Developmental Variation in the Dermal Glands and Wax Secretions of the Mealybug, *Phenacoccus fraxinus* (Hemiptera: Coccoidea: Pseudococcidae)[1]

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Abstract A microscopic study of the dermal glands and wax secretions of the mealybug *Phenacoccus fraxinus* Tang demonstrated that the type, structure, size, quantity and distribution of dermal glands varied with life stage. These variations resulted in different quantities and morphology of waxy secretions with development. The apertures of the trilocular pores were initially small, irregular narrow seams on 1st instars. By the 3rd nymphal stage, these apertures were large and figure-8 shaped. Slender wax filaments were produced in the 1st stage, whereas wax filaments produced in the 3rd stage had an indentation along the middle. These tilocular pores were distributed on the dorsum and the venter of both stages, corresponding to the shape of the aperture. Quinquilocular pores were characterized by a thick rim and were scattered on the ventral surface, but these were not distributed in spiracle furrows as observed with other scale insects. Tubular ducts were composed of 2 layers—outer and inner ductiles—that resulted in the wax secreted from them being long, hollow filaments that served as a framework for the cocoon and ovisac structures. Wax production from these tubular ducts occurred at two distinct times during development. One occurred in late autumn and was produced by the older 2nd -instar nymphs. These waxy filaments were used to construct cocoons for the overwintering mealybugs. The other event occurred in early summer with adult females. These filaments were used to construct the ovisac. During other times of the year, these tubular ducts produced no additional wax.

Key Words Pseudococcidae, *Phenacoccus fraxinu*, dermal glands, wax secretion, scanning electron microscopy, mealybug

The mealybug, *Phenacoccus fraxinus* Tang (Hemiptera; Coccoidea: Pseudococcidae), is an important forest pest in northern China. Damage caused by this pest is both direct (e.g., suction of plant juices) and indirect (e.g., production of honeydew which supports black sooty mold growth that reduces the tree photosynthesis). It is difficult to control this mealybug due to the protection provided by the waxy substances secreted by dermal glands located on the surfaces of their bodies. We have observed several types of dermal glands on *P. fraxinus*, including trilocular pores, queinquelocular pores and tubular ducts.

Phenacoccus fraxinus is univoltine and sexually dimorphic with females and males displaying different life cycles. Females have 3 nymphal stages—1st, 2nd and 3rd instars—before developing into adults. Males have 2 nymphal stages—1st and 2nd

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instars—followed by a prepupal and pupal stage from which alate adult males emerge. Dermal glands on the surface of the mealybugs vary in type, structure, quantity and distribution during development, thus affecting the structure, quantity and function of the waxy secretions they produce. Understanding these variations, their cause, and their impact may prove important in developing efficacious tactics for managing these pests.

Although dermal glands and waxy secretions of the scale insects are well-known, only a few studies report the ultrastructure and wax-secretion processes in Pseudococcidae. To date, these include *Ferrisia virgata* (Cockerell), *Phenacoccus manihoti* (Matile-Ferrero), *Planococcus citri* (Risso) (Cox and Pearce 1983), *Maconellicoccus hirsutus* (Kumar et al. 1997), and *Rhizoecus hibisci* (Kawai & Takagi) (Jansen 2001). Thus, the objective of our study was to identify the characteristics of the dermal glands and waxy secretions of *P. fraxinus* through its development to provide basic knowledge for the management of this pest.

Materials and Methods

Specimens of *P. fraxinus* used in this study were collected in 2006-2007 from the host plant, *Fraxinus chinensis* Roxb growing along roadsides in Taiyuan, Shanxi Province, China.

Specimens including 200 nymphs, 80 adult females and 80 adult males. These were collected each month from March to October. Collected specimens were transported to the laboratory for microscopic examination. Intact specimens were selected for use in the study.

For basic morphological examinations, whole specimens of different developmental stages were mounted on glass slides using methods as described by Kosztarab (1967). These were examined, measured and recorded with light microscopy at 20 X and 40X, including 30 individual specimens for each of the 1st, 2nd and 3rd instars, adult females and adult males.

Ultrastructural components and features of the wax glands and their secretions were observed with scanning electron microscopy (SEM). Specimens representing the different life stages were fixed in 3% glutaraldehyde prepared in 0.05 M phosphate buffer at pH 6.8 for 1 h at room temperature, washed 3 times in distilled water, dehydrated in graded series of acetone, critically dried in the critical point dryer, mounted onto copper stubs, and then coated with gold in the sputter coater. Twenty individual specimens were scanned at different angles using the scanning electron microscope (JSM-35C, JEOL, Japan) at 25kv.

Results

Nymph characteristics. The body of the 1st instar was flat, oval, and yellowish in color, and approx. 0.3 mm long and 0.2 mm wide. First instars began secreting waxy substances from the dermal glands soon after settling down and starting to feed. The wax first appeared on the dorsum and the margins of the body (Fig. 1A). Wax was secreted by the 14-15 pairs of cerari that were distributed around the dorsal margin of the body as well as the 1 pair of cerari on the dorsal surface of each body segment. Each cerarium consisted of 2 thick conical setae and 1 trilocular pore. Other waxes covering the dorsum were secreted from the trilocular pores distributed in a single line on the submarginal areas and in 2 horizontal lines on the median area of dorsum

(Fig. 1B). Every trilocular pore consisted of 3 loculi arranged in a triangle. The trilocular pores were 3.2 µm (range 2.9-4.4 µm) diam with each loculi pore approx. 0.8 µm (range 0.7-1.0 µm) diam (Fig. 1C). Wax substances extruded from each loculi of the trilocular pores accumulated on the dorsal surface. Wax on the posterior end of the body was produced by the anal ring and the anal ring setae. The ventral surface of the mealybug possessed a line of quinquelocular disc-pores, which secreted thin 0.5 µm (range 0.4-0.5 µm) diam and curly-shaped wax filaments. Some smaller diameter (0.4 µm; range 0.3-0.4 µm) wax filaments also appeared over the ventral surface and curled into a ring-shaped form.

The body of the 2nd instar was oval, light brown in color, and 1.0 mm long and 0.6 mm wide. The number of cerari on the body increased from the 14-15 pairs observed on the 1st instar to 16-18 pairs on the 2nd instar. Each cerarium, with the exception of the posterior pair, consisted of 2 thick conical setae and 2-3 trilocular pores. The posterior pair had 4-5 trilocular pores. Approximately 200 additional trilocular pores were observed scattered on the middorsum area. More of these pores (399; range 366-430) were observed on the 2nd instar than on the 1st instars (92; range 82-114). This resulted in greater wax secretion and accumulation on the 2nd instars than on the 1st instars. White wax filaments accumulated on the dorsal margins and middorsal area of the body correlating with the observed distribution of cerari (Fig. 2A).

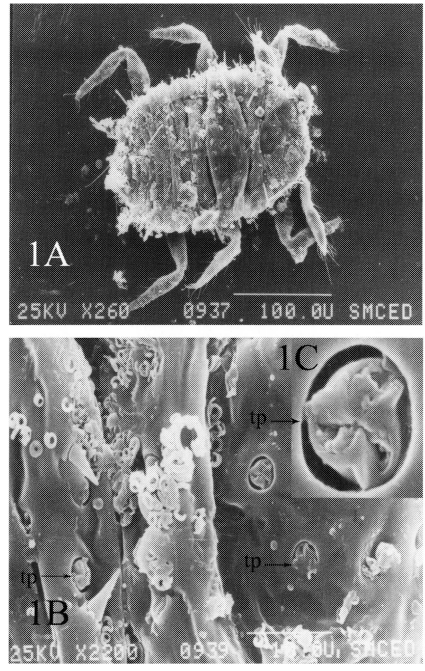

Fig. 1. Dorsal view of the 1st-instar nymph of *P. fraxinus* showing (A) the dorsum [260X], (B) two lines of trilocular pores (tp) on the head, prothorax and mesothorax [2200X], and (C) the 3 loculi arranged in a triangle within the trilocular pore (tp) [9400X]

Curly-shaped filaments were secreted from every loculi (Fig. 2B). The opening of each loculi in the trilocular pore was narrow, 1.0 μm (range 0.9-1.2 μm) long and 0.4 μm (range 0.3-0.5 μm) wide and was arranged in a spiral shape (Fig. 2C). Openings of the tubular ducts were 3.1 μm (range 2.7-3.5 μm) in diam. The quinquelocular disc-pores were not arranged in a line, as had been observed on the 1st instars, but were

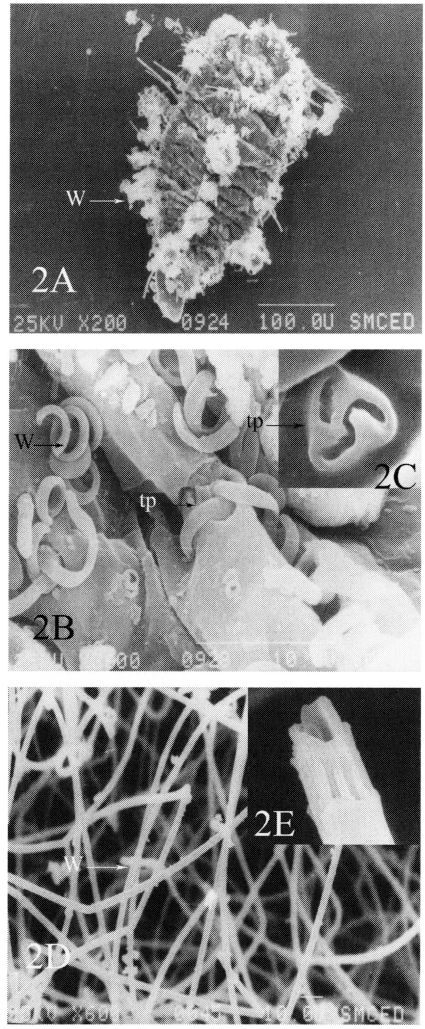

Fig. 2. Dorsal view of the 2nd-instar nymph of *P. fraxinus* showing (A) wax (w) accumulations on the mid and marginal areas of the dorsum [200X], (B) wax filaments (w) secreted by the trilocular pores (tp) [3600X], (C) a trilocular pore (tp) [9400×], (D) hollow wax filaments (w) secreted by the tubular ducts used in constructing the cocoon structure for overwintering 2nd instars [600X], and (E) hollow wax filaments with internal longitudinal ridges [9400X]

scattered over the ventral surface. These secreted wax filaments were 0.6 μm (range 0.5-0.7 μm) in diam. In the late stages of the 2nd instars, the mealybugs moved from the leaves to aggregate on the underside of plant branches, twigs and other stems. At that time, each nymph secreted filamentous wax to form a cocoon in which it overwintered (Fig. 2D). The wax cocoons were oval-shaped, 2-3 mm long and 1 mm wide, with a hole in one end. These cocoons were constructed from hollow wax filaments with longitudinal ridges on their internal surfaces that were secreted by the tubular ducts (Fig. 2E).

The body of 3rd instar females was oval, light brown in color, and approx. 1.8 mm long and 1.2 mm wide. Greater quantities of wax were observed on the dorsum of the 3rd instar body than were observed on the previous two instars. The trilocular pores produced filamentous wax that curled after emerging from the openings of the pores on the body surface (Figs. 3A, 3B). The cerari had 4-9 trilocular pores more than observed in earlier nymphal stages, and the loculi were enlarged with openings that were figure-8 shaped and arranged in spiral pattern. The tubular ducts were 3.6 μm (range 2.8-3.9 μm) diam; most did not secrete wax during this nymphal stage, thus, no wax was observed at their openings (Fig. 3C). At the posterior end of the mealybug, there were several thick and straight wax sticks that were wrapped by many thin wax filaments (0.2-0.3 μm diam) secreted by the anal ring and the anal ring setae (Figs. 3D,3E). The quinquelocular disc-pores distributed on the ventral surface were 6.3 μm (range 5.4-7.2 μm) in diam with a 2.0 μm (1.7-2.3 μm) thick rim in which each loculi was 0.9 μm (range 0.8-1.1 μm) in diam. Wax filaments were observed being secreted from these structures (Fig. 3F).

Female adult characteristics. The body of adult female had distinct segments, was reddish-brown in color, oval in shape, and approx. 6.0 mm long and 3.6 mm wide. A thin layer of white wax covered the dorsal surface, whereas only a small amount of wax was observed between adjacent segments. There were 18 pairs of cerari, each with about 10 trilocular pores. The number of trilocular pores (2560; range 2190-2894) and tubular ducts (413; range 368-459) was greater than the number observed on earlier nymphal stages. Trilocular pores were scattered on the dorsal surface, whereas tubular ducts were observed on the submarginal areas, head, mesothorax, and terminal segments. Wax on the dorsal surface was secreted mainly by the trilocular pores, whereas the tubular ducts did not secrete any waxy substances until the female adults began to oviposit. Three types of dermal glands were observed on the ventral surface, including the quinquelocular disc-pores on the head and thorax that secreted filamentous wax, and the multilocular disc-pores on the abdomen, particularly in the vulva area, that produced small, curly-shaped wax filaments that appeared to prevent conglutination of the eggs. Tubular ducts were scattered between the previous two glands. When the female adults prepared to oviposit, the tubular ducts secreted long, hollow wax filaments to construct the ovisac (Figs. 4A, 4B). Tubular ducts could be seen only as a 2-layered spherical opening on the surface of body; the tube structure extending from the opening on the surface into the interior of the body which could only be seen by dissection (Fig. 4C). Each tubular duct was composed of an outer ductile (9.6-14.0 μm long, 3.2-4.5 μm diam) and an inner ductile (5.5-8.2 μm long, 1.3-2.1 μm diam) (Fig. 4D). The hollow wax filaments produced by these tubular ducts, corresponding with distribution of tubular ducts on the body surface, were used to form the ovisac which first appeared on the middorsal area of the thorax and the anterior margin of the prothorax and head (Fig. 4E). Eventually the long ovisac was formed over the abdomen leaving the prothorax exposed (Fig. 4F).

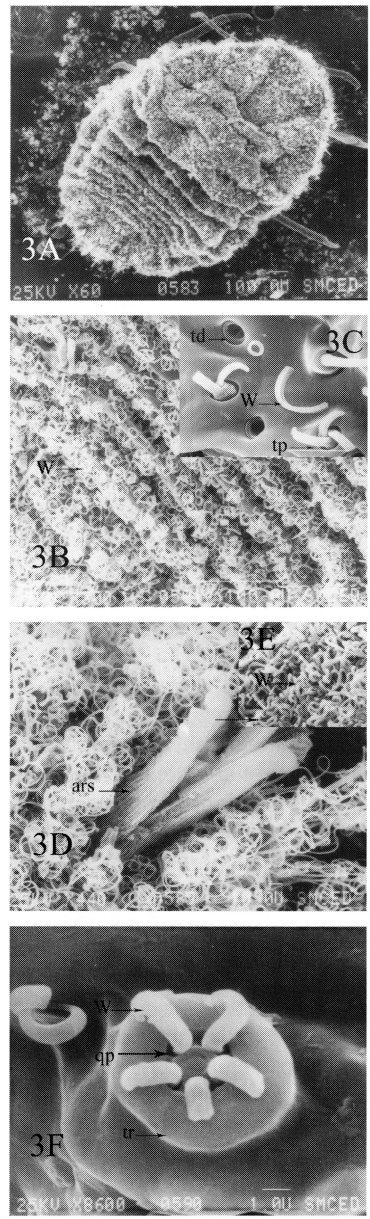

Fig. 3. Magnified views of the 3rd-instar nymph of *P. fraxinus* showing (A) the dorsum [60X], (B) filamentous wax (w) curled over the dorsum [300X], (C) wax filaments (w) secreted by trilocular pores (tp) and the 2-layered opening of the tubular ducts (td) [2600X], (D) anal ring setae (ars) [440X], (E) anal ring setae and associated wax (w) [6600X], and (F) a quinquelocular pore (qp) with thick rim and secreting waxy filaments (w) [8600X]

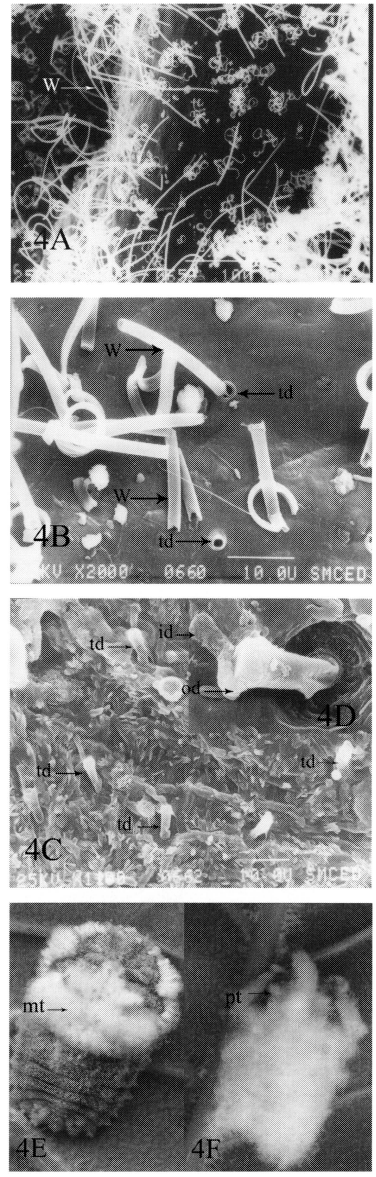

Fig. 4. Details of waxy secretions that comprise the ovisac produced by *P. fraxinus* adult females. (A) long wax filaments (w) constituting the ovisac [300X], (B) hollow structure of the wax filaments (w) secreted by tubular ducts (td) comprising the ovisac [2000X], (C) view of tubular ducts (td) on the inner surface of the integument [1100X], (D) outer and inner ductiles (od and id) of the tubular duct [6000X], and development of the ovisac showing wax over (E) the mesothorax and (F) the entire body except the prothorax

Male pupal and adult characteristics. The male mealybugs spent their prepupal and pupal stages in the wax cocoons constructed at the end of the 2nd instar stage. Therefore, there were only a few wax pores on their bodies with little production and accumulation of wax. The adult male was yellowish-brown in color, approx. 3.5 mm long, and had a distinct head, thorax, and abdominal body regions. The antennae were moniliform with many hairlike sensory setae. There were 3 pairs of simple eyes located on the dorsal, ventral and lateral surfaces of the head. Legs were well-developed with many setae. The anterior wings were semitransparent, whereas the posterior wing was vestigial and was termed a hamulohalter. Four long, straight wax filaments, 52 μm diam (range 45-60 μm), were observed at the posterior end of body (Fig. 5A). These wax filaments were secreted by 2 pairs of pore clusters, with each cluster consisting of 28-50 pores on the 7th segment and 62-83 pores on 8th segment. The quinquelocular disc-pores observed on the abdominal segments were arranged in longitudinal lines resulting in 2 lines of wax occurring on the ventral surface (Fig. 5B).

Discussion

Trilocular pores have been observed on mealybugs from 2 Coccoidea families—Coccidae and Pseudococcidae. In the Ceroplastes (Coccidae), 2 types of trilocular pores have been reported—one with the 3 loculi arranged in a triangle and the other with the 3 loculi arranged parallel to each other (Xie et al. 2006). However, in

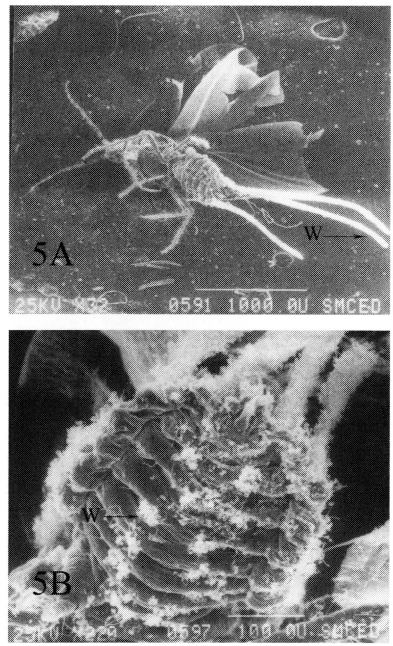

Fig. 5. Wax filaments (w) produced by *P. fraxinus* males (A) and the waxy secretions (w) of the quinquelocular pores on the ventral surface (B)

Pseudococcide, trilocular pores are characteristic of a large number of species. Our results show that the trilocular pores of *P. fraxinus* have different characteristics in size, number and distribution in relation to developmental stages. The openings of the trilocular pores of the 1st instars are small, irregular, and narrow seams from which wax filaments are secreted. However, in the 2nd instars, these pores were flat openings. In the 3rd instars and in adult females, the openings of the trilocular pores were larger and shaped as figure-8's. The narrow portion of the loculi created a middle marking or indentation on the wax filaments. The number of trilocular pores increased markedly with developmental stages resulting in increased amounts of wax production and accumulation on the insect's body.

Quinquilocular pores were commonly observed on the cuticle. These are generally distributed around the spiracles and along the spiracular furrows in most scale insects. Waxy substances secreted by these pores accumulated from the spiracle to the body margin and formed a channel for the scale insects for air exchange (Ben-Dov and Hodgson 1997). But, on *Dactylopius confuses* (Cockerell), quinquilocular pores were observed on the dorsal and ventral surfaces of the body (Foldi and Cassier 1985), whereas on *Pseudopulvinaria sikkimensis* Atkinson, quinquilocular pores are an exclusive type of gland distributed on the dorsal surface of the body, with a thick wax covering the body secreted by them (Ben-Dov and Hodgson 1997). However, the quinquilocular pores of 1st instar *P. fraxinus* are distributed along the submarginal area of the ventral surface in a line. In the 2nd and 3rd instars, quinquilocular pores were scattered on the ventral surface and on the abdominal segments. These quinquilocular pores were also characterized by a thick rim around the pore which differed from other previously studied species.

Tubular ducts were hollow pillar structures, which could not be seen by observing from the exterior surface of body with SEM because only the opening could be observed from the outer surface (Foldi and Lambdin 1995). We separated the dorsal cuticle and reversed it to observe internally, thereby locating and observing the tubular ducts that extended from integument through the cuticle and into interior portions of the body. These were composed of outer and inner ductiles; this double-layered structure forms long waxy filaments that are shaped like hollow tubes.

The tubular ducts produced and secreted wax only at two distinct times during its annual generation time—one occurring in late autumn that was secreted by the older second instars for cocoon production for overwintering, the other occurring in early summer that was secreted by adult females to produce ovisacs. The hollow structure of wax filaments produced by these ducts not only strengthened the cocoon and ovisac structures, but also did so with a decreased amount of wax. Kumar et al. (1997) indicated that the hollow, sticky filamentous wax in the cocoons and ovisacs of *Maconellicoccus hirsutus* Green are important in these structures. Certainly, they are also important in the construction of the cocoons and ovisacs of *P. fraxinus.* The sticky surface of these filaments also aids in attaching the ovisacs to environmental surfaces.

Our observations from this microscopic study have characterized the type, structure and number of dermal pores that produce and secrete waxy substances on the bodies of the various developmental stages of *P. fraxinus*, an important mealybug pest of forest trees in northern China. The structure and function of the waxy filaments produced differ with developmental stage and by the type of pore producing and secreting the wax. Whereas most of the wax produced by these pores serves as a protective covering for the insect, the wax produced by the tubular ducts is used to

construct structures in which either the insect overwinters or the female oviposits her eggs. Further knowledge of the function of these pores may prove helpful in the management of this serious pest.

Acknowledgment

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