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Morphometric, biological and behavioural differences between *Hemiptarsenus varicornis* (Hym., Eulophidae) and *Opius dissitus* (Hym., Braconidae) parasitoids of *Liriomyza trifolii* (Dipt., Agromyzidae)

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Abstract: The study of two parasitoids of the leaf-miner *Liriomyza trifolii* (Burgess), *Opius dissitus* Muesebeck and *Hemiptarsenus varicornis* (Girault), was conducted in the laboratory. It focused on the morphological and biological differences between the two species, and the production of females when confronted with a population of leaf-miner larvae at different stages and sizes.

1 Introduction

Liriomyza are small Diptera from the Agromyzidae family. They mine the leaves of a large number of floral and vegetable species (CHANDLER and VILLALON, 1989). For many years, chemical pest control was the only efficient means of controlling these miners. However, this caused strains of *L. trifolii* (Burgess) that were resistant to the most-commonly used insecticides to appear. Programmes for integrated control were thus undertaken to wipe out swarms of this crop-destroying insect. As *L. trifolii* was the natural host to a large number of parasitoids (SCHUSTER et al., 1991), pest control through the release of other insect species had an important place in these programmes. The aim of this study was to improve our knowledge of the biology of *Opius dissitus* Muesebeck and *Hemiptarsenus varicornis* (Girault), the natural parasitoids of *L. trifolii* (JOHNSON and HARA, 1987; SCHUSTER et al., 1991); few such studies had been made before.

2 Materials and methods

2.1 Biological material

The strain of *L. trifolii* used in this study originates from the island of Réunion. It was reared on beans and regenerated periodically by bringing in subjects from the fields. *H. varicornis* had the same origin, and is one of the principal antagonists of the leaf-miner in that region. It is originally from the Holarctic zone, the region situated between the northern hemisphere and the Tropic of Cancer. The strain of *O. dissitus* under study came from Senegal where it was introduced in 1982 by the FAO (Food Agricultural Organization) from the CIBC (Commonwealth Institute of Biological Control) in Trinidad (COLY, 1984). These two parasitoids were reared on the *L. trifolii* larvae in their last stage according to the technique used in the laboratory (BORDAT et al., 1992).

The different manipulations of this study were carried out in an air-conditioned cell with the following conditions: temperature, 25 ± 1°C; relative humidity, 75 ± 5%; and 12 h/12 h light and dark cycle.

2.2 The morphometric and biological differences between the two entomophages

A dozen bean leaves which had been pre-infested with the larvae of *L. trifolii* (larval stages L2 and L3) were exposed daily to the laying females of the two parasitoids for two hours. They were then placed in an air-conditioned cell to allow the development of the parasitoids. Each day, dissections of the larval and pupal stages of the host were made in order to take the measurements of 100 indiv. at each stage for each species.

2.3 Time spread of the different biological stages of the two entomophages

Bean leaves infested with the larvae of *L. trifolii* at stages L2 and L3 were left in the laying-box in the presence of parasitoid females for 24 h instead of 2 h. Observations were made every day in order to obtain 100 indiv. at each stage for each species.

2.4 Influence of the larval stage of *L. trifolii* on the parasitism of the two entomophages

For each species of parasitoid, 10 males and 10 females were introduced into a 450 mm cubic cage made of transparent plastic, the top of which was covered with a very fine meshed material to allow for air. In this container we had previously placed two lots of bean leaves, carrying *L. trifolii* larvae at stages L1, L2 or L3. The number of larvae presented was purposely large in order to avoid the risk of 'superparasitism' by the parasitoids. Subsequently, the female adults were counted as they hatched out. Ten replicates were carried out for each larval stage and each of the two species of Hymenoptera, and for each repeat, the *L. trifolii* larvae were left in the presence of the females for 24 h. The results obtained were submitted to a Newman–Keuls test.

2.5 Influence of the number of *L. trifolii* larvae on the parasitism of the two entomophages

Three batches of about 30, 100 and 200 *L. trifolii* larvae at stage L3 were presented to groups of 10 males and 10 females

Table 1. *Dimensions of adult* O. dissitus *and* H. varicornis *(average of 50 individuals of any species and of any sex)*

	O. dissitus		*H. varicornis*	
	Male	Female	Male	Female
Average length (mm)	1.50	1.49	1.63	1.80
Length range (mm)	1.12–1.88	1.16–1.88	1.12–1.84	1.36–2.16
Average span (mm)	3.42	3.46	2.53	3.06
Span range (mm)	2.56–4.08	2.60–4.24	1.68–2.96	2.24–3.52

of the two parasitoid species. After 24 h, these batches were taken away and put into the air-conditioned cell in order to allow the development of the implanted eggs to take course. The number of resulting adults was counted. In this experiment, six repeats were carried out. As before, the results were submitted to a Newman–Keuls test.

3 Results

3.1 Morphometric differences between *H. varicornis* and *O. dissitus*

The adults of *H. varicornis* are a metallic blue–green in colour. Their length varies considerably but it is an average of 1.63 mm for the males and 1.80 mm for the females (table 1). The species can be distinguished sexually by its antennae: the male has pectinated antennae whereas the female has long, thin ones. Their length represents about 50% of the insects overall body length (THIERY, 1982). Its feet are white, almost translucent.

The imagos of *O. dissitus* are black all over. Males and females are almost the same size—an average of 1.50 and 1.49 mm, respectively (table 1). There are no sexual differences visible to the naked eye and it is difficult to see the female's ovipositor without the help of a binocular magnifying glass. The antennae are long, black and thin, and almost as long as the body.

The eggs are oblong shaped, those of the *H. varicornis* being a translucent creamy white. In *O. dissitus* one end of the egg is slightly more swollen. Eggs of *H. varicornis* are slightly longer than those of *O. dissitus*. They are an average of 0.28 and 0.22 mm respectively (table 2). Throughout the dissections we managed to observe up to 5 eggs per *L. trifolii* larva and this was the case for both of the entomophagous species (FARRET, 1991).

The *H. varicornis* L1 are cylindrical, slightly ringed in shape, and translucent, apart from the central part where there is an opaque, yellow area. They are an average of 0.51 mm long (table 2). In *O. dissitus*, the first larval stage is also translucent but its morphology is completely different. The L1 has a very distinctive head with strong buccal parts consisting of two sharp hooks and the body is slightly ringed and ends abruptly in a small caudal appendix. The size varies between 0.25 mm for the smallest and 0.76 mm for the biggest. In both species the presence of two L1 per larva or pupa host was observed several times (FARRET, 1991).

The *H. varicornis* L2 is also cylindrical in shape but longer and more streamlined than the L1. Its ringed appearance has practically disappeared and it is a bright orange colour, except for the ends, which are translucent. In the dissections, we never found more than one L2 per host larva. It is variable in size, between 0.34 and 2.40 mm (table 2). The *O. dissitus* L2 is very different from the L1. It has become worm shaped, has lost its buccal hooks, and now occupies the whole of the host pupa's length. It has turned a creamy white colour (FARRET, 1991). Its length varies between 0.60 and 1.70 mm (table 2).

The prepupae can be distinguished from the L2 by a swelling at one end, giving the appearance of segmentation. *Hemiptarsenus varicornis*'s prepupae is white, whereas the prepupae of *O. dissitus* is creamy-beige, turning yellow. They are both about the same size, 1.41 mm and 1.47 mm for *O. dissitus* and *H. varicornis*, respectively (table 2).

At the pupal stage, protrusions for the feet and antennae can easily be distinguished. The different parts of the body, the head, thorax and abdomen are quite visible. After oxidation of the tegument, the pupae vary in colour throughout their transformation. On formation, they are pale yellow in colour, but they gradually darken, the eyes turning distinctly red, then brown. By the end of the pupal stage, they are black all over. The *H. varicornis* pupae are slightly bigger, 1.61 mm compared to 1.52 mm for the *O. dissitus* pupae (FARRET, 1991).

Table 2. *Length in mm of different biological stages of* O. dissitus *and of* H. varicornis *(average of 100 individuals of any stage)*

	O. dissitus		*H. varicornis*	
	Average	Range	Average	Range
Eggs	0.22	0.16–0.30	0.28	0.14–0.38
L1	0.47	0.25–0.76	0.51	0.28–0.96
L2	0.99	0.60–1.70	1.34	0.34–2.40
Prepupae	1.41	1.02–1.84	1.47	0.96–2.08
Pupae	1.52	0.79–1.78	1.61	1.04–2.10

3.2 Biological differences between *H. varicornis* and *O. dissitus*

In both species, the adults copulate as soon as they emerge and can begin laying the very same day. However, the parasitism systems of the two Hymenoptera are different. *H. varicornis* is a larval ectoparasite whereas *O. dissitus* is a larvo-pupal endoparasite. In fact, after copulation, the *H. varicornis* females lay one or several of their eggs near a *L. trifolii* larva that she has paralysed previously. The paralysis of the larva and the egg laying take place in two distinct phases. Sometimes the female cannot relocate the larva that she has paralysed, and, in this way, about 20% of *L. trifolii* larvae die without being parasitized. When it hatches out, the *H. varicornis* larva latches onto the host, and gradually empties it out during the days that follow. Pupal formation takes place beside the remains of the

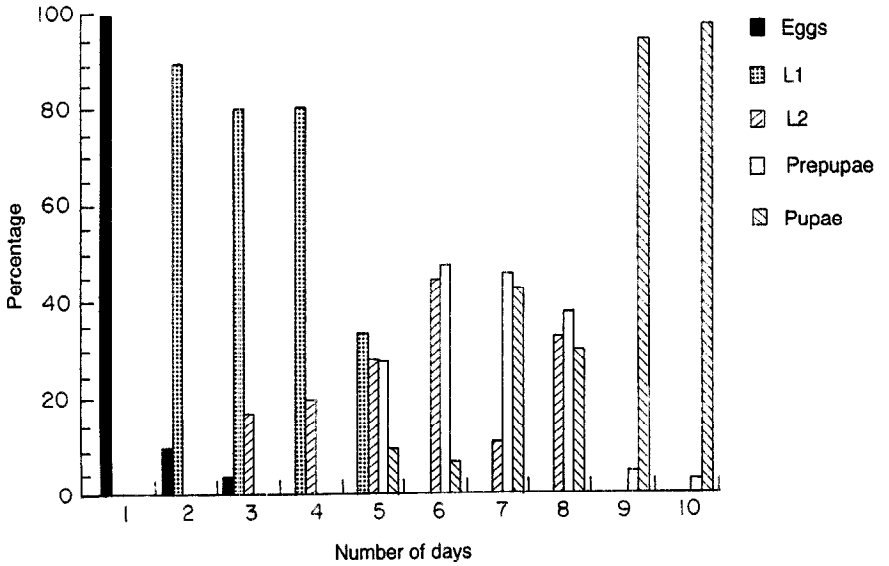

Fig. 1. Duration of the preimaginal stages of O. dissitus *reared at 25° C*

host in the gallery hollowed out by the larval leaf miner. Once the adult has formed, it emerges from the plant after piercing the epidermis of the leaf. Thus, the complete biological cycle of *H. varicornis* takes place inside the plant.

The *O. dissitus* females lay their eggs directly inside the larva's body cavity without anaesthetizing it beforehand. The host larva continues to eat normally, then leaves the plant in order to become a pupa, taking with it the egg or the young larva. *Opius dissitus* then develops inside the *L. trifolii* pupa, taking advantage of the latter's protective cuticle. The imago comes out of the pupa, which can usually be found on the ground. Unlike *H. varicornis*, most of *O. dissitus*'s cycle takes place outside the plant.

3.3 Time spread of the two parasitoids different biological stages

In both species, the eggs's incubation period varies a great deal. It can be as long as 7 days for *H. varicornis* and 3 days maximum for *O. dissitus* (fig. 1 and fig. 2). The duration of the L1 stage is also different in each species. On the second day after laying, 90% of *O. dissitus* are at the L1 stage, 80% on the third and fourth days and 35% on the fifth day (fig. 1). In *H. varicornis*, 40% are noted on the second day after laying, rising to 90% on the third day; after this, the percentage decreases gradually each day, and it is below 5% on the eight day after laying (fig. 2).

The duration of the L2 stage is the same in both species, that is to say, 6 days. In *H. varicornis*, the maximum number of L2 are present on the fourth and fifth days after laying, with 59% and 68% respectively; this proportion decreases steadily to 50% on the sixth day, 25% on the seventh day, 19% on the eighth day and 5% on the ninth day (fig. 2). In *O. dissitus*, 17% of L2 appear 3 days after laying, the percentage increasing until the sixth day (20%, 27% and 45% for the fourth, fifth and sixth days respectively), before dropping to 11% on the seventh day and going back up to 33% on the eighth day (fig. 1).

In *O. dissitus*, the prepupae appear as early as the fifth day and thereafter up to the tenth day with maximum numbers on the fifth, sixth, seventh and eighth days

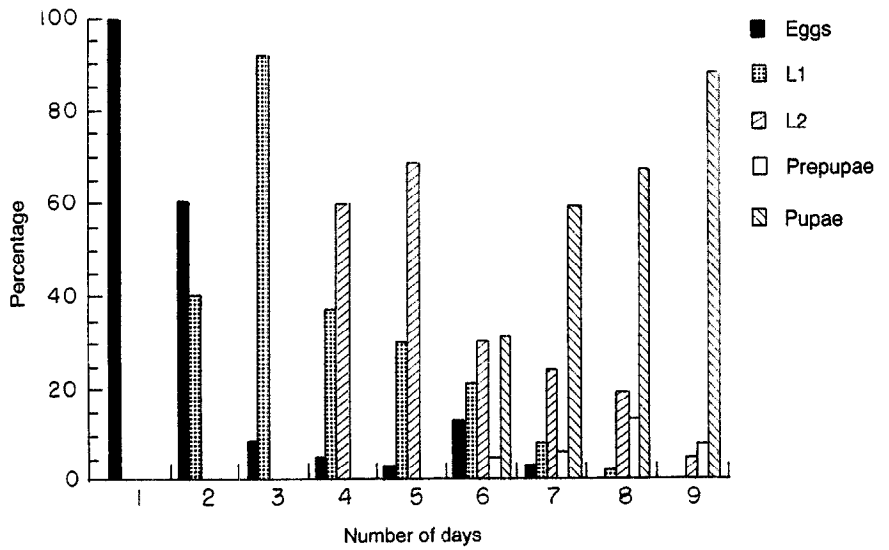

Fig. 2. Duration of the preimaginal stages of H. varicornis *reared at 25° C*

Table 3. *Number of adults produced by 10 females of* O. dissitus *and 10 females of* H. varicornis *parasitizing different stages of larvae of* L. trifolii *(10 repeats)*

Larval stage of *L. trifolii*	Number of adult *O. dissitus*	Number of adult *H. varicornis*
L1	0[a]	33[a]
L2	53[b]	187[b]
L3	401[b]	535[c]

[a], [b], [c] and [d] are different at the 5% level (Newman–Keuls test)

with 28%, 50%, 45% and 28% respectively (fig. 1). In *H. varicornis* prepupae can be found between the sixth and ninth days, but in very small quantity: 5%, 6%, 14% and 8%, respectively (fig. 2).

In *O. dissitus*, the first pupae appear right from the fifth day, but it is not until the ninth and tenth days that there are the highest counts – 95% and 98% (fig. 1). With *H. varicornis* we see the first pupae on the sixth day and the percentage increases steadily until the ninth day (30%, 59%, 67% and 88%, respectively) (fig. 2).

3.4 The influence of the larval stage of *L. trifolii* on the parasitism of the two entomophages

The host's larval stage has an influence on the production of females in the two parasitoids. In *O. dissitus*, the production resulting from the first larval stage of *L. trifolii* was 191 adults, 353 for the L2 and 401 for the L3 (table 3). It should also be noted that the Newman Keuls test detected no significant difference, at 5%, between the number of adults coming from L2 and L3.

In *H. varicornis*, the production coming from L1 was 33 adults, 187 for L2 and 535 for L3 (table 3). The number of adults coming from the three stages of the *L. trifolii* are significantly different, by 5%, according to the Newman Keuls test.

3.5 The influence of the number of *L. trifolii* larvae on the parasitism of the two entomophages

When the two parasitoid species are in competition, the population size of *L. trifolii* larvae has a considerable influence on the production of females. With a small population, the production of *O. dissitus* adults was only one individual, whereas a large proportion was identical to a control group – 586 and 576 individuals, respectively (table 4). These two latter figures were not significantly different at 5%, according to a Newman–Keuls test.

Table 4. *Number of adults produced by 10 females of* O. dissitus *and 10 females of* H. varicornis *in competition on a small (30) and large (200) population of larvae of* L. trifolii *(6 repeats)*

	Number of adult *O. dissitus*	Number of adult *H. varicornis*
Control	576[a]	426[a]
30 larvae	1[b]	26[d]
200 larvae	586[a]	349[c]

[a], [b], [c] and [d] different at the 5% level (Newman–Keuls test)

Hemiptarsenus varicornis was also affected by host population size. Twenty-six adults were produced in the case of the small population, 426 in the control group and 349 for a large population (table 4). The figures concerning the number of adults produced with the control group and with the large population were not significantly different, at 5%, according to the Newman–Keuls test.

4 Discussion

One of the main biological differences between the two species is the ability of *H. varicornis* to prolong the incubation period for its eggs to seven days. This spreading out of incubation over time could be the reason for the emergence, by instalments, of the following generation's adults. We must point out, however, that it was not possible to determine whether the older eggs laid had been fertilized or not, for, once they have been disturbed and uncovered, the eggs die. However, this phenomenon is not possible in the case of *O. dissitus* as the egg that develops inside a non-paralyzed larva cannot have too long an incubation period, otherwise the subsequent larva would be at risk, due to the possible metamorphosis of the host.

The two species also differ in the number of prepupae and the duration of the prepupal stage. In fact, at the time of dissecting, quite a large number of *O. dissitus* were observed over 6 days, whereas only a very small number of *H. varicornis* were found, over 4 days only. We may consider that, the prepupae of *O. dissitus* is protected by the cuticle of the *L. trifolii* pupa, whereas *H. varicornis* has no protection in the gallery hollowed out by the host larva, and this explains the low percentage of prepupae found, and the very short duration of pupal formation, in the latter species.

Opius dissitus can survive in the L1 of *L. trifolii*, its larvae using their host's growth as a model for their own. By contrast, as the larvae parasitized by *H. varicornis* are paralysed, they can no longer grow and only provide limited food for the parasitoid. This causes the death of a great many larvae and the emergence of very small individuals.

In the case of competition between the two species, on a small population of host larvae, *H. varicornis* seems to have the advantage over *O. dissitus*, which does not seem to be able to ensure new generations. If we were to suppose that a larva could be parasitized by both species at once, it is probable that the *O. dissitus* larva would die, as the larva, paralysed by *H. varicornis*, would not be able to change into a pupa. It is likely, however, that *H. varicornis* would be able to complete its cycle on a larva that had been partially eaten by *O. dissitus*.

Thus, it seems that these two species could cohabit, providing the populations of *L. trifolii* were large enough. However, as *O. dissitus* is able to live on the leaf-miner in its very early stages, it would seem to be more efficient at the onset of attacks by *L. trifolii*

Résumé

Différences morphométriques, biologiques et comportementales entre *Hemiptarsenus varicornis* (Hym., Eulophidae) et *Opius dissitus* (Hym., Braconidae) parasitoïdes de *Liriomyza trifolii* (Dipt., Agromyzidae).

Opius dissitus et *Hemiptarsenus varicornis* sont deux parasitoïdes naturels de *Liriomyza trifolii*, mouche mineuse des feuilles. Des études en laboratoire montrent que *O. dissitus* peut, à l'inverse d'*H. varicornis*, se multiplier dans des stades L1 de *L. trifolii*. Par contre, sur de faibles populations de larves hôtes et lorsque les deux espèces de parasitoïdes sont en compétition, seul *H. varicornis* parvient à se multiplier.

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