996 \pm 105c	21.5 \pm 1.1b	oval, white, vitellogenic
4	1568 \pm 69c	994 \pm 58c	28.7 \pm 1.8b	chorion light tan, subtle hatch line
5	1590 \pm 63c	926 \pm 30d	68.6 \pm 4.0c	chorion dark brown, obvious hatch line
6	1556 \pm 8c ²	907 \pm 13d ²	76.8 \pm 1.8c	laid eggs taken from <i>Acer negundo</i> leaves

¹ N = 25 eggs/stage unless otherwise noted; means sharing a letter within a column are not significantly different at $P < 0.05$ in the Tukey-Kramer HSD test.

² N = 15 eggs/stage

width was substantial in linear regression analysis (Fig. 1, regression formula of egg width = $0.14 + 0.51 \times (\text{egg length})$, r^2 of 0.89). When all egg stages were graphed together, stage 2 eggs formed a discrete collection of data points and there were no intermediates between stage 2 and stage 3 eggs. This suggests that the process of vitellogenesis and egg material uptake occurs very rapidly. In addition, stage 3 and 4 eggs were somewhat variable and overlapped in size. However, stage 5 eggs consistently were located in the center of the general "cloud" of stage 3 and 4 eggs. As the chorion hardened and darkened in stage 5, the eggs became compressed, possibly by the elimination of water because protein levels still were increasing between stage 4 and 5 (data given below). Eggs laid on substrates (stage 6) were not significantly different in size from stage 5 eggs. No attempt was made to determine fertilization rates in this study.

Protein content analysis. Analysis of protein levels for eggs at all stages was performed with the Lowry Assay. This assay detects free amino acids after digestion, but does not hydrolyze proteins that have been cross-linked into either chorion or the exoskeleton of the developing embryo. Preliminary data indicated that levels of protein found in stage 1 eggs (under $1 \mu\text{g}/\text{egg}$) were too low to measure reliably so this stage was not analyzed fully. An ANOVA revealed that protein content increased from stage 2 eggs to stage 5 eggs (Table 1). Stage 6 (laid eggs) had a slightly higher protein content but this value was not significantly different from stage 5 protein levels. Stage 3 and 4 eggs overlapped in detectable protein in the Lowry Assay.

Protein levels increased as egg sizes (and provisioning rates) declined between stage 3 and stage 5 eggs. Thus, it is possible that non-protein nitrogen sources already present in stage 3 eggs were being incorporated into amino acids/proteins detectable by the Lowry Assay as the eggs progressed to stage 5.

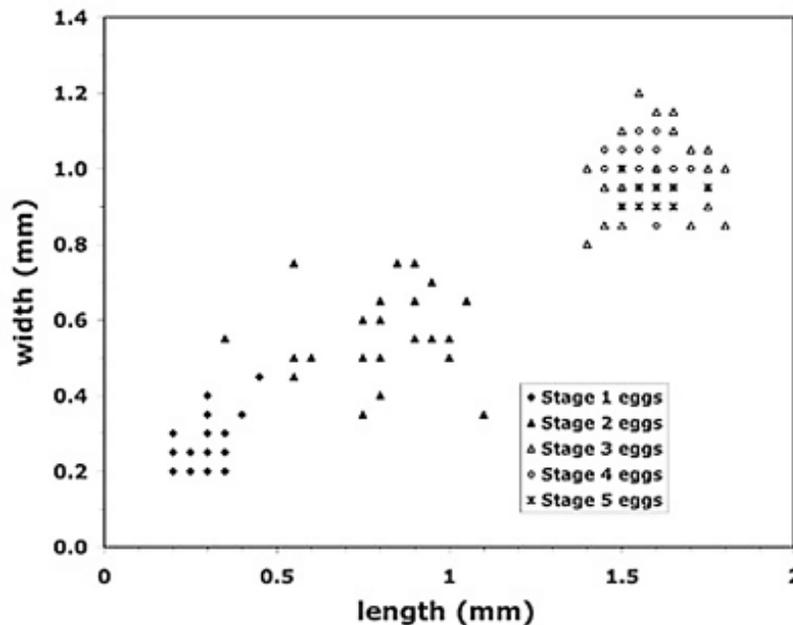


Figure 1. *Boisea trivittata* egg sizes identified by stage (N = 25 for each stage)

In addition, general increase in protein synthesis during these stages must outpace proteins lost into chorion formation. Hatchling protein content dropped below stage 6 by 30% (data not shown), possibly due to protein digestion or to cross-linking of proteins during exoskeleton formation.

Melanization study. Newly molted adults progress through a series of identifiable stages before becoming completely melanized and indistinguishable from older adult animals. The scale began with non-melanized newly eclosed adults that possessed orange bodies and incompletely expanded wings. The next steps (in order) were the full expansion of wings, the appearance of a ring around the ocellar spot, body coloration darkening from orange to brown to black, ventral head area fully darkened, and the sub-buccal area becoming fully darkened. Smith and Shephard (1937) noted which areas of the bugs became melanized, but no order for the process was given.

Newly molted boxelder bugs (N = 6) progressed to full melanization in approximately 5-6 hours (at $27 \pm 2^\circ\text{C}$). Thus, any field-collected insect that lacked fully darkened coloration on the underside of the head was considered incompletely melanized (molted within the past 24 hours). Animals collected in the last stage prior to full melanization were dissected, and their eggs were classified as to stage and size. Most eggs were in stage 1 of development. Of the 66 insects analyzed, only 3% (2/66) contained any stage 2 eggs, which constituted about 1% (7/676) of the total egg number. These data indicate that melanization occurred prior to egg provisioning.

Egg kinetics. Preliminary seasonal analysis of egg stages indicated that boxelder bugs approaching overwintering (September and October collection dates, N = 10 for each date) contained only stage 1 eggs (100%) and had abundant fat body stores when dissected. Animals emerging during early spring had depleted fat body stores (N = 10) and no eggs in advanced egg stages of development (stage 3 or beyond). Numbers of more mature eggs (stages 3, 4 and 5) substantially increased within females collected 5-7 days later (N = 10), approximately three weeks after the first spring emergence dates. Females at this time contained abundant fat body material, presumably due to spring feeding. The mobilization of resources from the fat body to the ovary is by inference; the vitellogenesis process has not yet been characterized.

Mating pairs collected in the field were analyzed for presence of vitellogenic eggs at stage 3 or greater. Of twelve mating pairs thus analyzed, 11 females (92%) had advanced eggs; the remaining female possessed only stage 1 and 2 eggs. Although these data suggest that fully provisioned eggs are not an absolute requirement for the mating process, the precise relationship between mating and onset of vitellogenesis has yet to be determined.

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