Phytoprotection

phytoprotection

Morphological determination of the larval instars of *Eupeodes americanus* (Diptera: Syrphidae)
Discrimination morphologique des stades larvaires d'*Eupeodes americanus* (Diptera : Syrphidae)

Arlette Fauteux, Noémie Gonzalez, António O. Soares and Éric Lucas

See table of contents

Explore this journal

Cite this article

Article abstract

In North America, the potential of the American hoverfly *Eupeodes americanus* (Wiedemann, 1830) (Diptera: Syrphidae) as a biocontrol agent has been demonstrated, particularly against the foxglove aphid *Aulacorthum solani* Kaltenbach, 1843 (Hemiptera: Aphididae). Since no information is available to distinguish the larval instars of this species, the present study used the semi-continuous observation (time-lapse photography and stereo microscope) of the three larval stages to build a table of morphological traits and a dichotomic key for discriminating the larval instars by observation under stereo microscope. Discriminating traits are black hairs at first instar and fused posterior breathing tubes at third instar.

érudit

Morphological determination of the larval instars of *Eupeodes americanus* (Diptera: Syrphidae)

Arlette Fauteux[1✉], Noémie Gonzalez[1], António O. Soares[2], and Éric Lucas[1]

In North America, the potential of the American hoverfly *Eupeodes americanus* (Wiedemann, 1830) (Diptera: Syrphidae) as a biocontrol agent has been demonstrated, particularly against the foxglove aphid *Aulacorthum solani* Kaltenbach, 1843 (Hemiptera: Aphididae). Since no information is available to distinguish the larval instars of this species, the present study used the semi-continuous observation (time-lapse photography and stereo microscope) of the three larval stages to build a table of morphological traits and a dichotomic key for discriminating the larval instars by observation under stereo microscope. Discriminating traits are black hairs at first instar and fused posterior breathing tubes at third instar.

Keywords: black hairs, moult, posterior breathing tubes, time-lapse photography, biocontrol.

[Discrimination morphologique des stades larvaires d'*Eupeodes americanus* (Diptera : Syrphidae)]

En Amérique du Nord, le potentiel du syrphe d'Amérique *Eupeodes americanus* (Wiedemann, 1830) (Diptera: Syrphidae) comme agent de lutte biologique a été démontré, notamment contre le puceron de la digitale *Aulacorthum solani* Kaltenbach, 1843 (Hemiptera: Aphididae). Aucune information n'étant disponible pour distinguer macroscopiquement les stades larvaires de cette espèce, la présente étude a utilisé l'observation semi-continue (photographie time-lapse et observation à la loupe binoculaire) des trois stades larvaires pour construire un tableau de traits morphologiques et une clé dichotomique pour discriminer les stades larvaires par observation à la loupe binoculaire. Les traits discriminants sont la présence de poils noirs au premier stade et la fusion des tubes respiratoires postérieurs au troisième stade.

Mots-clés : poils noirs, mue, tubes respiratoires postérieurs, photographie time-lapse, lutte biologique.

1. Laboratoire de lutte biologique, Département des sciences biologiques, Université du Québec à Montréal, 141 avenue du Président-Kennedy, Montréal (Québec), Canada H2X 3X8. ✉ fauteux.arlette@courrier.uqam.ca
2. cE3c-ABG – Centre for Ecology, Evolution and Environmental Changes and Azorean Biodiversity Group, Faculty of Sciences and Technology, University of the Azores, 9501-321 Ponta Delgada, Portugal

Hoverflies (Diptera: Syrphidae) have proven to play an important role in the ecosystem due to their dual services as pollinators (adult) and biological control agents (larvae) (Dunn *et al.* 2020). Since 2014, works from the Biocontrol laboratory of University of Quebec in Montreal (UQAM) on the American hoverfly, *Eupeodes americanus* (Wiedemann, 1830) (Diptera: Syrphidae), have shown that this species is able to be active at low temperatures (12-14-18 °C), e.g., for: (i) flight, (ii) oviposition, and (iii) feeding activities (Bellefeuille *et al.* 2017, 2019). Due to those characteristics, this species shows great potential as a biological agent, even when the temperature is low. *Eupeodes americanus* can feed on more than 25 aphid species (Rojo *et al.* 2003; Vockeroth 1992), including several major pests in Quebec greenhouses such as the green peach aphid *Myzus persicae* Sulzer, 1776 (Hemiptera: Aphididae), the foxglove aphid, *Aulacorthum solani* Kaltenbach, 1843 (Hemiptera: Aphididae), the pea aphid *Acyrthosiphon pisum* Harris, 1776 (Hemiptera: Aphididae) and the melon aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae).

While *E. americanus* appears to be a promising biological control agent, many biological traits remain to be explored such as its voracity, development, and hibernation habits. Regarding their morphological traits, only adults are well described, notably with identification keys (Skevington *et al.* 2009; Vockeroth 1992). However, for most hoverfly species, larval instars have not been differentiated in most of the earlier works. According to Joshi and Ballal (2013) and Rotheray and Gilbert (2011), this is probably due to the fact that exuviae are not easily visible by being very thin, transparent, and often crumpled and damaged. In Europe, the morphological description of syrphid larvae is more advanced with notably a key for the third instar larvae of most European genera (Láska *et al.* 2013) and a colour guide (Rotheray 1993). Some studies tend to describe all three larval instars, but they often only provide information on variable traits such as colour and body length (Bergh and Short 2008; Davidson 1919). The full development of the posterior breathing tubes is usually a fundamental criterion to identify the third instar larvae of Syrphidae (Hartley 1961; Rotheray and Gilbert 2011), but it has not been confirmed for *E. americanus*. Ouattara *et al.* (2022) showed that, like most hoverflies (Rotheray 1993; Skevington *et al.* 2019), *E. americanus* has three larval instars. However, the identification of the larval instars through morphological characters remains incomplete. This lack of knowledge is a barrier to further studies on this species. Notably, identification of instars is necessary to evaluate the syrphid's development or voracity since aspects like development time, mortality, or number of preys consumed vary between instars. This study aims to provide a dichotomous key of discriminating morphological traits to differentiate the three larval instars of *E. americanus*.

Insect rearing. Insect rearing was carried out at UQAM in the Biocontrol laboratory. The melon aphids, *A. gossypii*, were reared on cucumber, *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae), the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus, 1758), on barley plants, *Hordeum vulgare* L. (Poales: Poaceae), and the pea aphid, *A. pisum*, on broad bean plants *Vicia faba* L. (Fabales: Fabaceae) (25 °C, 16L:8D photoperiod, and 60% RH). American hoverfly, *Eupeodes americanus*, rearing was done as described in Bellefeuille *et al.* (2019). Adults were fed with pollen and sugared water and larvae were fed with *R. padi* reared on barley.

Photography methods for first to second instar differentiation. Eleven larvae were observed from egg hatch to the second instar (during approximately three days) using a time-lapse photography technique to visualize moulting, usually difficult to observe. Eggs were allowed to hatch on a broad bean leaf placed on humid cotton in a 5 cm diameter opened Petri dish. After hatching, larvae were transferred to cucumber or barley leaves placed on agar gel in the same type of opened Petri dish (21 °C, 9L:15D photoperiod, and 45% RH). Polytetrafluoroethylene insect barrier, PTFE Plus (Formica®) was used to prevent larvae from escaping. Larvae were fed *ad libitum* either with melon aphids or bird cherry-oat aphids. The Petri dish was kept under ambient light during the day and under an LED lightbulb (120 volts, 150 mA, Luminus® PLYB1305D) in a basic reflector lamp with a red filter that was continuously on at night. ISO and exposition time were the same during day and night (ISO-400 and ¼ second exposition time). Time-lapse was set at a 5 min delay between each shot and a Canon EOS 50D® with a 4x optical zoom was used. The camera was placed at approximately 65 cm above the Petri dish. Photos were observed twice a day. The moulting was identified by the behaviour of the larva (standing still for a moment and then stepping out of its exoskeleton, leaving it on the leaf) and by observing the exuviae using a binocular microscope. Less than 24 h after moulting (after confirming that no other moult took place between first moult and observation), larvae were observed under a binocular microscope. A preliminary descriptive grid from the first to the second instar was created assessing principal morphological traits for each instar. ImageJ (an open-source image processing program developed by the National Institutes of Health, Bethesda, Maryland, USA) software was used to measure the length of all larvae less than 10 min after hatching or moulting ($n = 13$ for the first instar and $n = 11$ for the second instar).

Observation method for second to third instar differentiation. To confirm if the finding of Rotheray and Gilbert (2011) could be applied to *E. americanus* third instar larvae, ten new second instar larvae were placed individually in Petri dishes and fed with *A. pisum* (24 °C, 16L:8D photoperiod and 40% RH). Time-lapse photography could not be used since third instar larvae were too mobile. Larvae were observed twice a day under a stereo microscope until moult. The posterior breathing tubes development was described. Moult of larvae was determined by observation of the exuviae. Less than 24 h after moulting, larvae were measured using ImageJ. Thanks to this experiment, the descriptive grid was completed up to the third instar.

Confirmation of E. americanus larval instars descriptive grid. The grid was then confirmed with the daily observation of 13 larvae from 24 h after hatching until the third instar, reared in controlled conditions (25 °C, 16L:8D photoperiod and 60% RH), and given *ad libitum A. gossypii* and *R. padi* aphids.

Results allowed the determination of primary and secondary traits (Table 1). Primary traits are constant over time and among individuals and are used to discriminate the larval instars of *E. americanus.* Secondary traits are more descriptive and may vary significantly over time (at the same instar) or according to the individual.

Two primary traits have been established. The colour of the dorsal hairs serves to differentiate the first instar from the two others. Noticeably, only the first instar has long black hairs which become translucent in the second and third instars (Fig. 1). The fusion of posterior breathing tubes, forming two contiguous circles, is the main trait that discriminates the third from the other instars. In fact, posterior breathing tubes are clearly spaced from each other in the first and second instars (Fig. 2).

Several secondary traits differ between the three instars (Fig. 3). For example, the number of spicules on the surface of the integument increases and gradually forms a pattern (forming dense groups of spicules distributed regularly among the dorsal surface of the integument). The thickness of the white stripes visible on the dorsal part of the larva also gradually increases over time. The stripes of the third instar larvae can be up to four times as wide as the stripes of the first instar larvae. The appearance of the integument also changes during development, going from glossy and translucent to mast and partially opaque. Finally, the length of the larvae can be used as a general indicator of the instar but varies greatly among individuals and depending on rearing conditions. Less than 24 h after hatching or moulting, first, second, and third instar larvae measure respectively about 1.23 ± 0.06 mm 3.08 ± 0.09 mm, and 7.43 ± 0.37 mm.

The present results allow to clearly discriminate the three larval instars of the American hoverfly, by using morphological traits. Even if it was not used as a discriminatory character, the change in the colour of hairs from the first to the second instar was previously described with *Eupeodes luniger* (Meigen, 1822) (Diptera: Syrphidae), by Bhatia (1939) and uncoloured hairs were also reported for species of the genus *Scaeva* Fabricius, 1805 (Diptera: Syrphidae) by Láska *et al.* (2006). The fused breathing tubes at the third instar were also observed in numerous species (Bhatia 1939; Hartley 1961). Other secondary characters from our descriptive grid were also reported in the literature. The presence of nine transverse rows of hairs at all stages was ubiquitous among the seven Nearctic, Palearctic and Holarctic species studied by Bhatia (1939). The appearance of the integument transitioning from transparent to opaque among stages as well as the presence of spinules or spicules at the third stage was also reported in numerous species (Bhatia 1939; Hartley 1961; Láska *et al.* 2006). Nonetheless, variation in those traits is evident as Bhatia (1939) showed that *Episyrphus balteatus* De Geer, 1776 (Diptera: Syrphidae) and *Sphaerophoria rueppellii* (Wiedemann, 1830) (Diptera: Syrphidae) integuments were transparent and shiny even at the third instar.

Table 1. Morphological traits grid for discrimination of the three larval instars of *E. americanus*. The primary traits allow the differentiation of the three larval instars by observation under the stereo microscope. The secondary traits are additional information and may vary slightly depending on the individual.

		First instar larvae	Second instar larvae	Third instar larvae
PRIMARY TRAITS	**Hairs**	Dorsal view: 9 transverse rows of 8 **long black hairs** each except for the rows of the two first segments containing 4 to 6 very small **black hairs**. Hairs of the first transverse row are not all the same size.	Translucent hairs at the same places as the 1st instar.	Translucent hairs at the same places as the previous instars.
	Posterior breathing tubes	Separated pale brown circular breathing tubes.	Bigger and lithely darker brown circular breathing tubes. Appearance of more bumps and still separated at the base.	**Larger and darker brown circular breathing tubes fused at the base.**
SECONDARY TRAITS	**Appearance of the integument**	Glossy and translucent.	Progressively more mast and opaque.	Mast and partially opaque.
	Longitudinal dorsal view	Very thin transverse white stripes if they are visible.	Still very thin but more defined transverse white stripes.	Thicker transverse white stripes. Sometimes joined with yellowish stripes.
	Spicules at the surface of the integument	No or few very small black spicules evenly distributed over the entire integument except the ventral part.	Few to many black spicules evenly distributed over the entire integument except on the ventral surface. No obvious pattern.	Densification of black spicules in certain places forming defined black spots in a regular pattern.
	Body length after moult (mm)	1.21 ± 0.06	3.08 ± 0.09	7.43 ± 0.37

The description of these primary and secondary traits of *E. americanus* larvae broadens the morphological knowledge on each instar of this species and makes it possible to easily differentiate them, either in the laboratory or in the field, with a hand lens. Being a promising biological control agent, it is important to assess fundamental characteristics for its utilization like its voracity, larval development, and mortality rate. Since these characters differ between the instars, it is essential to differentiate them. It is also useful for optimizing the mass rearing of *E. americanus*. Moreover, time-lapse

photography has proven to be a useful technique for monitoring the larval development of hoverflies. It allows knowing exactly when the moulting process takes place without having to find the exuviae which can be very difficult to see otherwise (Joshi and Ballal 2013; Rotheray and Gilbert 2011). Thanks to the high definition of the pictures, time-lapse photography allows observing details on a very small scale while requiring very little data storage compared to video. This little-used method would benefit from being exploited more.
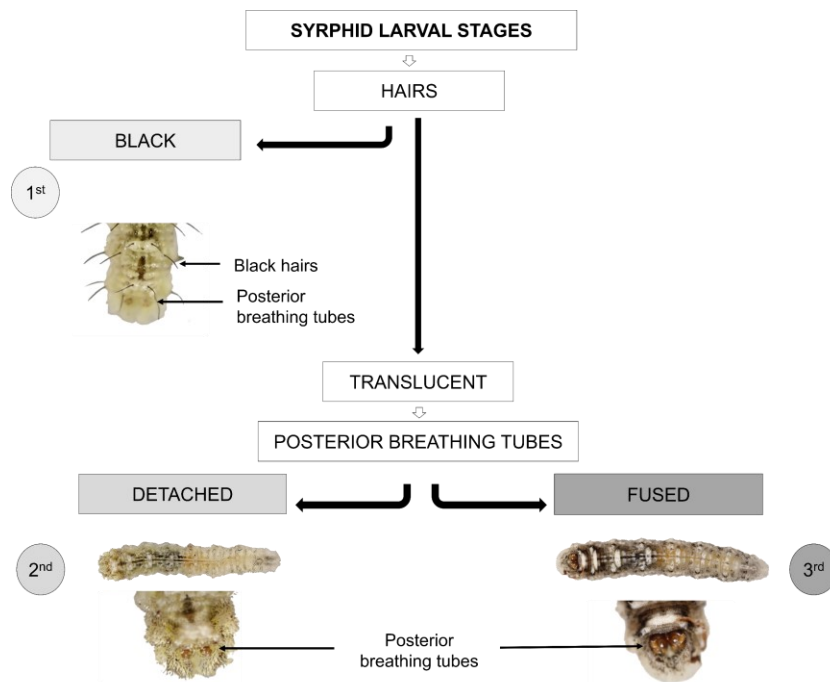

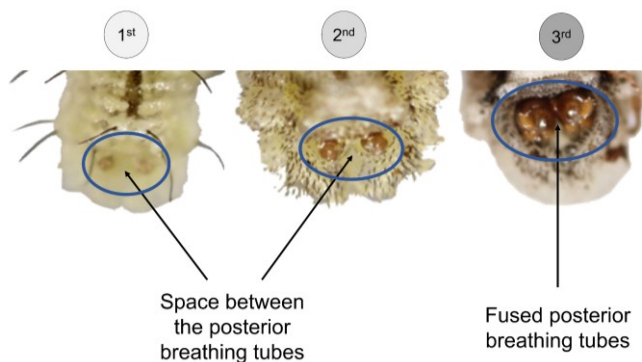**Figure 1. Dichotomous key for larval instars identification of *E. americanus***

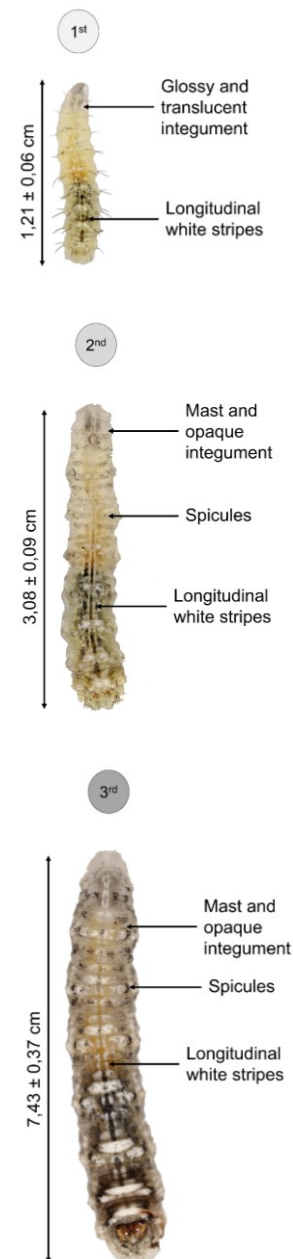**Figure 2. Detailed pictures of the posterior breathing tubes of *E. americanus* larvae**

Figure 3. Secondary morphological traits of *E. americanus* three larval instars

REFERENCES

Bellefeuille, Y., M. Fournier, and E. Lucas. 2017. *Eupeodes americanus* and *Leucopis annulipes* as potential biocontrol agents of the foxglove aphid at low temperatures. IOBC-WPRS Bull. 124: 62-66.

Bellefeuille, Y., M. Fournier, and E. Lucas. 2019. Evaluation of two potential biological control agents against the foxglove aphid at low temperatures. J. Insect Sci. 19: Article 2. doi:10.1093/jisesa/iey130

Bergh, J.C., and B.D. Short. 2008. Ecological and life-history notes on syrphid predators of woolly apple aphid in Virginia, with emphasis on *Heringia calcarata*. BioControl 53: 773-786. doi:10.1007/s10526-007-9114-0

Bhatia, M.L. 1939. Biology, morphology and anatomy of aphidophagous syrphid larvae. Parasitology 31: 78-120. doi:10.1017/S0031182000012646

Davidson, W.M. 1919. Notes on *Allograpta fracta* O. S. (Diptera: Syrphidae). Can. Entomol. 51: 235-239. doi:10.4039/ent 51235-10

Dunn, L., M. Lequerica, C.R. Reid, and T. Latty. 2020. Dual ecosystem services of syrphid flies (Diptera: Syrphidae): pollinators and biological control agents. Pest Manag. Sc. 76: 1973-1979. doi:10.1002/ps.5807

Hartley, J.C. 1961. A taxonomic account of the larvae of some British syrphidae. Proc. Zool. Soc. Lond. 136: 505-573. doi:10.1111/j.1469-7998.1961.tb05891.x

Joshi, S., and C.R. Ballal. 2013. Syrphid predators for biological control of aphids. J. Biol. Control. 27: 151-170.

Láska, P., L. Mazánek, and V. Bičík. 2013. Key to adults and larvae of the genera of European Syrphinae (Diptera, Syrphidae). Acta Mus. Sil., Sci. Nat. 62: 193-206.

Láska, P., C. Pérez-Bañón, L. Mazánek, S. Rojo, G. Ståhls, M.A. Marcos-García, V. Bičík, and J. Dušek. 2006. Taxonomy of the genera *Scaeva*, *Simosyrphus* and *Ischiodon* (Diptera: Syrphidae): descriptions of immature stages and status of taxa. Eur. J. Entomol. 103: 637-655. doi:10.14411/eje. 2006.085

Ouattara, T.Y., M. Fournier, S. Rojo, and E. Lucas. 2022. Development cycle of a potential biocontrol agent: the American hoverfly, *Eupeodes americanus*, and comparison with the commercial biocontrol agent *Aphidoletes aphidimyza*. Entomol. Exp. Appl. 170: 394-401. doi:10.1111/ eea.13152

Rojo, S., F. Gilbert, M.A. Marcos-Garcia, J.M. Nieto, and M.P. Mier Durante. 2003. A world review of predatory hoverflies (Diptera, Syrphidae: Syrphinae) and their prey. Centro Iberoamericano de la Biodiversidad, Alicante, Spain. 219 pp.

Rotheray, G.E. 1993. Colour guide to hoverfly larvae (Diptera, Syrphidae) in Britain and Europe. Derek Whiteley, Sheffield, England. 156 pp.

Rotheray, G.E., and F. Gilbert. 2011. The natural history of hoverflies. Forrest Text, Tresaith, United Kingdom. 333 pp.

Skevington, J.H., M.M. Locke, A.D. Young, K. Moran, W.J. Crins, and S.A. Marshall. 2019. Field guide to the flower flies of Northeastern North America. Princeton University Press, Princeton, NJ, USA. 512 pp.

Vockeroth, J.R. 1992. The flower flies of the subfamily Syrphinae of Canada, Alaska, and Greenland (Diptera: Syrphidae). Agriculture Canada, Ottawa, Canada. 455 pp.