Egg Morphology of Key Stored-Product Insect Pests of the United States

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ABSTRACT Eggs of Carpophilus hemipterus (L.) (Coleoptera: Nitidulidae), Lasioderma serricorne (F.) (Coleoptera: Anobiidae), Ephesia elutella (Hübner) (Lepidoptera: Pyralidae), and Amyelois transitella (Walker) (Lepidoptera: Pyralidae) were imaged using scanning electron microscopy to explore how respiratory openings on the chorion surface may affect the relative efficacy of fumigation. Each C. hemipterus egg had two aeropyles and no micropyles; A. transitella and L. serricorne eggs had many aeropyles and several micropyles; and each E. elutella egg had many aeropyles and a single micropyle. Our data suggest that gases, including fumigants, differentially diffuse into the eggs of these species, with penetration through aeropyles and micropyles likely occurring to a greater extent in L. serricorne, E. elutella, and A. transitella than in C. hemipterus. Although confirmatory measurements of fumigant diffusion into eggs are needed, these findings suggest that species-specific ovicidal efficacies are related, at least in part, to the surface morphology of eggs and that chorionic respiratory structures differentially affect fumigant penetration and/or uptake.

KEY WORDS egg respiratory system, aeropyle, fumigant efficacy, diffusion, tolerance

Postharvest management of insect pests is essential for the maintenance of a safe food supply, and fumigation has a long history as a successful control measure against stored-product insects. The U.S. dried fruit and nut, grain, processed food, and tobacco industries critically rely on postharvest chamber fumigation to disinfect incoming products during harvest, stored products amenable to reinfestation, and outgoing product subject to a required phytosanitary treatment. Given the declining use of methyl bromide (MeBr or CH₃Br) caused by regulatory phase out, it is increasingly important to understand how various chemicals such as sulfuryl fluoride (SF or SF₂O₂), phosphine (hydrogen phosphide or PH₃), propylene oxide (PPO or C₃H₆O), ethylene oxide (PPO or C₂H₄O), and sulfur oxides (SO₂, SO₃) are optimized to control key stored-product insect pests (Fields and Scheffrahn 1990, Bell and Savvidou 1999, Baltaci 2002, White 2002, Thorne et al. 2003, Johnson et al. 2012).

It is generally accepted that eggs of stored-product insect pests are the most fumigant-tolerant life stage and require higher concentration × time exposures for control compared with other stages (Bell 1976, Su and Scheffrahn 1990, Bell and Savvidou 1999, Baltaci et al. 2009, Bonjour et al. 2011, United Nations Environment Programme [UNEP] 2011, Athanassiou et al. 2012). Unlike other developmental stages (i.e., larvae, pupae, and adults) where the uptake of physiologically active gases (e.g., oxygen, fumigant) occurs through the tracheal system, which opens to the ambient environment via spiracles, gases enter insect eggs through respiratory openings such as aeropyles and micropyles found on the chorion, the outermost proteinaceous covering of the egg (Hinton 1963, 1981; Nation 2008). Aeropyles are microscopic holes in the chorion that extend radially inward, creating a network that allows, at least in theory, gas exchange with the ambient environment via the interstices of the inner chorion region (Tuft 1950, Hinton 1963, Daniel and Smith 1994, Trougakos and Margaritis 2002). A micropyle is a surficial invagination, through which sperm and gases enter the egg, located in various quantities at the anterior end, often in morphologically differentiated chorion termed the micropylar area (Tuft 1950, Outram 1967, Arbogast et al. 1980, Arbogast and Byrd 1981, Margaritis 1983, Marvaldi 1999). Gases can also diffuse directly into the egg through the chorion (Tuft 1950, Outram 1967, Trougakos and Margaritis 2002).

The external morphology of insect eggs has been studied using the scanning electron microscope (SEM) in the context of differentiating species and tracing origins of infestations in domestic and international trade (Arbogast et al. 1980; Arbogast and Byrd 1981; Kucžerová and Stejskal 2002, 2010; Hasbani et al. 2008; Dutra et al. 2011). However, little or no work has been conducted to investigate the possible mechanis-
tic relationship between respiratory openings on the chorion surface that facilitate gas exchange and the various environmental, physiological, and toxicological factors that influence the relative tolerance of insect eggs to fumigants. In relation to effectively managing key stored-product insect pests using SF, an increasingly used postharvest MeBr alternative in the United States, eggs of *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae) require ≈30-fold higher concentration of SF for control at the LD₉₀ level compared with eggs of *Ephesia elutella* (Hubner) (Lepidoptera: Pyralidae) and *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) at ≈15.6°C (Baltaci et al. 2009, Walse et al. 2009, UNEP 2011), and approximately fourfold higher concentration compared with eggs of *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) at ≈26.7°C (Su and Scheffrahn 1990). Therefore, we compared the abundance, distribution, and location of respiratory openings in the chorions of these four species as the first step in quantifying consequences of egg morphology on the relative tolerance of insect eggs toward fumigants.

**Materials and Methods**

**Insects.** Eggs were obtained from adult females of *C. hemipterus*, *A. transitella*, *E. elutella*, and *L. serricorne* reared in the insectary at the U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS), San Joaquin Valley Agricultural Sciences Center, Parlier, CA. Voucher specimens of *C. hemipterus*, *L. serricorne*, *E. elutella*, and *A. transitella* adults that laid the eggs used in this study were preserved in 95% ethyl alcohol and deposited at the K. C. Emerson Entomology Museum at Oklahoma State University under lot numbers 138, 140, 142, and 143, respectively. Rearing conditions for these insects were 27 ± 0.10°C (mean ± SE), 60 ± 0.24% relative humidity (RH; mean ± SE), and a photoperiod of 16:8 (L:D) h. *C. hemipterus* were reared on ripened banana on top of soil substrate in 946-ml glass jars; *L. serricorne* on rice bran diet in 946-ml glass jars; and *A. transitella* and *E. elutella* on red flaky wheat bran diet in 3.8-litre glass jars. The *C. hemipterus* culture was originally obtained in 1978 from the Italian Swiss Colony Winery in Fresno county; *L. serricorne* in 1967 from an unknown source; *A. transitella* in 1966 from the University of California, Berkeley; and *E. elutella* in 1969 from the USDA Tobacco Investigations Laboratory, Richmond, VA (U.S. Department of Agriculture [USDA] 2012).

**Egg Collection.** *C. hemipterus*. Freshly laid eggs, no older than 24 h, were obtained by placing 75–100 adults in a 237-ml glass jar. Glass slides (25 by 75 by 1 mm) were prepared as substrate for egg laying. A thin smear of coddling moth bean agar diet was spread down the center (the 75-mm midline) of each slide onto which two wax paper strips (25 by 75 mm) folded in half were placed on each side with the crease folds toward the center, that is, covering the whole slide surface. Two slides were then bound together using a rubber band (#16) in such a way that the wax papers (four of them) were sandwiched between the glass slides and the edges of the slides were aligned. Four prepared slide units were placed in a 237-ml glass jar along with egg-laying females. The glass jar was covered with a moist filter paper, a wire screen (U.S. #40 mesh), and a final moist filter paper, and all these were secured using a threaded metal ring. Jars were placed in a holding room at 27 ± 0.01°C and 60 ± 0.24% RH for 12–18 h. The eggs were processed as described below for analysis using SEM (S-3500N Hitachi, High Technologies America, Pleasanton, CA).

*L. serricorne*. One hundred to 200 adults were aspirated into a 237-ml glass jar. The glass jar was covered with a wire screen (U.S. #40 mesh) and secured using a metal ring. The jar containing adults was inverted onto a 9-cm glass petri dish lined with a filter paper and spaced (≈2–3 mm) from the filter paper using a large paper clip. We used rice bran diet around the edge of the petri dish to stimulate oviposition. The setup was then placed in a holding room maintained at 27 ± 0.01°C, 60 ± 0.24% RH, and a photoperiod of 16:8 (L:D) h for 2–3 d. After 3 d, 0- to 3-d-old eggs were collected and analyzed using SEM.

*E. elutella*. One hundred to 200 adults were aspirated into a 1.9-liter glass jar that was covered with a wire screen (U.S. #40 mesh; USDA 2007). The jar containing adults was inverted over an old culture jar to discard any eggs and left on its side for few minutes. The jar was then inverted on top of a large paper clip spacer, which sat on a petri dish (90 by 20 mm) lined with a filter paper. The egg layers were placed in a holding room maintained at 27 ± 0.01°C, 60 ± 0.24% RH, and a photoperiod of 16:8 (L:D) h for 2–3 d. After 3 d, 0- to 3-d-old eggs were harvested from the filter paper and analyzed using SEM.

*A. transitella*. One hundred fifty adults were aspirated and transferred to a 2.3-liter coffee can, and the can opening was covered with wax paper that was secured to the container with a rubber band (#18). The setup was then placed on its side on an incubator shelf with the wax paper facing a night light (4-W bulb) in a holding room that was maintained at 27 ± 0.01°C, 60 ± 0.24% RH, and 16:8 (L:D) h for 2–3 d. SEM analysis was conducted on 0 to 3-d-old eggs.

**Scanning Electron Microscopy.** Freshly collected eggs (100–150) were mounted on double-sided carbon tabs (Ted Pella, Inc., Redding, CA) on aluminum stubs using a soft brush. *C. hemipterus*, *E. elutella*, and *L. serricorne* eggs were first attached to a piece of single-sided tape. Subsequently, the tape containing eggs was attached to a double-sided sticky carbon tab on an aluminum stub such that the eggs were exposed. For *A. transitella*, the wax paper containing eggs was carefully cut into small pieces and attached to a double-sided sticky carbon tab on an aluminum stub such that the eggs were exposed. To prevent specimen charging and increase secondary electron signals, eggs of all species were sputter coated with gold (SPI Module Sputter Coater, SPI Supplies, West Chester, PA) at 5 mA for 40 s at 90° to the target, followed by 40 s at 45° to the target. The samples were then viewed under an SEM, and digital images were taken at 5.00 kV. When possible, the numbers of aeropyles on exposed
surfaces of individual eggs were counted. In addition, pictures of entire eggs, chorion sculpture, aeropyles, and micropyles were taken; ~30–40 pictures each of different eggs, aeropyles, and micropyles were taken for each species. Measurements made on digital images using ImageJ software (National Institute of Health, Bethesda, MD) included length and diameter (at widest point) of the egg, and diameter and cross-sectional area of each aeropyle opening. Each measurement was considered a replicate. External surface area and surface area-to-volume ratio (SA:V) were calculated. Parameters such as number of aeropyles per unit area of an egg and total aeroplar surface area were estimated, and where applicable, error was reported based on the propagation of SEs (Lehrter and Cebrian 2010).

To test the null hypothesis that the diameters of aeropyle openings, external surface areas, or SA:V of eggs were equivalent across insect species, an analysis of variance (ANOVA) was conducted for each using Statistical Analysis System software (Version 9.2, SAS Institute, Cary, NC; SAS Institute 2010) and the PROC GLM model. If the null hypothesis was rejected, the ANOVA was followed by Tukey honestly significant

Fig. 1. SEM of a C. hemipterus egg (100×) (A), a bumpy texture of the egg chorion (900×) (B), anterior end of the egg with aeropyles indicated by arrows (450×) (C) and at 1,200× (D), a magnified aeropyle (12,000×) (E), and a cross-section of the freeze-fractured chorion: ol is the outer layer, ml the middle layer, and il the inner layer (F) (9,000×).
Cryofracturing *C. hemipterus* Eggs. A Hitachi S-3500N SEM (Hitachi High Technologies, America, Pleasanton, CA) equipped with a CT-1500 C cryo-unit (Quorum Technologies, East Grinstead, United Kingdom) was used to study the interior details of the chorion of cryofractured *C. hemipterus* eggs. Cryofracturing is a method previously applied to image cell interiors using SEM (Bozolla and Russell 1992). The eggs were aligned on a sticky tape and placed on the cryo-specimen holder, a copper stub with a groove along its diameter filled with a mixture of colloidal graphite and Tissue-Tek. The eggs were then cryo-fractured by being transferred in the frozen state to the cryostage of the SEM where they were subsequently fractured, sublimated (15 min at 90°C), sputter coated with gold at 7 mA for 2 min. The fractured eggs were transferred to the cryostage of the SEM where they were analyzed at 15 kV and −178°C.

### Results

**General Egg Morphology.** *C. hemipterus.* Eggs are cylindrical in shape (Fig. 1A), 891.7–1,164.3 μm long, and 206.2–305.9 μm in diameter at the widest point (Table 1). Both anterior (narrower) and posterior (broader) ends of each egg are bluntly pointed. The surface of the chorion is smooth under low magnification (100x; Fig. 1A). However, at higher magnifications (≥450x), the chorion surface has a bumpy texture (Fig. 1B–D), which is more pronounced at the anterior end. Two aeropyles, each located at the tip of the anterior end (Fig. 1C and D; Table 1), are funnel shaped and lack collars (Fig. 1E). A collar is thickened and raised chorion forming the rim of an aeropyle opening (Arbogast et al. 1980); it can be well developed or less distinct. Collars can be useful morphological features to facilitate identification of eggs of various insect species (Arbogast et al. 1980). The chorion has three distinct layers: a thick outer layer, a spongy middle layer, and a thin innermost layer (Fig. 1F).

**L. serricorne.** Eggs are ovate in shape (Fig. 2A), 347.2–433.2 μm long, and 179.1–247.5 μm in diameter at the widest point (Table 1). The egg gradually narrows toward the anterior and posterior ends, which are bluntly rounded (Fig. 2A and B). The egg surface, except the micropylar area prominently located at the tip of the anterior end (Fig. 2B and C), is marked by numerous tubercle-like projections (inconspicuous protuberances) that look granulated at lower magnification (250–400x) (Fig. 2A and B), but are clearly observed at higher magnifications (≥1,500x) (Fig. 2C and D, indicated by a circle). In addition to these tubercle-like projections, the anterior end has pillar-like projections that are arranged in a pentagonal pattern (Fig. 2B, indicated by a square), which at higher magnification (8,000×; Fig. 2D) are observed as a fibrous bundle that rises to a height of 11.21 ± 0.46 μm (mean ± SE) from the surface of the chorion. All chorionic projections, the tubercle-like projections, as well as pillar-like projections, have an aeropyle or aeropyles at the tips (8,000× and higher; Fig. 2D and E; Table 1). The aeropyles lacked collars and were distributed over the egg surface. The micropylar area is located at the tip of the anterior pole and has 7–10 micropyles (Fig. 2C and F; Table 1).

**E. elutella.** Eggs are nearly globate in shape (Fig. 3A), 415.5–535.6 μm long, and 344.7–421.3 μm in diameter at the widest point (Table 1). The chorion is marked by numerous tubercle-like winding ridges that were joined at termini but did not have an angular pattern (Fig. 3A–C). Aeropyles, one or several, were present at the termini (Fig. 3C–E) and were distinctly characterized by a broad collar (Fig. 3E). Termini were distributed over the entire egg surface, but those with aeropyles were localized near the ends (Fig. 3C and D). At a high magnification (20,000×), the aeropyle opens to an inner chorionic meshwork (Fig. 3E). Approximately 10% of the observed eggs had a nipple-like projection formed by chorionic folds at the tip of the anterior end, which masked the presence of the micropylar area and a single micropyle opening (Fig. 3A, indicated by a white arrow). The ridges of the micropylar area were appressed, and there were 14–24 primary cells in a micropylar rosette that opened at the center (Fig. 3F). The primary cells are distinct loop-like or petal-like structures that have distinct ridges or carinae, and radiate out from the central depression at the apical end and give a flower-like appearance to the micropylar region of the egg (Baker et al. 2012).

**A. transitella.** Eggs are ovate in shape (Fig. 4A), 619.4–695.4 μm long, and 428.8–552.4 μm in diameter at the widest point (Table 1). The chorion is marked

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**Table 1. Parameters (mean ± SE) and descriptions of eggs and location, abundance, and attributes of respiratory openings of *C. hemipterus*, *L. serricorne*, *E. elutella*, and *A. transitella* eggs (n = 30)**

| Attribute               | *C. hemipterus* | *L. serricorne* | *E. elutella* | *A. transitella* |
|-------------------------|-----------------|-----------------|---------------|------------------|
| Length (μm; range)      | 1063.2 ± 12.5 (891.7–1164.3) | 400.2 ± 3.7 (347.2–433.2) | 488.1 ± 4.6 (415.5–535.6) | 651.1 ± 4.6 (619.4–695.4) |
| Diameter (μm; range)    | 264.9 ± 3.6 (206.2–305.9) | 210.5 ± 3.1 (179.1–247.5) | 373.5 ± 3.6 (344.7–421.3) | 476.9 ± 6.5 (428.8–552.4) |
| L/W ratio               | 4.03 ± 0.07     | 1.91 ± 0.03     | 1.31 ± 0.02   | 1.37 ± 0.02      |
| Surface of the chorion  | Smooth          | Basal tubercle-like projections; pentagonal pattern of pillar-like projections at the anterior end. | Tubercles joined together by ridges at the termini | Reticulate pattern of polygons |
| Distribution of aeropyles | Anterior end     | Distributed over the surface of egg | Distributed at ends; predominant at the posterior end | Distributed over the surface of egg; predominant at ends |
| Micropyles              | Absent          | Present; 7–10 micropyles | Present        | Present; 1–5 micropyles |
with protuberances that rise to form a reticulate pattern of polygons (Fig. 4A and B). Aeropyles were found at the vertices of polygonal structures and were distributed across the egg surface, although localized at the ends (Fig. 4C–E). Aeropyles were characterized by distinct broad collars and open to an inner chorionic meshwork (15,000×; Fig. 4D). The polygonal ridges were prominent all over the egg surfaces except the micropylar area at the anterior end where 1–5 micropyles were centered at a rosette formed by 17–23 primary cells (Fig. 4E and F).

**Fig. 2.** SEMs of an *L. serricorne* egg (250×) (A), the view of an egg from the anterior end showing chorionic sculpture (400×) (B), the anterior end with micropyles; note the nine micropyles (1,500×) (C), an aeropyle at the tip of a chorionic projection indicated by a white arrow (8,000×) (D), aeropyles at the tip of a chorionic projection indicated by white arrows (40,000×) (E), and a magnified micropyle (15,000×) (F).

**External Surface Area and Volume.** External surface areas and SA:V ratios of eggs varied across species (*F* = 513.2, df = 3,116, *P* < 0.0001 and *F* = 503.9, df = 3,116, *P* < 0.0001, respectively; Table 2). The average external surface area of a *C. hemipterus* egg was significantly larger relative to *A. transitella*, *E. elutella*, and *L. serricorne* eggs (Table 2). Likewise, the average external surface area of the *A. transitella* egg was significantly larger relative to *E. elutella* and *L. serricorne* eggs, and the external surface area of the *E. elutella* egg was significantly larger than the *L. serricorne* egg.
Among the insect eggs studied, L. serricorne had the smallest eggs (Tables 1 and 2). However, L. serricorne had the largest SA:V ratio compared with C. hemipterus, A. transitella, and E. elutella (Table 2). Similarly, the C. hemipterus SA:V ratio was significantly larger relative to A. transitella and E. elutella, whereas A. transitella had a larger SA:V ratio than E. elutella. However, our estimate of L. serricorne external surface area is likely lower than the actual external surface area because we did not account for the elaborate chorionic sculpturing on the eggs (Fig. 2A–D).

**Respiratory Structures. Aeropyles.** Diameters of the aeropyle openings varied across species (\( F = 74.81; \text{df} = 3,116; P < 0.0001; \text{Table 3} \)). The diameter of C. hemipterus aeropyles did not differ from those of E. elutella and A. transitella but was larger than those of L. serricorne (Table 3). The numbers of aeropyles per square micrometer in L. serricorne, E. elutella, and A. transitella, estimated by dividing the average number of aeropyles per egg by the average external surface area of an egg, were \( \approx 435,000 \), 15-, and fivefold greater, respectively, than in C. hemipterus.

**Fig. 3.** SEMs of an E. elutella egg (200×) (A), sculpture of the posterior-end chorion (400×) (B), the posterior end of the egg with aeropyles indicated by black arrows (700×) (C), aeropyles atop a single chorionic projection (5,000×) (D), a magnified aeropyle showing the inner chorionic meshwork (20,000×) (E), and the anterior end of the egg with a micropyle indicated by a white arrow and aeropyles indicated by black arrows (1,500×) (F).
**C. hemipterus** eggs had only two aeropyles each, which had an opening of 1.24 ± 0.11 μm (mean ± SE) in diameter (Table 3). The number of aeropyles per square micrometer was $2.0 \times 10^{-6} \pm 3.5 \times 10^{-8}$ (mean ± SE; Table 3). The combined area of all the aeropyle openings per egg, the total aeropylar surface area, was $2.16 \pm 0.19 \mu m^2$ (mean ± SE), based on estimation by multiplying the average cross-sectional area of an aeropyle and the average number of aeropyles per egg (Table 3). Relative to the other species, the area of chorion open to ambient atmosphere (total aeropylar area + micropylar area) was smallest in **C. hemipterus** and was ≈491.9-, 9.6-, and 2.54-fold less than that for **L. serricorne**, **E. elutella**, and **A. transitella** eggs.

**L. serricorne** had 291,200 ± 13,145 (mean ± SE) aeropyles per egg. These aeropyles were 0.10 ± 0.01 μm in diameter (Table 3), which is significantly smaller than **C. hemipterus**, **E. elutella**, and **A. transitella** aeropyles (Table 3). The estimated number of aero-
Materials and Methods section.

The diameter of each micropylar opening of an aeropyle was not calculated directly from average diameter or radius of the aeropyles but as described in the

### Table 2. Surface area and volume parameters (mean ± SE) of *C. hemipterus, L. serricorne, E. elutella*, and *A. transitella* eggs (n = 30)

| Insect species | External surface area of an egg (µm²) | SAV ratio² |
|----------------|---------------------------------------|------------|
| *C. hemipterus* | 9.9 ± 0.09a by 1.3 ± 0.01b (10³) | 2.9 ± 0.06 | 1.07 ± 0.04b (10³) |
| *L. serricorne* | 3.4 ± 0.07a by 2.6 ± 0.02b (10³) | 2.9 ± 0.06 | 0.76 ± 0.03b (10³) |
| *E. elutella* | 5.5 ± 0.2 by 0.8 ± 0.05c (10³) | 17.0 ± 1.1 | 1.07 ± 0.04b (10³) |
| *A. transitella* | 6.8 ± 0.8 by 1.2 ± 0.0b (10³) | 17.0 ± 1.1 | 1.1 ± 0.04b (10³) |

¹ Mean within a column followed by different letters are significantly different (α = 0.05).
² Mean within a column followed by different letters are significantly different (α = 0.05).

### Table 3. Aeropyle parameters (mean ± SE) of *C. hemipterus, L. serricorne, E. elutella*, and *A. transitella* eggs (n = 30)

| Insect species | Diameter of aeropyles (range (µm)) | Cross-sectional area of an aeropyle (µm²) | No. aeropyles per egg (n = 40) | No. aeropyles per unit area (µm²) of an egg | Total aeropylar surface area of an egg (µm²) |
|----------------|-----------------------------------|------------------------------------------|--------------------------------|---------------------------------|------------------------------------------|
| *C. hemipterus* | 1.24 ± 0.11ab (0.49–2.58) | 1.08 ± 0.15 | 2 ± 0.00 | 2.0 by 10⁻⁵ ± 3.5 by 10⁻⁶ | 2.16 ± 0.19 |
| *L. serricorne* | 0.10 ± 0.01c (0.034–0.204) | 0.0034 ± 0.0006 | 291.200 ± 13.145 | 0.87 ± 0.043 | 990.1 ± 180.4 |
| *E. elutella* | 1.40 ± 0.04a (0.82–2.55) | 1.19 ± 0.20 | 17.4 ± 0.79 | 2.9 by 10⁻³ ± 1.4 by 10⁻⁶ | 20.71 ± 1.39 |
| *A. transitella* | 1.07 ± 0.04b (0.71–1.66) | 0.76 ± 0.05 | 7.24 ± 0.36 | 1.06 by 10⁻³ ± 5.6 by 10⁻⁷ | 5.59 ± 0.34 |

¹ Mean within a column followed by different letters are significantly different.
² Cross-sectional area of aeropyles was not calculated directly from average diameter or radius of the aeropyles but as described in the Materials and Methods section.

### Discussion

The current study provides, for the first time, quantitative information on morphological and physical factors that influence gas exchange in the eggs of key stored-product insect pests (*C. hemipterus, L. serricorne, E. elutella*, and *A. transitella*), such as the number of aeropyles present, aeropylar area, external surface area, the percentage of external surface area covered by chorion, and estimated egg volume. Descriptions of the external morphology of *E. elutella* and *L. serricorne* eggs are generally consistent with those previously described by Arbogast et al. (1980) and Kučerová and Stejskal (2010), respectively. However, we note that whereas Kučerová and Stejskal (2010) indicated there were no aeropyles present in *L. serricorne* eggs, our examination of these eggs showed that they have aeropyles. This discrepancy could be a result of differences in the strains of *L. serricorne* studied and the level of magnification used in these two studies. In the current study, aeropyles in *L. serricorne* were observed at a magnification of 8,000 and higher (Fig. 2D and E), whereas Kučerová and Stejskal (2010) used a magnification of 3,700×. Descriptions of external morphology of eggs from the family Pyralidae were similar to that described above for *E. elutella* and *A. transitella*, having a sculptured chorion marked with tubercles that are joined together to form an angular pattern or winding ridges without any angular pattern, a micropylar area surrounded by cells that form a rosette-like structure, and several aeropyles with distinct collars distributed all over the surface of the chorion but predominant at the ends (Arbogast et al. 1980, Arbogast and Byrd 1981, Baker et al. 2012). There are currently no published SEM descriptions of eggs from the family Nitidulidae, which includes *C. hemipterus*. Descriptions of external egg morphologies of various stored-product pests are useful tools in identifying species and tracing origins of infestations in domestic and international trade (Kučerová and Stejskal 2010).

Morphological and physical characteristics of eggs were quantified to estimate the relative potential con-
tribution of chorionic respiratory structures to gas penetration and/or uptake. Respiratory structure-normalized surface areas (aeropylar and micropylar areas) were calculated to estimate the amount of egg surface area available for passive uptake of gases, which precludes the diffusion of gas through the chorion. The average diameter of the opening of aeropyles for the four species studied ranged from 0.1 to 1.39 μm, at least three orders of magnitude greater than the size of O₂ (1.2 Å), MeBr (2.49 Å), SF (2.99 Å), PH₃ (2.05 Å), ozone (O₃: 1.42 Å), and PPO (2.11 Å), and are expected to easily accommodate the mean free path of gas molecules (1,000 Å) (Hinton 1963) under conventional fumigation conditions. The total aeropylar surface area to route passive gas exchange (Tuft 1950) was highest in L. serricorne eggs and lowest in C. hemipterus. For L. serricorne, the micropylar area is 7% of the aeropylar area, suggesting that passive gas diffusion via this route would be concomitantly less. For the other three species, micropylies are not likely an appreciable route for passive gas diffusion.

While diffusion of gases through the chorion of insect eggs has not been directly probed, critical insight into the mechanism of gas diffusion through lipidic and cellulosic films (surfaces) can be extracted from literature detailing the kinetics of gas diffusion into fruits (Walse et al. 2013). Diffusion through the chorion would be a function of many factors, including a physiochemical interaction between the gas and the chorion, the thickness of the chorion, the surface area of the chorion, and the SA:V ratio of the egg. Presumably, in eggs with fewer or no respiratory openings, the contribution of diffusion through the chorion to the overall uptake of gases would be greater compared with eggs with relatively larger aeropylar and micropylar areas.

Enumerating the relative contribution of chorionic diffusion vs. respiratory structure-mediated uptake of gases is critical to understanding how egg morphology could be related to the relative ovicidal tolerance of insect species toward fumigants. Future research will be aimed at systematically and quantitatively exploring the role of egg morphology and molecular diffusivity, using microscopic and molecular marker techniques, in the context of species-specific fumigant efficacies.

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