External morphology and developmental changes of tarsal tips and mouthparts of the invasive spotted lanternfly, *Lycorma delicatula*

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Introduction and Objectives

External structures of insects contribute to the ability of herbivores to select and feed on their host plants. The invasive spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae) is an economically important and polyphagous insect pest in the eastern US which causes substantial damage to many woody plants. Lanternfly nymphs switch host plants during their development. However, little is known about relationship between the lanternfly and its plant hosts, and particularly about morphological adaptations of the lanternfly to host plant usage at each developmental stage of the pest. In this study, ***we focused*** on assessing changes in morphology of (a) **the lanternfly mouthparts** (stylets and labium), and (b) **the lanternfly tarsal tips** (arolia and tarsal claws) at each developmental stage, using both scanning electron microscopy and morphometric analysis. ***We hypothesized*** that the lanternfly mouthparts and tarsal tips would be highly adapted to various host trees and to undergo structural changes throughout the development.

Methods

We collected about 50 insect individuals (approx. 10 individuals per developmental stage) from 13 different host trees at three locations in Berks County, PA in July 2018. The insects were immediately preserved in ethanol. The mouthparts and tarsi were isolated under the dissecting microscope. Then, isolated mouthparts and tarsi were transferred to a fixation solution for scanning electron microscopy.

Tissue fixation was done using modified hexamethyldisilizane drying technique. The morphology of the stylets and tarsal tips were investigated, size measurements were taken (Fig. 1), and all the morphological structures were photographed.

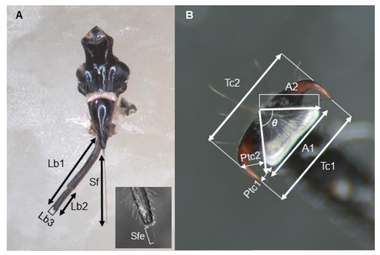


Fig 1. Morphometric characteristics measured for the labium, the stylet fascicle and the tarsal tip of *Lycorma delicatula*. (A) Labium and stylets. Lb1, distance from the labial tip to the base of the first labial segment; Lb2, distance from the labial tip to the base of the last labial segment; Lb3, maximum width of the last labial segment; Sf, distance from the tip of the stylet fascicle to the base of the stylets; Sfe, distance from the apex of stylet fascicle extended from labial tip to the labial tip. (B) Tarsal tip, the dorsal view. Tc1, distance between tarsal claw tips; Tc2, distance between bending centers of the external arcs of the tarsal claws; Ptc1, distance between the lateral margin of the arolium and tarsal claw tips; Ptc2, distance between the lateral margin of the arolium and bending centers of the external arcs of the tarsal claws; A1, the maximum anterior width of the arolium; A2, length of the lateral margin of the arolium; θ, the angle between the lateral margins of the arolium.

Results

The labium consists of four segments in 1-4th nymphal instars and five segments in adults; the third segment is the longest one in instars, and the fourth one is the longest labial segment in adults (Fig. 2).

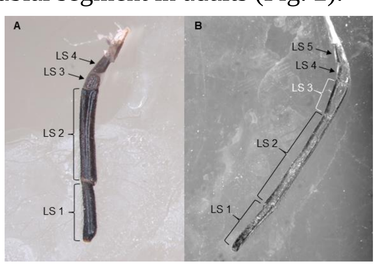


Fig 2. Labium and labial segments of *Lycorma delicatula*. (A) 4th instar nymph; (B) Adult male. LS1-LS5, labial segments; LS3 in adults (white) is the 5th (extra) segment.

Results (cont.)

Mouthparts: Labium + Mandibular Stylets

At each developmental stage, the tip of the labium is divided into two lobes by the labial groove: each lobe carries one ventral and one dorsal sensory field which have numerous sensilla. Six types of sensilla are surrounded by cuticular processes (Fig.3). The length of labium and stylets exponentially increases by 4th-instar nymph and the adult stage (Fig. 4).

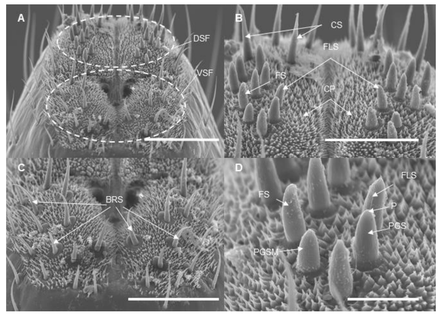


Fig 3. SEM of the labial tip of *Lycorma delicatula* (Third instar nymph). (A) Sensory fields. DSF, dorsal sensory field; VSF, ventral sensory field. (B) and (D) Dorsal sensory field. CS, clavate sensilla; FS, forticate sensilla, FLS, finger-like sensilla; CP, cuticular process; PGSM, multiporous peg sensilla; PGS, peg sensilla; P, pore.. Bars: (A) = 50 µm; (B) = 30 µm; (C) = 40 µm; (D) = 10 µm.

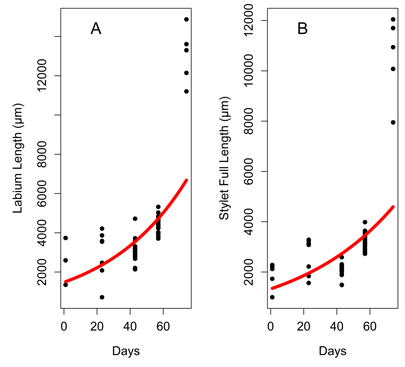


Fig 4. Growth curves for the labium and stylet length during the lanternfly development. (A) Labium length changes, exponential model ($y = 1465.57e^{0.02x}$, $R^2 = 0.49$). (B) Stylet length changes, exponential model ($y = 1326.1e^{0.01x}$, $R^2 = 0.43$). Axis labels: Day, days of the lanternfly development; day 0, hatching of the 1st nymphal instar; day 74, appearance of the adults

Tarsal Tip: Tarsal Claws + Arolium

Each tarsal tip in the spotted lanternfly carry two tarsal claws and an arolium. We observed that in adults the tarsal claws are more spread out than that in the nymphal instars; in the early instars (1st and 2nd) the claws are located very close to the arolium, then the distance between tarsal claws exponentially increases by the adult stage, (Fig. 6 and 7).

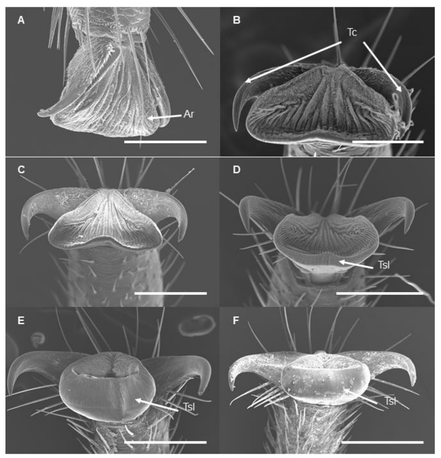


Fig 6. SEM of the tarsal tips of the forelegs of *Lycorma delicatula* at each developmental stage. (A) First instar nymph. (B) Second instar nymph. (C) Third instar nymph. (D) Fourth instar nymph. (E) Adult female. (F) Adult male. Ar, arolium; Tc, tarsal claw; Tsl, terminal sticky lip. Bars: (A) and (B) = 100 µm; (C) = 200 µm; (D) = 300 µm, (E) and (F) = 400 µm.

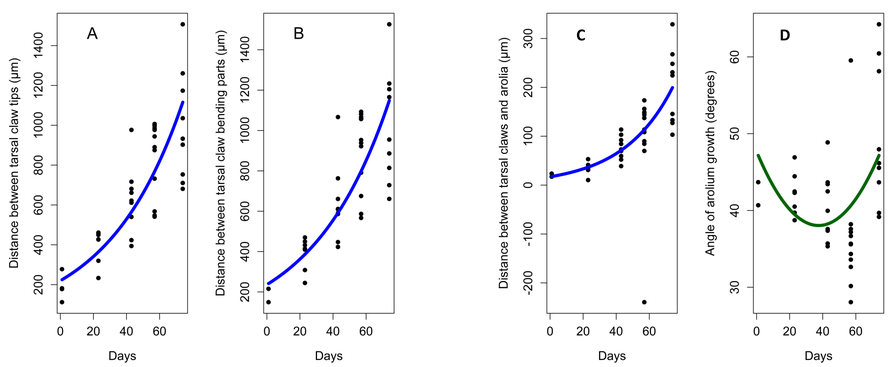


Fig 7. Growth curves for distance between tarsal claws and arolium, and the angle of the arolium growth during the lanternfly development. (A) Distance between tarsal claw tips, exponential model ($y = 219.2e^{0.02x}$, $R^2 = 0.72$). (B) Distance between bending centers of the external arcs of the tarsal claws, exponential model ($y = 235.09e^{0.02x}$, $R^2 = 0.69$). (C) Distance between tarsal claws and arolium, exponential model ($y = 17.11e^{0.03x}$, $R^2 = 0.76$). (D) Angle of the arolium growth, quadratic model ($y = 47.67 – 0.51x + 0.007\,x^2$, $R^2 = 0.16$). Axis labels: Day, days of the lanternfly development; day 0, hatching of the 1st nymphal instar; day 74, appearance of the adults.

At each developmental stage, tips of mandibular stylets possess four oval prominences and several longitudinal striations (in 4th nymphal instars and adults only) on the outer surface; while the inner surface of the stylets is smooth (Fig. 5).

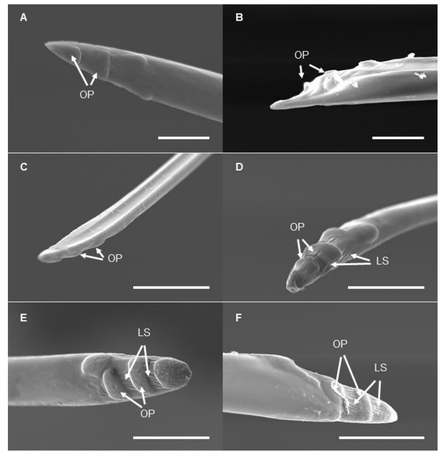


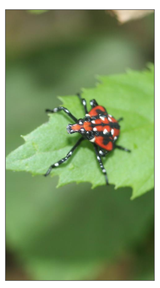
Fig 5. SEM of the mandibular stylets of *Lycorma delicatula* at each developmental stage. (A) First instar nymph. (B) Second instar nymph. (C) Third instar nymph. (D) Fourth instar nymph. (E) Adult female. (F) Adult male. OP, oval prominences; LS, longitudinal striations. Bars: (A) and (B) = 10 µm; (C) and (D) = 30 µm, (E) and (F) = 50 µm.

Discussion and Conclusions

Our study revealed several interesting **developmental patterns** which potentially allow *L. delicatula* to better attach to a host plant and deeper penetrate to the host plant tissues at the late nymphal stages and adult stage:

- The labium in adults consists of five segments whereas the nymphs have four labial segments;
- The labium and stylet length, as well as the tarsal claw dispersal from the arolium, exponentially increase by 4th - instar nymph and adult stage;
- Mandibular stylets possess four indentations on the outer surface of the stylet apical part which become more evident in 4th - instar nymphs and adult;
- Longitudinal striations between indentations are present on mandibular stylets of 4th-instar nymphs and adults;
- Arolium surface becomes wrinkled in late instars and adults;
- Changes in the angle of arolium growth follow the quadratic growth curve; the angle becomes obtuse in adults which potentially causes increase of the arolia basal width.

Additionally, we have found six morphological types of sensilla which are present at the labial tip at each developmental stage of *L. delicatula*; which potentially indicates the lanternfly ability to effectively explore the host plant suitability at each developmental stage. We suggest that observed patterns are adaptations to host plant usage and correspond with a type of host plants utilized at each developmental stage.



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