DISTINGUISHING LARVAL INSTARS OF THE VEGETABLE LEAFMINER, *LIRIOMYZA SATIVAE* (DIPTERA: AGROMYZIDAE)

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ABSTRACT

Measurements of either the length of mouth hooks or the cephalopharyngeal skeleton showed clear separation between larval instars of *Liriomyza sativae* Blanchard. Mouth hooks of first instar larvae also differed morphologically from those of later instars. Larval instars were distinguished under a dissecting microscope at 200x magnification, enabling rapid separation of freshly-collected specimens.

The growth ratio of cephalopharyngeal skeletons between first and second instar larvae was higher (1.75) than between second and third instar larvae (1.56). Growth ratios calculated from published reports on other *Liriomyza* species were typically very similar to those of *L. sativae.*

RESUMEN

Medidas del largo de los ganchos bucales o del esqueleto cefalofaringeal demostró una clara separación entre los estadios larvales de *Liriomyza sativa* Blanchard. Los ganchos bucales también fueron distintos morfologicamente de aquellos en los estadios siguientes. Los estadios larvales se separaron bajo un microscopio de disección a una magnificación de 200X, lo que permitió separar rápidamente los especimen recién colectados.

La taza de crecimiento de los esqueletos cefalofaringeales entre el primer y segundo estadio, fue mayor (1.75) que entre el segundo o tercer estadio larval (1.56). La taza de crecimiento calculada en reportes publicados sobre otras especies de *Liriomyza* fue típicamente muy similar a aquellos de *L. sativae.*

Liriomyza leafminers cause extensive damage to a variety of economically important vegetable and ornamental crops (Parrella & Keil 1984, Minkenberg & van Lenteren 1986). Consequently, they are major targets of both chemical (Parrella et al. 1982, Mason et al. 1987, Leibee 1988) and biological control programs (Frijters et al. 1986, Westerman & Minkenberg 1986, Parrella et al. 1987). These programs would likely benefit from more detailed physiological or ecological studies conducted with particular larval instars. Larval instars of cyclorrhaphous Diptera have shown differences in their ability to escape parasitism and encapsulate parasitoid eggs (van Alphen & Drijver 1982), and in their susceptibility to pesticides (Parrella et al. 1982).

Larval instars of cyclorraphous Diptera are generally distinguished on the basis of the size and/or morphology of the cephalopharyngeal skeleton, which is characteristic in this group (Teskey 1981). The cephalopharyngeal skeleton consists of the mouth hooks and the hypopharyngeal and tentoropharyngeal sclerites (Teskey 1981). The size and/or morphology of mouth hooks (Bodenstein 1950, Kamali & Schulz 1973, Robinson & Foote 1978, Lawrence 1979), and/or the length of the entire cephalopharyngeal skeleton (Beri 1974, Ipe 1974, Hendrickson & Barth 1978, Lawrence 1979) have been used to distinguish larval instars.

In this study, larval instars of *Liriomyza sativae* Blanchard were clearly distinguished using measurements of mouth hook or cephalopharyngeal skeleton length on slide-mounted larvae using a compound microscope. The measurements were used also

to develop a method to distinguish instars using a dissecting microscope equipped with an ocular micrometer. Being able to conduct instar determination under a dissecting microscope is desirable because it considerably reduces processing time for samples containing many larvae.

Growth ratios of *L. sativae* mouth hooks and cephalopharyngeal skeletons were compared both across instars and with other *Liriomyza* species. Constant growth ratios were expected within a species according to the Brooks-Dyar rule of geometric growth (Hutchinson & Tongring 1984, Daly 1985).

Materials and Methods

Experimental Procedure

Bush lima beans (*Phaseolus limensis* var. *limenanus* Bailey 'Henderson') were grown in a greenhouse in 15 cm diameter pots at two plants per pot in a steam-pasteurized peat-vermiculite mixture (Speedling Inc., Sun City, FL) and watered with a modified Hoaland solution (Petitt 1988). Plants 10-14 d old were placed for 6 h in a 1.9 x 1.1 x 0.6 m cage containing a colony of adult *L. sativae*. Thereafter, plants were held in environmental chambers at 20 or 25 ± 1°C and a 14 h photophase beginning at 0700 hours.

Larvae were collected from primary leaves every 6 and 12 h at 25 and 20°C, respectively, beginning the third day after oviposition and preserved in a 70% ethanol:water mixture. Larvae were cleared in a 10% solution of potassium hydroxide in 70% ethanol for 2-12 h, depending on larval size. The lengths of mouth hooks and cephalopharyngeal skeletons (hypopharyngeal and tentoropharyngeal sclerites only) of 560 larvae were measured on a compound microscope at 400x using an ocular micrometer (Fig. 2). Measurements were made to the nearest micrometer unit which was equal to 2.5μm. Length differences between the smallest mouth hook or cephalopharyngeal skeleton measured for an instar and the largest of the preceding instar were used to determine the magnification necessary for distinguishing instars using a dissecting microscope. Two hundred larvae, some of which were from each stadium, were classified using a dissecting microscope with an ocular micrometer to test the method and to determine the time required for sample processing.

Data Analysis

The natural logarithms of the length of mouth hooks and the cephalopharyngeal skeleton were regressed against presumed instar number (independent variable) to confirm that no instars were missed (Daly 1985). Growth ratios were calculated by dividing the size of a structure in an instar by the size of the equivalent structure in the preceding instar.

Voucher Specimens

Specimens of adult *L. sativae* were sent to K. A. Spencer (Univ. of Exeter, Exwell Farm, Bray Shop, Callington, PL17, 8QJ, Cornwall, United Kingdom) and their correct identification was verified. Voucher specimens were deposited in the Florida State Collection of Arthropods in Gainesville, Florida.

Results and Discussion

Clear separation among the three larval instars of *L. sativae* occurred using either mouth hook or cephalopharyngeal skeleton length (Fig. 1; Table 1). No abrupt deviation

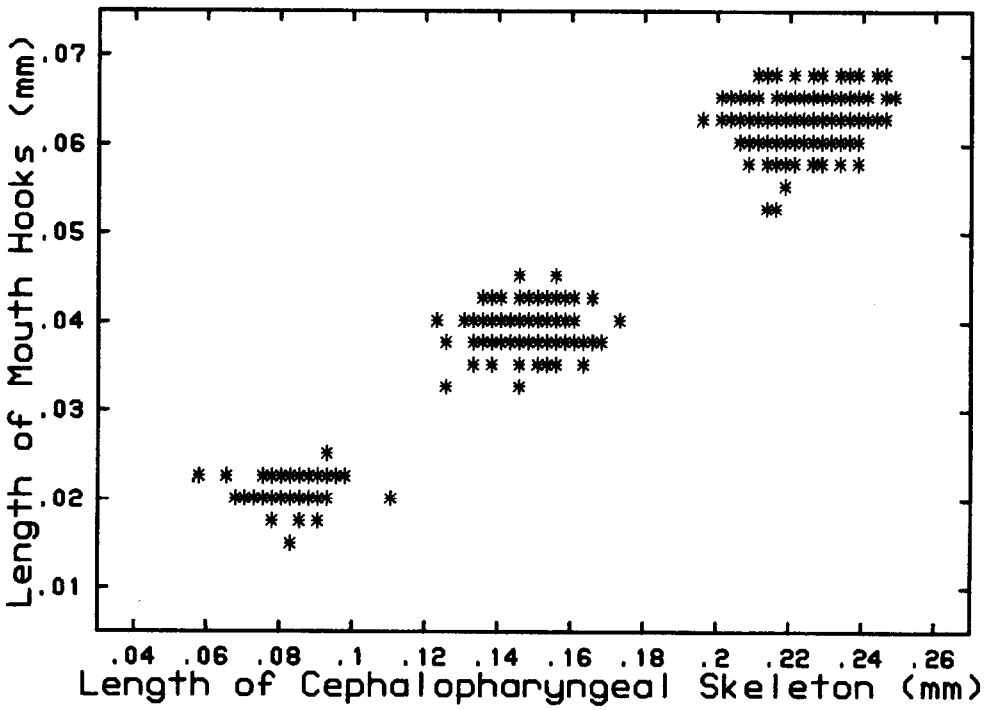

Fig. 1. Relationship between lengths of mouth hooks and cephalopharyngeal skeleton for first, second, and third instar *L. sativae* larvae.

occurred when presumed instar number was regressed with the natural logarithms of mouth hook (r = 0.99) and cephalopharyngeal skeleton (r = 0.99) length, indicating that no instar was missed (Daly 1985). The mean cephalopharyngeal skeleton lengths of first, second, and third instar *L. sativae* (0.09, 0.15, and 0.23 mm, respectively) were very similar to the mouth hook lengths reported by Oatman & Michelbacher (1958) for *L. pictella* Thomson. These authors apparently used the term "mouth hooks" to describe the cephalopharyngeal skeleton. *L. pictella* of Oatman & Michelbacher (1958) actually may have been *L. sativae* but the absence of voucher specimens makes positive identification impossible (see Spencer 1981).

The mouth hooks of second and third instar larvae are morphologically similar, but the first instar is recognizably different (Fig. 2). The second and third instar mouth hooks were less than one-half as wide as long (including teeth) in side view, and had two distinct pairs of alternating, ventrally curved, terminally-pointed teeth. The right side of third instar mouth hooks was longer than the left in front view, as is usual for

TABLE 1. LENGTH OF MOUTH HOOKS AND CEPHALOPHARYNGEAL SKELETON FOR LARVAL INSTARS OF *L. SATIVAE*.

		x̄ length (S.E.M.) (mm) and Range			
Instar	n	Mouth Hooks		Cephalopharyngeal Skeleton[a]	
1	133	0.021 (0.0001)	(0.015-0.025)	0.085 (0.0006)	(0.058-0.111)
2	193	0.039 (0.0002)	(0.033-0.045)	0.149 (0.0006)	(0.123-0.173)
3	234	0.063 (0.0002)	(0.053-0.068)	0.233 (0.0007)	(0.196-0.249)

[a]Cephalopharyngeal skeleton measurement included the hypopharyngeal and tentoropharyngeal sclerites.

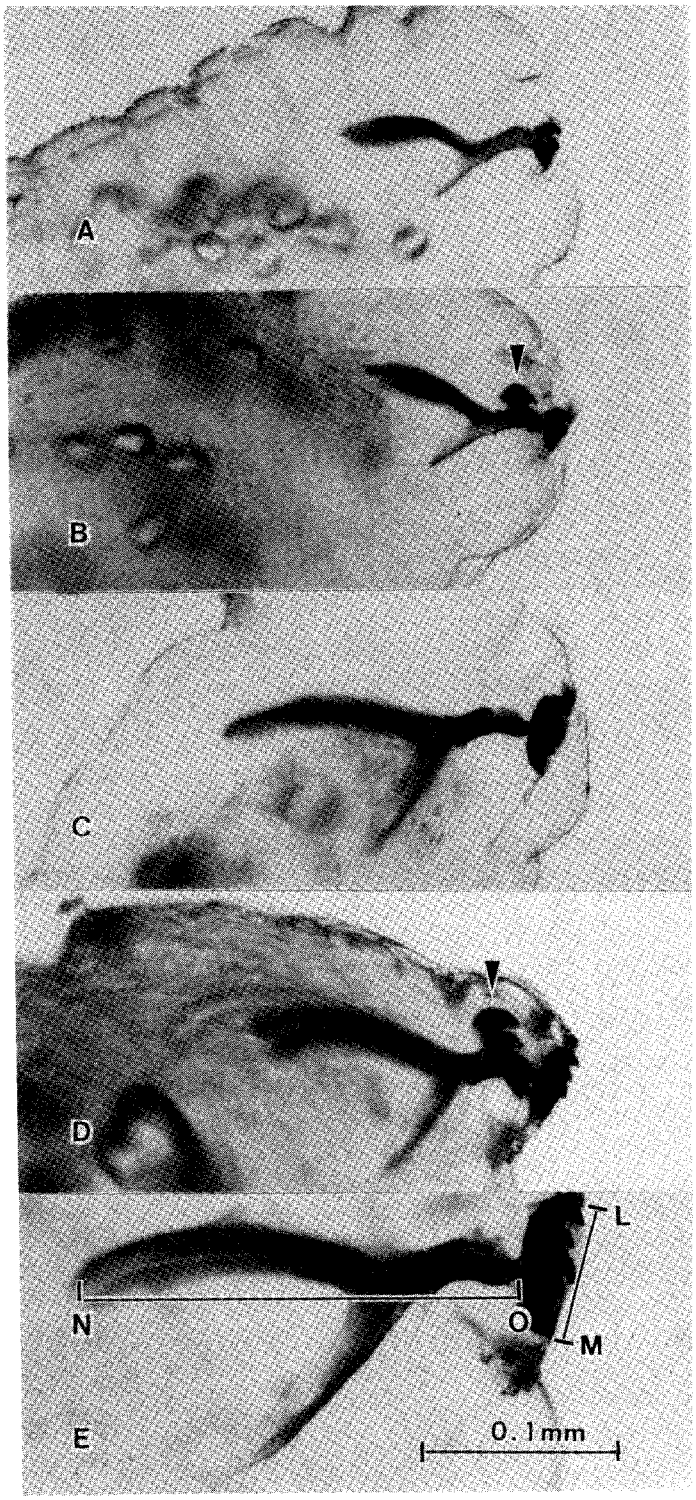

Fig. 2. Cephalopharyngeal skeleton including mouth hooks of first (A, B), second (C, D), and third (E) instar larvae of *L. sativae*. Partially formed mouth hooks (arrows) posterior to the functioning mouth hooks in B and D indicate the onset of ecdysis to the next instar. Length of mouth hooks (L-M), and length of the cephalopharyngeal skeleton (N-O), as measured in this study, are shown in E. The same magnification was used in A-E.

TABLE 2. GROWTH RATIOS OF THE LENGTH OF THE CEPHALOPHARYNGEAL SKELETON OF *L. SATIVAE* AS COMPARED WITH LARVAE OF OTHER *LIRIOMYZA* SPECIES.

Ratio of Instars	Ratio of Length of Cephalopharyngeal Skeleton			
	L. sativae	*L. pictella*[a]	*L. trifolii*[b]	L. brassicae[c]
2/1	1.75	1.72	1.75	1.98
3/2	1.56	1.55	1.55	1.56

[a]Oatman & Michelbacher 1958.
[b]Parrella & Bethke 1988.
[c]Beri 1974.

larval Agromyzidae (Beri 1983). The relative size of each mandible could not be distinguished in earlier instars. The first instar mouth hooks were one-half or more as wide as long, and only a pair of apical teeth was apparent. Occurrence of partially formed mouth hooks along the hypopharyngeal sclerite indicated the onset of ecdysis to the next instar (Fig. 2B and 2D).

As in other agromyzids (Beri 1973), first instar *L. sativae* apparently were metapneustic, having only the posterior pair of spiracles. The anterior spiracles of second and third instar *L. sativae* are stalked with numerous spiracular bulbs.

The growth ratio of cephalopharyngeal skeletons was 1.75 between first and second instar *L. sativae* and 1.56 between second and third instars. The ratios were consistent for the species *L. sativae, L. pictella, L. brassicae* (Riley) and *L. trifolii* (Burgess), with the exception that the ratio between first and second instars was larger in *L. brassicae* (1.98) than in the other species (Table 2; Oatman & Michelbacher 1958, Beri 1974, Parrella & Bethke 1988).

The average growth ratios between first and second and second and third instar *L. sativae* mouth hooks were 1.86 and 1.62, respectively. Growth ratios between second and third instar mouth hooks in *L. andryalae* Hering and *L. strigata* Meigen were each 1.60 (Beri 1971). The constancy of the growth ratios across species may be related to their similar mode of feeding on leaf mesophyll tissue (Hutchinson & Tongring 1984).

Athough measurements of the mouth hooks and cephalopharynqeal skeletons reported in this study were made on cleared, slide-mounted larvae under a compound microscope, 200 larvae also were classified by measurements of these structures under a dissecting microscope. At 200x total magnification, all instars could be distinguished by measuring either the length of the mouth hooks or cephalopharyngeal skeleton. Because the larvae became more opaque with time in ethanol, only recently collected larvae could be measured without clearing. Samples of 20 larvae required ca. 5 min to classify using this method.

Rapid instar determination under the dissecting microscope makes immediate processing of samples possible. Cohorts may be subsampled frequently, if necessary, to determine when all individuals have reached the desired stadium for use in experimentation. Availability of this method will enable studies to be conducted with particular larval instars of *L. sativae*.

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RESPONSES OF *HYLASTES SALEBROSUS* TO TURPENTINE, ETHANOL, AND PHEROMONES OF *DENDROCTONUS* (COLEOPTERA: SCOLYTIDAE)

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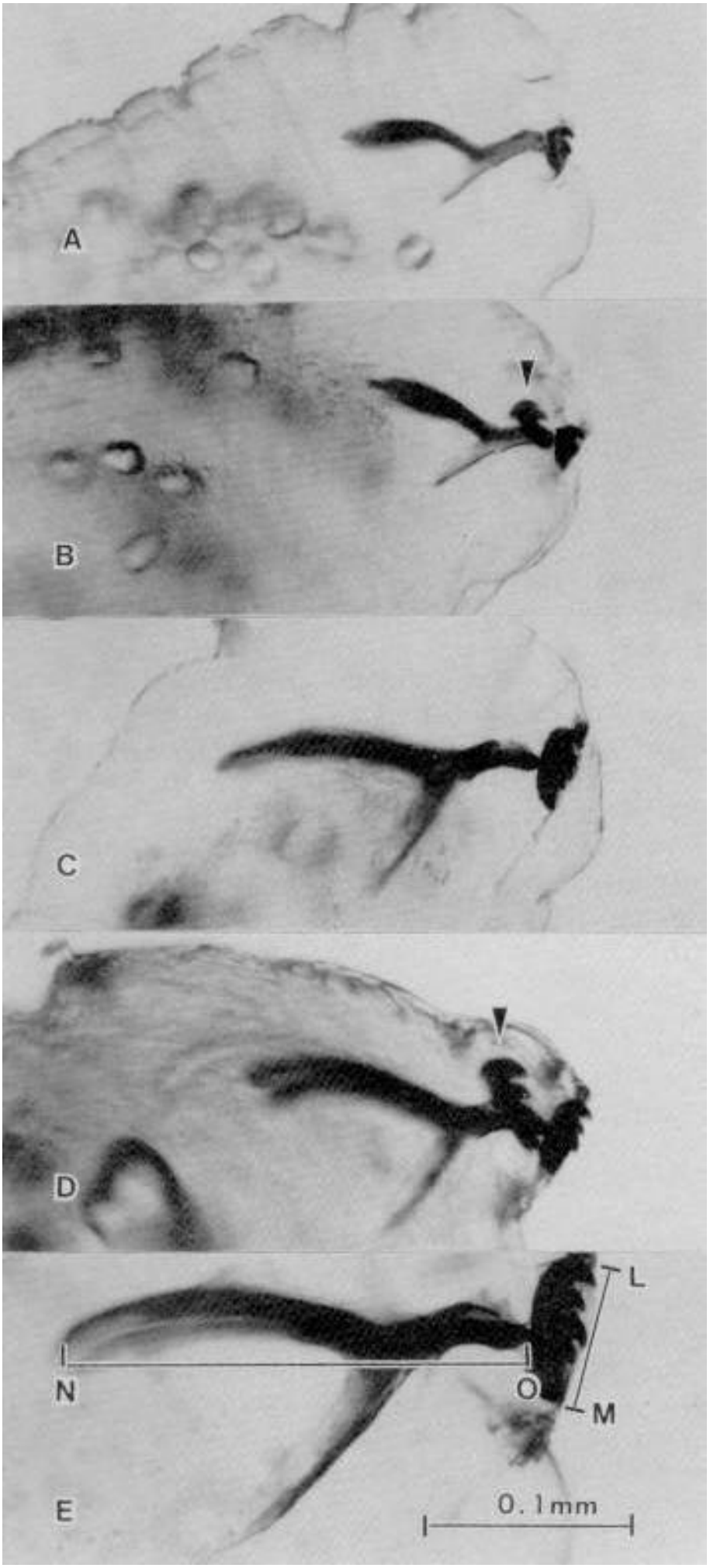
ABSTRACT

Attraction of the pine bark beetle *Hylastes salebrosus* Eichhoff to the host chemicals turpentine and ethanol, and the *Dendroctonus* pheromones frontalin and *exo*-brevicomin, were assessed in four field trapping experiments. *H. salebrosus* were attracted to turpentine alone, and the addition of ethanol, whether mixed with the turpentine or deployed at any of three different release levels, elicited a significant increase in attraction. In another experiment incorportaing all semiochemicals tested, beetles were more attracted to traps baited with the combination of the turpentine:ethanol mix, frontalin, and *exo*-brevicomin, than to traps with the turpentine:ethanol mix only. A final pair of experiments determined that *exo*-brevicomin, and not frontalin, deployed with turpentine was important for eliciting attraction. *H. salebrosus* may produce and use *exo*-brevicomin as an attractive pheromone, but the data also suggest that *H. salebrosus* can exploit *exo*-brevicomin as a kairomone from other scolytid species that colonize the same pine resource.

RESUMEN

Atracción del escarabajo descortezador de pino *Hylastes salebrosus* Eichhoff a las químicas producidas por el huésped, aguarraz y etanol, y a las feromonas de *Dendroctonus* frontalina y *exo*-brevicomina, se ensayó en cuatro experimentos de trampeo en el campo. *H. salebrosus* se atrayeron a aguarraz solo, y la adición de etanol, ya sea mezclado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