Head Capsule Widths of Nymphal Instars of the Cotton Fleahopper¹

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Reinhard (1926) provided guidelines for distinguishing the five nymphal instars of the cotton flea hopper, *Pseudatomoscelis seriatus* (Reuter), based on the shape and relative size of wing pads. While training student workers to distinguish instars, several inexperienced observers had difficulty discerning the wing pads on earlier instars, particularly on nymphs that had recently molted or were about to molt. Consequently, these individuals often could not confidently determine the instars of nymphs. Reinhard (1926) reported the average head capsule width for each instar as an additional guide, but did not provide ranges or estimates of variation within instars. Reported herein are the means (± S.D.) and ranges of head capsule widths within instars observed for laboratory-reared and field-collected nymphs, and comments regarding the use of head capsule widths to distinguish instars.

Laboratory-reared nymphs originated from diapausing eggs in woolly croton plants, *Croton capitatus* Michaux., that were collected in Burleson Co., TX, during February through March of 2006. To obtain nymphs, plants infested with diapausing flea hopper eggs were submerged in water for 15-30 min every other day and were held at 29.4 ± 1°C and a photoperiod of 13:11 (L:D) h to terminate egg diapause (Breene et al. 1989). Newly eclosed nymphs (<18-h-old) were placed individually in 20-ml glass vials capped with nylon organdy cloth, and each nymph was provisioned with a 3- to 4-cm long section of green bean, *Phaseolus* spp. The ends of each green bean section were previously dipped in low melting point parafilm wax to prevent nymphs from crawling inside the sections and to secure the bean sections to the bottom of the vials in an upright position. Nymphs were held at 29.4 ± 1°C and a photoperiod of 13:11 (L:D) h. At least twice daily, vials were examined under a dissecting microscope for the presence of exuviae (indication of molt). When an exuvia was detected, the exuvia was removed and the respective green bean section was replaced. Each nymph was tentatively assigned an instar based on the number of molts. When the desired instar was attained, respective nymphs were killed with ethyl acetate to assist in the measurement of head capsule widths. Prior to measurement, wing pads of each nymph were examined to confirm instar. On rare occasions, the number of molts and wing pad characteristics did not indicate the same instar. In such cases, instar classification was based on wing pad characteristics. For each instar, 25 nymphs were measured <18 h after molting (or hatching in the case of first instars), and a different set of 25 nymphs were measured between 18 and 36 h after molting.

Field-collected nymphs were obtained from horsemint plants, *Monarda* spp., in Brazos Co., TX, during 2 May through 12 June 2006, and from cotton, *Gossypium hirsutum* L., in Burleson Co., TX, during 8 through 26 June 2006. On each day of

¹ Hemiptera: Miridae
collection, portions of plants infested with fleahopper nymphs were clipped, placed in plastic sealable bags, and returned to a laboratory. Plant materials were vigorously shaken over a white collection bin and dislodged nymphs were collected with an aspirator. Representatives of all five instars were usually obtained from both plant sources on each collection date. Collected nymphs were killed with ethyl acetate, and the instar of each nymph was determined (by the author) based on wing pad characteristics. Sixty nymphs of each instar from horsemint, and 51 to 60 nymphs of each instar from cotton, were measured.

Head capsule widths of nymphs were determined at a magnification of 40x using a dissecting microscope equipped with an ocular micrometer (0.025-mm divisions @ 40x, Model GSWH10x-H-2, Olympus America, Melville, NY). Head capsule width was determined by placing the nymph on its back and measuring the distance from the outside margin of one eye to the outside margin of the other eye. Each nymph was measured twice by different individuals to the nearest half-division mark, and the average was recorded. Head capsule widths were compared among nymph sources (greenbeans, horsemint, and cotton) and instars using a two-way ANOVA (PROC GLM, SAS Institute 2002). Differences among pair-wise treatment comparisons were identified using the ADJUST = TUKEY option of the LSMEANS statement (SAS Institute 2002). To assess the reliability of using head capsule widths to distinguish instars, data were pooled across nymph sources to establish a robust range of head capsule widths for each instar, and the ranges among instars were examined for overlap.

Head capsule widths differed significantly among nymph sources ($F=194.46; df=2, 804; P<0.001$) and instars ($F=16,269.50; df=4, 804; P<0.001$). However, the source-by-instar interaction indicated differences between sources varied among instars ($F=23.34; df=8, 804; P<0.001$). In general, nymphs collected from horsemint plants tended to have the greatest head capsule widths, while nymphs collected from cotton and those reared on green beans usually possessed similar head capsule widths (Table 1). On average, head capsule widths of first, second, third, fourth, and fifth instars were 0.26, 0.34, 0.42, 0.51, and 0.60 mm, respectively. While differences in mean head capsule widths were detected among nymph sources, ranges of head capsule widths for each instar appeared to be similar among sources. Also, no overlap of ranges was observed among instars within each source (Table 1). When data were pooled across nymph sources, head capsule widths of first instars ranged from 0.200 to 0.280 mm, second instars were between 0.313 and 0.375 mm, third instars were between 0.381 and 0.463 mm, fourth instars were between 0.450 and 0.569 mm, and fifth instars were between 0.550 and 0.669 mm. Although there was a slight overlap of ranges between those of third and fourth instars and between those of fourth and fifth instars, tips of wing pads on fourth instars extend to the second abdominal segment whereas those of fifth instars extend to the fourth or fifth abdominal segment. Consequently, wing pad characteristics can be used to easily distinguish these instars from each other and from third instars. In contrast, distinctions between wing pad characteristics of first, second, and third instars are rather subtle and wing pads on these earlier instars can be difficult to discern, particularly on nymphs that have recently molted or are about to molt. As such, inexperienced observers may require additional guidelines to accurately distinguish these instars. Given that the overall ranges of head capsule widths observed for first, second, and third instars did not overlap, the respective ranges reported herein should provide inexperienced observers additional guidelines for distinguishing the earlier nymphal instars of the cotton fleahopper.
TABLE 1. Mean ± SD and Range of Head Capsule Widths of Cotton Fleaheopper Nymphs Reared on Green Beans, or Field-Collected from Horsemint and Cotton Plants.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Mean ± SD (mm)</th>
<th>Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green bean</td>
<td>Horsemint</td>
</tr>
<tr>
<td>1</td>
<td>0.263 ± 0.015</td>
<td>aA</td>
</tr>
<tr>
<td>2</td>
<td>0.340 ± 0.009</td>
<td>bAB</td>
</tr>
<tr>
<td>3</td>
<td>0.414 ± 0.013</td>
<td>cA</td>
</tr>
<tr>
<td>4</td>
<td>0.500 ± 0.015</td>
<td>dA</td>
</tr>
<tr>
<td>5</td>
<td>0.589 ± 0.015</td>
<td>eA</td>
</tr>
</tbody>
</table>

Means within a column followed by different lower case letters, and values within a row followed by different capital letters, are significantly different (α = 0.05, Tukey-Kramer test).

- Nymphs originated from diapausing eggs in woolly croton plants, and were reared at 29.4 ± 1°C and a photoperiod of 13:11 (L:D) h. Fifty nymphs of each instar were measured.
- Nymphs were collected from blooming horsemint plants in Brazos Co., TX, during 2 May through 12 June 2006. Sixty nymphs of each instar were measured.
- Nymphs were collected from fruiting cotton plants in Burleson Co., TX, during 8 through 26 June 2006. Fifty-one to 60 nymphs of each instar were measured.
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References Cited