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Behavior and superparasitization of *Edovum puttleri* Grissell (Hym. Eulophidae), an egg parasitoid of *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae). (*)

I. Introduction

Despite current economic problems which are unfavourable to the use of *Edovum puttleri* in the biological control of *Leptinotarsa decemlineata* (CPB), the possibility of its use in the future has induced the authors to collect as much information as possible on the biology of this parasitoid. In addition to wanting to acquire more knowledge per se in this regard, possible advantages deriving from its application have also been considered. In particular, the study intended:

1. further ascertaining the behavior of *Edovum* females (already described by Gardenghi et al., 1990, 1994) on egg masses of *Leptinotarsa* as, although generally standardized, this behavior has nevertheless been seen to differ depending on the conditions of the host eggs i.e., whether intact or not, parasitized or not, their number and the number of females simultaneously present on the egg mass;
2. determining the effects of such conditions with special reference to superparasitization, to the development of the host and to that of the parasitoid;
3. identifying the eventual signals capable of preventing superparasitization and the conditions which make it more frequent and perhaps also more advantageous.

II. Materials and methods

A. - Experiment "a" (overcrowding and egg dissection).

The trials entailed placing groups of four *Edovum* females in clear plastic Petri dishes (3.3cm in diameter and 1.1cm in height) on the bottom of which had been previously placed eight CPB host eggs and minute drops of honey on the lid. The dishes were kept for either 24 or 48 hrs in a climatic chamber with a photoperiod of 16L:8D, temperatures of 27 ± 1°C during the day and 20 ± 1°C during the night and a relative humidity of 80 ± 5%.

After 24 or 48 hrs in which the females had been kept together with the host eggs the latter were removed from the dish and placed on a slide with a drop of

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water. They were then opened one by one by means of needle pliers so as to determine the presence of *Edovum* eggs or larvae as well as their health conditions on the basis of their colour (white and clear for live eggs or spotted or black for dead ones). Larvae could also be live or dead.

The females used for the trials had been previously checked to determine their age and parasitizing capacity. Only females between one and two weeks old (corresponding to the most fertile age) and those which had already been active on host eggs during the first week, were chosen.

Eggs were collected from potato plants daily so as to have eggs no more than 24 hrs old.

B. - Experiment "b" (overcrowding and egg fate).

The procedure for this experiment was similar to that of the previous one, the only difference being that the host eggs were not opened but kept in the dish and placed in the climatic chamber to follow their fate. The fate of each host egg was then recorded according to whether *Edovum* developed, a *Leptinotarsa* larva hatched or to whether the egg simply collapsed.

C. - Experiment "c" (overcrowding and direct observation of activity).

These trials were conducted under the same conditions as for experiments "a" and "b". The purpose, however, was to directly observe the behavior of the females and their activity on the eggs. Observations were conducted by means of a stereomicroscope for time intervals of 2 hrs each, after which the females were removed from the dish. A drawing had been previously made of the precise position of each egg in the dish so as to permit identification during the trial and subsequently. It was therefore possible to precisely determine how each egg had been treated and its fate. During stereomicroscopy, the dish was kept at a temperature of about 26 °C. After each obervation period, the egg masses which had been visited by the parasitoid were kept in the rearing chamber and checked every day. Their fate was then recorded and could consist in any of the following: emergence of the *Edovum*, death of the *Edovum* before emergence, hatching of the *Leptinotarsa* larva, abortion of the *Leptinotarsa*, or egg collapse.

D. - Experiment "d" (productivity trials).

This experiment consisted in leaving each group of four females in contact with 24 eggs for only a 24-hr period. The CPB eggs were then allowed to develop and each checked to ascertain whether the host or the parasitoid developed or whether unproductive.

E. - Experiment "e" (the female first visits the eggs on one side and then on the other).

This experiment was conducted in order to determine the signals which permit the female to identify eggs which have already been parasitized and to ascertain to what extent these signals are taken into consideration by the female.

On the basis of observations previously conducted on *Edovum* females which were active on an egg mass comprising at least 20 eggs for 2 to 3 hours, only 4 cases of double B behavior (and presumably of double oviposition) were reported

on the same host egg out of a total number of 533 B behaviors, corresponding to 0.75% of total parasitizations.

In order to explain how superparasitization had been avoided, it was initially presumed that after deposition of the *Edovum* egg a signal may be left external to that of *Leptinotarsa*. In fact, it was observed that after oviposition (behavior B) the female often rubs its abdomen against the CPB egg. This behavior was called by us "marking". Many cases, however, were observed in which this rubbing behavior was not performed after oviposition. On the basis of these observations, it may be assumed that marking is not so essential in order to leave an external signal. Moreover, an "engaged" signal internal to the parasitized egg detectable by the female with its ovipositor, could also be hypothesized.

In order to test this hypothesis, the activity of the parasitoid in the presence of only a small number of hosts (4 eggs) was observed. Under these conditions, the female is obliged to pass over the already parasitized eggs several times so that its behavior on the eggs can be observed more than once as it is almost certain that the female has not yet exhausted its daily reserve of mature oocytes.

So as to better assess the importance of the eventual internal signal with respect to the external one, four CPB eggs were placed lengthwise (that is, with the longer axis parallel to the support) close to each other and then turned over after having been parasitized. This procedure was adopted as it was assumed that by turning the eggs over the external signal would have been hidden from the female, which could thus only explore the side of the egg which had not yet been stung.

Two major problems had to be solved before these trials could be carried out, namely: 1) the risk that by turning the eggs over with pincers the side which had not been marked could also become marked by contact ; 2) the difficulty of perfectly turning them over by 180 degrees so that the female could come into contact with a part of the egg which had already been visited and marked. After several attempts, the eggs were placed inside a small slot cut out of a small piece of cardboard having the same thickness as the egg (fig.I). The cardboard with the CPB eggs was then placed on the bottom of a Petri dish (3.3cm in diameter and 1.1cm in height). Upon coming into contact with the eggs thus arranged, the activity of the parasitoid was seen to be perfectly natural. The cardboard was then turned over after the female had finished its activity and left the egg.

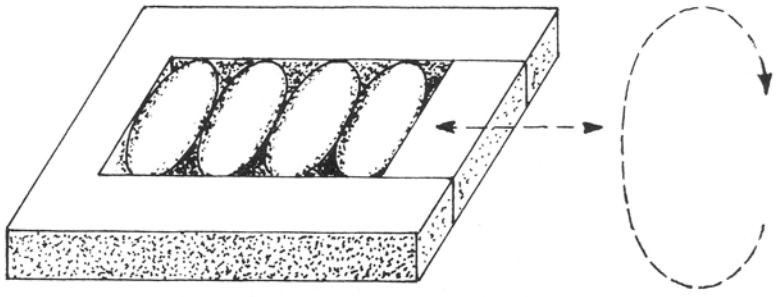


Figure I - The drawing shows the way the eggs were arranged in the piece of cardboard so as to be able to turn them over without touching them. The activity of the *Edovum* female was observed first on one side and then, after overturning, on the other.

Observations were made with a binocular and a VHS videorecording system was also employed. Temperature was kept constant at 26 to 27 °C.

The females employed in this experiment were grown in barrels (3.3cm in diameter and 7.5cm in height) in quantities of 5 to 10 for each barrel. Barrels contained strips of oiled paper on which had been placed minute drops of honey and into which CPB egg masses were introduced every 2 or 3 days. About 48 hours before each trial a few individuals between 4 and 13 days old were transferred into small boxes which did not contain any egg masses. It may therefore be presumed that upon the experiment being carried out, the 6 to 15 day-old females had already come into contact with CPB eggs and that they also possessed a reserve of mature oocytes.

The activity of the female was schematically recorded by taking note of its behavior and of the number of times it passed over an egg drumming it with its antennae but without stinging it. Several minutes were left to pass upon the female leaving the eggs before turning them over. Upon the same female returning to the egg, its behavior was once more recorded.

The *Leptinotarsa* eggs were then opened only after the female had terminated its activity on the other side of the eggs in order to ascertain whether oviposition had in effect occurred as a consequence of all the B behaviors observed.

III. Results

A. - Experiment "a" (4 females with 8 eggs for 24 and 48 hours and subsequent egg dissection).

Superparasitized and unparasitized eggs. As already mentioned, previous trials in which the activity of single females with at least 20 host eggs was observed for a period of 2 or 3 hours, showed that superparasitization almost never occurs (only in 0.75% of parasitizations). The present trials showed that in high parasitoid density conditions, superparasitization occurs very frequently, a single CPB egg being parasitized even up to 5 times (tab. 1).

Table 1 also shows that despite the high density of parasitoids, even after 48 hours the number of eggs which have not been parasitized is still very high (38.64%). Checking each egg individually revealed that unparasitized eggs were often to be found next to ones which had been parasitized several times. An unparasitized egg was even found next to one which had been parasitized five times.

A possible explanation of this irregular behavior on the part of the females was afforded by directly observing their activity in such overcrowded conditions (see trials of experiment "c", tab. 3). In fact, it has been observed that the female of this species is very often disturbed by the presence of other females on the egg mass even to the point of abandoning the egg on which it is active. It is not infrequent, therefore, that only behavior A (host feeding) is put into effect and that, even if behavior B has commenced, the female does not continue it if disturbed.

Moreover, the simultaneous presence of many females and of few host eggs may cause the parasitoid to become disturbed so that it is less responsive to the chemical marking signals.

It should however be noted that superparasitization is not always to be consi-

Table 1 - Results of the trials conducted by keeping groups of 4 *Edovum* females each in the presence of 8 CPB eggs in a capsule for 24 and 48 hours. Host egg dissection revealed that each *Leptinotarsa* host egg may be parasitized repeatedly, up to five times. After 24 hours, both parasitoid eggs and larvae were found in the host eggs.

CPB eggs	24 h		48 h	
	No.	mean	No.	mean
total	280 (8 eggs x 35)	8	616 (8 eggs x 77)	8
unparasitized	128 (45, 71%)	3,66 ± 1,89	238 (38, 64%)	3,09 ± 2,13
with 1 egg or larva of *Edovum puttleri*	110 (39, 29%)	3,14 ±1,75	247 (40, 10%)	3,21 ± 1,62
with 2 eggs or larvae of *Edovum puttleri*	33 (11, 79%)	0,94 ± 1,21	103 (16, 72%)	1,34 ± 1,34
with 3 eggs or larvae of *Edovum puttleri*	6 (2, 14%)	0,17 ± 0,38	23 (3, 73%)	0,30 ± 0,73
with 4 eggs or larvae of *Edovum puttleri*	3 (1, 07%)	0,09 ± 0,28	4 (0, 65%)	0,05 ± 0,22
with 5 eggs or larvae of *Edovum puttleri*	0	0	1 (0, 16%)	0,01 ± 0,11

dered as a defective behavior. In fact, this behavior may actually be normal for the parasitoid, that is put into effect even when it is capable of distinguishing the parasitized hosts from the unparasitized ones. As will be explained in the following section, the scarce availability of hosts and the simultaneous presence of many females may both be conditions which make superparasitization advantageous for the parasitoid.

Eggs and larvae of live and dead *Edovum*. Observation of the health conditions of parasitoid eggs and larvae within the host revealed the simultaneous presence of a live *Edovum* larva together with black or spotted eggs or dead larvae in most cases of superparasitization. Having verified by trial "c" (tab. 3) that no more than one adult ever emerges from superparasitized eggs, it may be inferred that one of the parasitoid larvae exerts an early inhibiting action against the development of other eggs laid subsequently or against that of other larvae hatched in the host.

The simultaneous presence of two live larvae in the same host after 48 hours of permanence of the parasitoid with host eggs was reported in only 3 cases of superparasitization out of 131 (2.29%). In these cases it may be assumed that the two *Edovum* eggs were deposited shortly one after the other and that the CPB eggs were opened soon after the larvae had hatched, that is before one of the two had had the time to eliminate the other.

Moreover, it should be noted that dark *Edovum* eggs or dead larvae are also to be found in many cases in which CPB eggs are parasitized only once. It should in fact be borne in mind that, as has already been observed, behaviors A (host fee-

Table 2 - Results of the trials conducted by keeping groups of 4 *Edovum* females each in the presence of 8 CPB eggs in a capsule for 24 and 48 hours. Unlike for the trials in table 1, *Leptinotarsa* eggs were not dissected but left to develop and their fate recorded.

CPB eggs	24 h		48 h	
	No.	mean	No.	mean
total	144 (8 eggs x 18)	8	256 (8 eggs x 32)	8
emerged *Edovum*	63 (43, 75%)	3,50 ± 2,48	72 (28, 12%)	2,25 ± 1,92
dead CPB eggs	81 (56, 25%)	4,50 ± 2,48	184 (71, 88%)	5,75 ± 1,99
hatched CPB	0	0	0	0

ding) and C (rapid sting without feeding or oviposition) tend to inhibit the development of *Leptinotarsa*, and this inhibition is all the more marked the greater their number. It may therefore be assumed that in overcrowding conditions host eggs are subject to a high number of stings, so much so as to also adversely affect the development of *Edovum*. It can therefore be concluded that a large number of parasitized eggs would not have led to the development of *Edovum* at all so that, even if CPB eggs had not been opened, the number of emerged *Edovum* would have been low and in any case much less than the overall number of CPB eggs actually parasitized. This conclusion was fully borne out by the results of the following experiment where only 28.12% of the parasitized eggs led to the emergence of *Edovum*.

B. - Experiment "b" (4 females with 8 eggs for 24 and 48 hours, and egg fate).

Almost total inhibition of *Leptinotarsa* development. Results of table 1 show that many eggs were not parasitized even after 4 females had been kept in contact with 8 eggs for 24 and 48 hours (45.71% and 38.64%, respectively). As can be seen from the results reported in table 2 and figure III, *Leptinotarsa* cannot develop even from these unparasitized CPB eggs. It may therefore be safely concluded that the host eggs had been subject to many behaviors A and C, which are therefore lethal for the development of the *Leptinotarsa*.

Drop in *Edovum* emergence rate.

The assumptions made on the basis of the health conditions of *Edovum* eggs and larvae in the previous experiment were confirmed in this experiment, and that is that not only the host but also the parasitoid is damaged when the CPB eggs are stung a high number of times. In fact, after 24 hours' permanence of the females with the host eggs the number of *Edovum* emerged was equal to 43.75%, dropping even further to 28.12% after 48 hours' permanence (tab. 2 and fig.III).

It may therefore be concluded that the high density of *Edovum* is a factor which limits not only the host but also the parasitoid population.

Table 3 - Results of the trials conducted by keeping groups of 4 *Edovum* females each in the presence of 8 CPB eggs in a capsule for 2 hours, with direct observation of female activity and a record of their behavior. Failed ovipositions = female, after the oviposition behavior, loses its egg as the latter remains attached to the tip of the ovipositor and is therefore dragged out and released outside the host egg; behavior A = host-feeding; behavior C = rapid sting without feeding or oviposition; aborted CPB = albeit commencing, *Leptinotarsa* development is interrupted before hatching following on behavior A and/or C.

Edovum behavior	CPB eggs	emerged *Edovum*	dead *Edovum*	hatched CPB	aborted CPB	dead CPB eggs
1 oviposition	51 (53,13%)	45	2	-	-	4
2 ovipositions	12 (12,50%)	11	-	-	-	1
3 ovipositions	1 (1,04%)	1	-	-	-	-
failed oviposit.	4 (4,17%)	-	-	-	-	4
behav. A only	7 (7,29%)	-	-	2	1	4
behav. C only	2 (2,08%)	-	-	-	-	2
behav. A + C	18 (18,75%)	-	-	1	1	16
no visit	1 (1,04%)	-	-	1	-	-
total	96 (8 x 12)	57	2	4	2	31
%		(59, 38%)	(2, 08%)	(4, 17%)	(2, 08%)	(32, 29%)
mean		4,75 ± 1,96	0,17 ± 0,39	0,33 ± 0,49	0,17 ± 0,39	2,58 ± 2,02

C. - Experiment "c" (4 females with 8 eggs for 2 hours, and observation of female activity and egg fate).

High percentage of emerged *Edovum*. The trials entailed placing four females together with eight eggs for a period of only two hours each. As can be seen from the results reported in table 3, this time is more than enough for the majority of CPB eggs to be parasitized (66.67% of parasitized eggs).

The number of A and C behaviors undergone by the eggs was still relatively low under these conditions so that parasitoid development was not adversely affected. In fact, emergence was around 59.38%, that is greater than that reported for previous trials. The slight possibility for *Leptinotarsa* to develop should also be noted (4.17% of hatched CPB, tab. 3 and fig.III).

Superparasitized eggs. A high number of superparasitizations was also observed in this experiment. In fact, 12 eggs had undergone two behaviors B (12.50%), while one egg had undergone three (1.04%). *Edovum* emergence was observed in all these cases, but never more than one individual per host. On the basis of these results, the assumption made in esperiment "a", and that is, that the development of a parasitoid in a superparasitized egg is normal and that it inhibits that of other *Edovum* eggs or larvae present in the same host egg, would therefore seem to be confirmed.

Mutual interference of the females. Direct observation of the activity of the females in this experiment showed their behavior not to be completely normal as compared to that of a female acting alone on the egg mass. In fact, the proximity of more females with each other causes interference so that a behavior,

Table 4 - Results of trials conducted by keeping groups of 4 *Edovum* females each in the presence of 24 CPB eggs in a capsule for 24 hours. Fate of the host eggs was recorded.

CPB eggs	24 h	
	No.	mean
total	336 (24 eggs x 14)	24
emerged *Edovum*	253 (75,30%)	18,07 ± 4,97
dead CPB eggs	82 (24,40%)	5,86 ± 4,87
hatched CPB	1 (0,30%)	0,07 ± 0,27

albeit initiated, is not brought to completion, while in other cases the female was seen to stop on an egg for a considerable period of time without acting or even to abandon the egg mass by jumping and flying away.

The four failed behaviors B observed, that is the failure of the parasitoid to introduce its egg into the host while merely depositing it on top of the CPB egg, may also be taken as a symptom that the females were disturbed. It should however be noted that this event can sometimes be observed even in the case of a female acting alone.

D. - Experiment "d" (4 females with 24 eggs for 24 hours and egg fate).

Having observed that an excessive density of *Edovum* is also detrimental to the development of its offspring, an experiment was conducted to determine the ideal conditions for maximum parasitoid production. Findings showed that this experimental design led to the greatest number of emerged *Edovum* thus far recorded (75.30% of the total number of host eggs provided) (tab. 4 and fig.III).

The ideal ratio of host egg to parasitoid female for maximum parasitoid production would therefore seem to be 1 female to 6-8 eggs a day. This ratio also permits to practically inhibit the development of the CPB eggs which have eventually not been parasitized. This result may be useful in determining the ideal conditions for optimum parasitoid rearing and for its eventual use in the biological control of *Leptinotarsa*.

E. - Experiment "e" (one female with 4 eggs and revisitation of the 4 overturned eggs).

The external signal of a parasitized egg. The results of this experiment showed first and foremost that the *Edovum* female is capable of distinguishing intact eggs from those which it itself has parasitized shortly before. As can be seen in table 6 (which refers to side "a" only) and figure II, the females almost always, parasitized intact eggs (95.65%), while, upon the first visit, only rarely those already parasitized (6 cases out of 88).

Table 5 - Results of trials conducted with 4 CPB eggs arranged so that one *Edovum* female could visit first one side (side "a") and then, after overturning, the other side (side "b") (see also figs. I and II). The various parasitization levels were recorded and ranked with scores from 0 (unparasitized eggs) to 5 (eggs parasitized 5 times).

side	CPB eggs	unparasit.	1 oviposit.	2 oviposit.	3 oviposit.	4 oviposit.	5 oviposit.
a	92 (4 eggs x 23)	3 (3, 26%)	70 (76, 09%)	18 (19, 56%)	1 (1, 09%)	0	0
b	92 (4 eggs x 23)	20 (21, 74%)	68 (73, 91%)	4 (4, 35%)	0	0	0
a+b	92 (4 eggs x 23)	1 (1, 09%)	21 (22, 82%)	50 (54, 35%)	17 (18, 48%)	2 (2, 17%)	1 (1, 09%)

In most cases (74 out of 88) the already parasitized egg was abandoned after a brief drumming of the egg with the antennae. It may therefore be concluded that with behaviour B (oviposition) the female leaves a signal on the surface of the CPB egg, a signal which it is then able to perceive by means of its antennae and which induces it to abandon the egg.

The importance of marking. After termination of behavior B, the female generally rubs its abdomen against the CPB egg, a behavior we have called "marking."

As previously observed by us, this behavior is often not performed after oviposition (behavior B without marking). Also in this experiment, the female was seen on several occasions not to mark the egg after oviposition. More precisely, out of 172 behaviors B observed, 71 (41.28%) were not followed by marking.

As can however be seen in table 6, the signal which permits recognition of the fact that the egg has already been parasitized does not always require rubbing. In fact, even eggs which had undergone behavior B without marking were usually abandoned (in 73.81% of cases) by the female upon encountering them again for the first time.

Marking by rubbing does however appear to be important for reinforcing the external signal denoting that the egg has already been parasitized. In fact, as can be seen in table 6, the marked eggs were parasitized upon being visited for the first time only in one case out of 46 (2.17%) and only in 6 cases (13.04%) out of all the times they were visited, as against 5 times (11.90%) and 13 times (30.95%) out of the 42 cases in which the egg had been parasitized but not marked.

A casual event offered the definitive demonstration that rubbing with the abdomen after behavior B is performed in order to mark the CPB egg so as to avoid superparasitization. In this case it was seen that after oviposition a female accidentally marked an adjacent egg which had not yet been visited. When this latter egg was subsequently visited, it was explored with the antennae several times but even though intact it was not parasitized, and this was the only case out of 92 (tab.6). It was parasitized only after it had been turned over. It may therefore be safely concluded that by rubbing its abdomen against the CPB egg after behavior

B the female releases a chemical substance on the CPB egg so that it becomes repellant to the female.

The internal signal and behavior C. The hypothesis that the female may be able to recognize that the host has been parasitized also by means of its ovipositor may be considered to have been confirmed by the trials of experiment E. It was in fact seen that on various occasions the female abandoned the egg already parasitized in the course of previous visits after having performed on it only a single behavior C. For instance, a single behavior C was observed on side "a" in 12 eggs which had been subject to behavior B without marking and in 4 eggs which had been marked, whereas it was never observed on intact eggs. Moreover, a single behavior C was also observed on side "b" of 13 eggs which had been visited and parasitized only on the other side.

Till now the C behavior has only been considered as representing a terminal activity on the egg mass once the female has exhausted its supply of mature oocytes. The purpose of this behavior has been thought to be that of killing off the

Table 6 - Results of trials conducted with 4 CPB eggs visited by one *Edovum* female. These trials were conducted in order to observe parasitoid behavior on already visited host eggs depending on the activity they had been subject to during the first visit, the conditions of the eggs being one of the following: intact, parasitized without marking, or parasitized with marking. A distinction was made between the behavior upon the first visit in the different conditions and that observed for total subsequent visits to the same egg.

initial egg condition	CPB eggs	behavior	first visit	total No. of visits
non stung	92	drumming only	1 (1, 09%)	1 (1, 09%)
		A and/or C	3 (3, 26%)	2 (2, 17%)
		parasitized	88 (95, 65%)	89 (96, 74%)
parasitized without marking	42	drumming only	31 (73, 81%)	16 (38, 10%)
		A and/or C	6 (14, 29%)	13 (30, 95%)
		superparasitized	5 (11, 90%)	13 (30, 95%)
parasitized with marking	46	drumming only	43 (93, 48%)	38 (82, 61%)
		A and/or C	2 (4, 35%)	2 (4, 35%)
		superparasitized	1 (2, 17%)	6 (13, 04%)

remaining intact eggs or it has been ascribed to newly emerged females or assumed to be an activity performed on eggs at an advanced stage of embryo development. Neither of these three possible conditions however were present in our experiment. Behavior C may therefore be assumed to have a further meaning and function, and that is that of exploring and eventually refusing an already parasitized host.

Turning over of the eggs however showed that in the absence of an external marking the internal signal alone is not sufficient to avoid superparasitization. In the majority of cases (70 out of 89, 78.65%) further oviposition was seen to occur in overturned eggs, even if already parasitized.

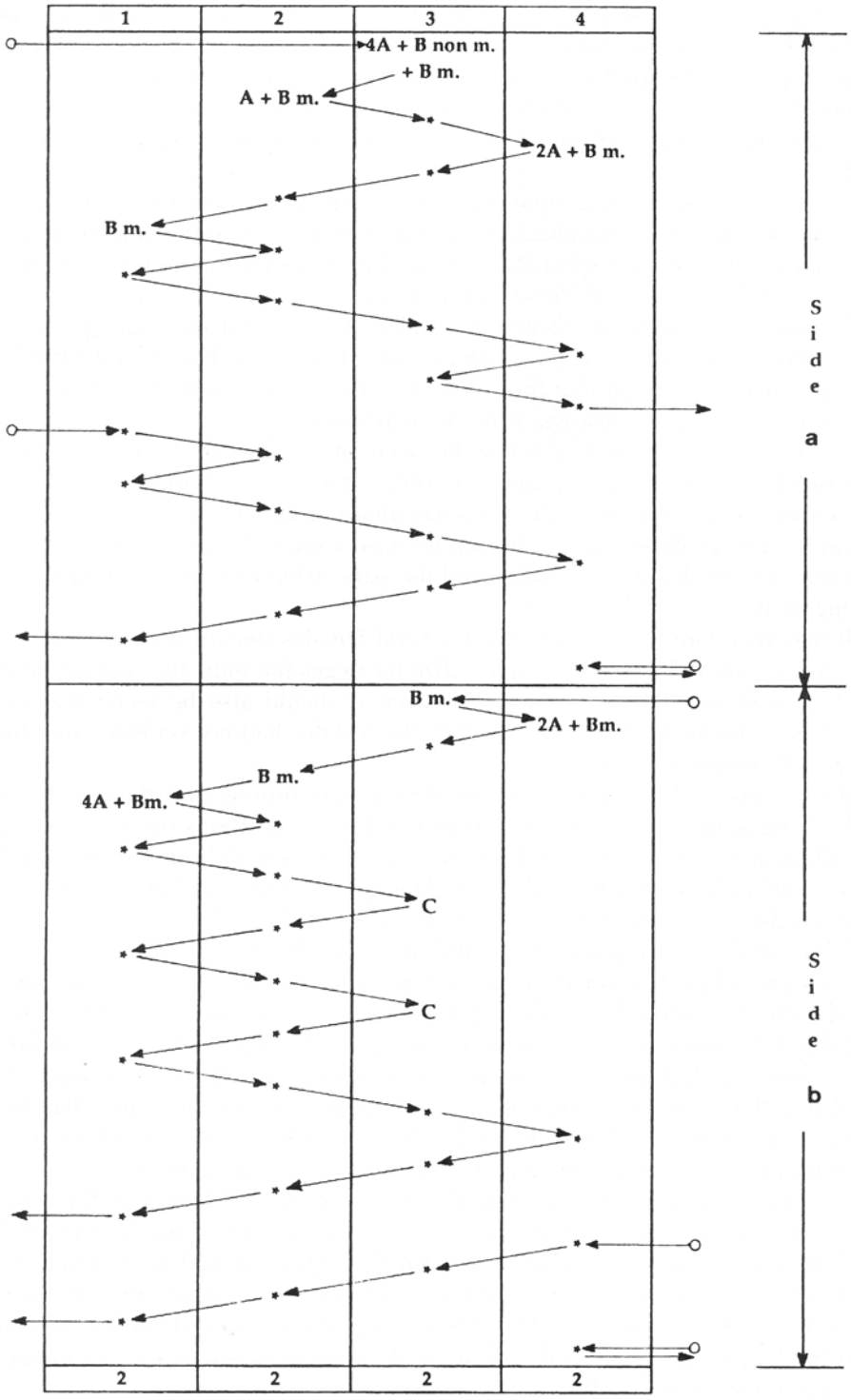
In conclusion, therefore, it may be said that *Edovum* avoids superparasitism essentially on the basis of its perception of the external signal given off by the CPB egg after oviposition even if at times refusal of the parasitized host seems to occur after exploration of the host egg with the ovipositor.

Superparasitization. As can be seen from table 5, 18 eggs (19.56%) underwent two parasitizations and 1 (1.09%) three parasitizations on side "a". These cases of superparasitization however almost always occurred after the female had tried to locate intact eggs in vain because unavailable, and after, during the course of this exploration, it had visited the parasitized eggs several times, abandoning them.

It may therefore be concluded that several females (for instance, 11 out of 23 on side "a") are induced to superparasitize host eggs but only after having unsuccessfully searched for intact ones. Moreover, it should also be noted that upon abandoning the host, at least on side "a", the females had not yet exhausted their reserve of mature oocytes.

As has already been mentioned, the stratagem of turning over the eggs to conceal the marking led the female to perform other ovipositions on the same eggs which had been parasitized shortly before. At the end of the experiment, therefore, as many as 70 eggs out of 92 (76.09%) had been parasitized at least twice, 17 of which three times, 2 four times and one even five times (tab.5).

Difficulty in oviposition. Final mention should be made of the sporadic cases in which two behaviors B were performed on the same egg one immediately after the other. The failure of the female to abandon an egg after having performed behavior B, as it normally does, and the repetition of this behavior on the same egg had already been observed in other experiments. It was hypothesized that these were not cases of superparasitism but that the female had been unsuccessful in oviposition at the end of the first behavior B, after which in fact no marking took place, and that it therefore was induced to make a further attempt before abandoning the egg. Dissection of the CPB eggs at the end of each trial proved this hypothesis to be correct. In fact, out of the 8 cases of this kind recorded, it was seen that only a single oviposition had taken place after the double B behavior in 7 cases, while in one case none at all. It is also worth noting that in 6 of these cases difficulty in oviposition was exhibited by the female at the beginning of the trial, that is on the first egg visited (see, for example, the case reported in fig. II).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IV. Conclusions

Van Alphen (1990) has extensively studied the issue of superparasitism in general. Our study concentrates only on the superparasitic behavior of solitary parasitoids, such as *Edovum*.

Having ascertained that the parasitized host is generally recognized by means of pheronomes released (marking) by the parasitoid on the external surface and/or inside the host during oviposition, the question which still remains to be answered is why superparasitization is so diffuse in nature. The hypothesis that such an occurrence is ascribable to an error on the part of scarcely capable females can today safely be discarded and it may be assumed that the phenomenon is in certain conditions advantageous and that it is therefore an adaptive strategy.

When a female oviposits in a host egg which it has itself already parasitized, the phenomenon is termed "self-superparasitism". On the basis of the findings of the present study, it was seen that the *Edovum* female is very skilled in distinguishing parasitized host eggs from intact ones. Despite this ability, it was seen, however, that under particular conditions self-superparasitization may still occur, in which case we are in the presence of a competition amongst siblings. As only one can survive, this behavior may at first sight appear not to be advantageous. There are however circumstances in which self-superparasitism may be advantageous to the parasitoid. One of these circumstances is given for example when there is a high density of parasitoids or a low number of host eggs available so that the risk of a host egg being parasitized by more than one female is high. It is in cases such as these that the females of the parasitoid compete amongst each other as the greater number of eggs deposited in the host by the first female increases their possibility of winning the competition against other parasitoid eggs subsequently deposited in the same host egg by another female (Van Alphen, 1990).

If these circumstances do not prevail, that is if, as in our experiments with *Edovum*, many CPB eggs are available, self-superparasitization is not advantageous.

The question may also be asked as to whether the parasitoids are capable of distinguishing hosts which contain their own offspring from those containing the eggs of other individuals, that is whether they are capable of distinguishing their own markings from those of other individuals of their species. This question has not yet been fully answered even though the females of several species, such as *Epidinocarsis lopezi*, a parasitoid of the *Phenacoccus manihoti* Homopter (casava mealybug), have been seen to deposit their eggs to a significantly greater extent in hosts parasitized by other females rather than in those parasitized by themselves

Figure II - Results of the trials conducted with 4 host eggs arranged so that the parasitoid could visit them only on one side (side "a"). The eggs were then turned over so that the other side (side "b") could be visited (see fig. I).
The arrows show the movements of the female on the 4 eggs. The asterisk shows where the female stopped and drummed the egg without stinging it; A = host-feeding; B = oviposition; C = rapid sting without feeding or oviposition; Bm = behavior B with marking; B non-m = behavior B without marking. The arrows outside the drawing show parasitoid entry and exit points from the egg mass. The numbers at the top are those given to the four host eggs, while those at the bottom correspond to the number of *Edovum* eggs found in each host egg.

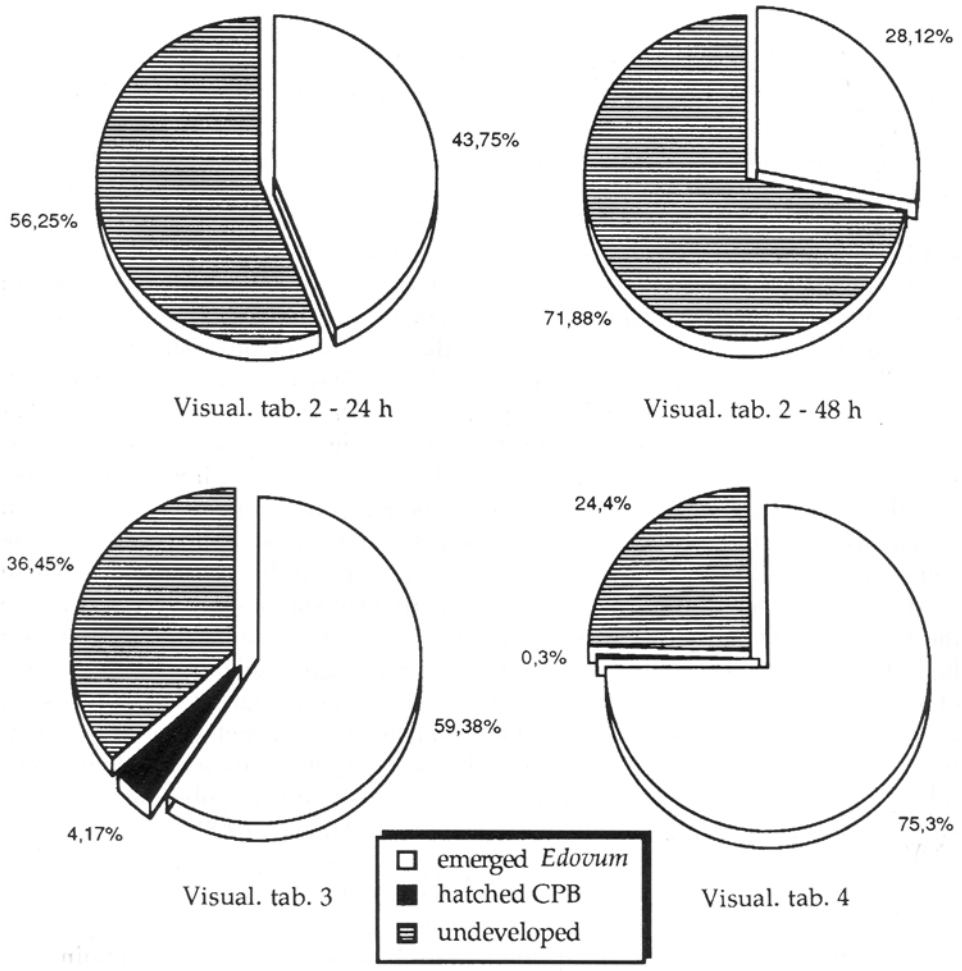

Figure III - Visualization of the results of experiments "b", "c", "d" and of the corresponding tables 2, 3, and 4.

(Van Dijken et al., 1990). In the case of *Edovum* there is no conclusive evidence to suggest that the female is capable of distinguishing eggs parasitized by itself from those parasitized by other females. In *Edovum*, therefore, the distinction between self-superparasitism and conspecific superparasitism put forward by Van Alphen does not appear to be applicable. It can only be noted that the main condition which induces superparasitism as such is the high number of parasitoids compared to the host eggs available (for example, a 1:2 ratio).

When competing individuals are simultaneously present in the same territory, two possible strategies may essentially be adopted i.e., 1) either that of fighting the intruder, which thus avoids superparasitization, or, alternatively, 2) that of permitting the intruder to enter the territory, which thus leads to superparasitism.

Our findings seem to suggest that *Edovum* is more likely to adopt the second strategy. It was in fact observed that, despite exhibiting irritation, more than one female may be active on the same egg mass and that superparasitization occurs more frequently as the density of *Edovum* females increases.

The presence of unparasitized eggs, which were in any case killed, next to superparasitized ones observed in several experiments, is more difficult to explain. Perhaps the presence of more than one *Edovum* female within the limited space of a small box hampers the efficient recognition of the already parasitized eggs.

As regards marking, a considerable difference in behavior was observed amongst the *Edovum* females depending on the individual as well as in the same individual over time. Overall, marking occurs in about 50% of all parasitized eggs. Notwithstanding, it would in most cases appear that the female avoids superparasitizing not only the marked eggs but also the unmarked parasitized ones. In other words, it would appear that the signal denoting "parasitized egg" is given off even if the egg has not been rubbed by the female. Marking, therefore, may be considered as merely reinforcing either the "parasitized egg" or some other chemical signal as yet not identified. As far as the duration of marking efficacy is concerned, our observations would seem to confirm that after 12 hours the female is still capable of recognizing an already parasitized egg, independently of whether it was parasitized by itself or by some other individual.

Moreover, it would seem that even inexperienced *Edovum* females are capable of distinguishing parasitized from unparasitized hosts so that it may be concluded that this behavior is not learnt.

Finally, it may be noted that when a parasitoid comes into contact with an already parasitized host, it is faced with a choice between two conflicting actions, that is either that of ovipositing or that of moving away from the unpleasant signal. Several factors come into play in inducing the parasitoid to either superparasitize or not, such as the need to oviposit, which will be all the more compelling the greater the number of mature eggs in its oviduct, the fact that it has already been able to easily find intact eggs or vice versa that it frequently encountered parasitized ones, and, finally, the eventual presence of other females which may be a disturbing factor, thus leading the parasitoid to be less responsive to chemical marking signals and more competitive towards conspecifics.

In view of the above considerations, it may be safely assumed that superparasitization is in fact an adaptive strategy and particularly advantageous to the female adopting it in competition with other females when the availability of unparasitized hosts is scarce.

Furthermore, even if a certain number of the parasitoid eggs are lost through superparasitization, this behavior is nevertheless also advantageous to the species as a whole, and not only to the individual. In fact, it is an important factor balancing the population dynamics between the host and its parasitoid. It should be remembered that killing off of all host eggs would be clearly disadvantageous to the parasitoid as it would lead to its own extinction. As already seen, in *Edovum* the high density of the parasitoid not only causes the death of the majority of host eggs but also leads to superparasitization associated with a high number of stings to the host eggs. This, in turn, leads to a high death-rate of the parasitoid itself,

thus causing the population density of the latter to drop rapidly and the host:parasitoid ratio to be re-equilibrated. Re-equilibration of course entails the loss of a certain number of parasitoid eggs and larvae, a phenomenon which is typical of population crises in general.

SUMMARY

The present study examines some aspects of the behavior of *Edovum puttleri* Grissell, an Eulophidae Hymenopter, with particular reference to superparasitization i.e., the deposition by the parasitoid of more than one egg within the same host egg. Experiments were conducted by bringing one or more *Edovum* females in contact with a reduced number of host eggs.

More precisely, groups of 4 females were placed together with 8 CPB eggs. The parasitoid was left with the host egg for periods of 2, 24 or 48 hours and its activity was directly monitored by means of a binocular or a videocamera. At the end of these times, the host eggs were either dissected to verify the eventual presence of *Edovum* eggs and/or larvae or allowed to develop so as to record their fate. The various trials showed that: 1. superparasitization is a frequent behavior and a single *Leptinotarsa* egg can be parasitized up to five times; 2. no more than one *Edovum* ever emerges from a superparasitized egg; 3. in case of overcrowding lasting a relatively long time (24 or 48 hours), the percentage of parasitization was high, while that of *Edovum* emergence was low, and all the lower the longer the time of permanence of the females with the host eggs (a finding which may be accounted for by the fact that the host eggs are subject to a high number of stings by the parasitoid, which are therefore detrimental to the development of the parasitoid itself); 4. unparasitized eggs may also be found together with superparasitized ones; 5. *Leptinotarsa* development is almost totally inhibited also in the unparasitized eggs (always due to the stings received from the parasitoid); 6. more than one female together tend to disturb each other, thus leading to reduced and irregular activity on the CPB eggs so that many of these are repeatedly stung but not parasitized while others are superparasitized.

In the experiment in which each group of 4 females were placed together with the highest number of host eggs (24) for 24 hours, the latter were allowed to develop fully. Even in this case, the development of *Leptinotarsa* was seen to have been almost totally inhibited, but the percentage of emerged *Edovum* was higher than that reported for previous experiments. In view of these findings, it may be concluded that for the purpose of parasitoid rearing and perhaps also of its field application, the best ratio of *Edovum* females to host eggs is probably 1 female to 6-8 eggs a day.

In another experiment, observations were conducted on the activity of individual females on groups of 4 host eggs. The eggs were arranged so that the parasitoid could only visit them on one side, after which they were turned over so as to permit the female to visit them on the other side as well. It was seen that: 1. the female is capable of distinguishing parasitized from unparasitized eggs (this is probably due to the fact that a chemical signal is left by the female on the host egg surface, which can subsequently be detected by the *Edovum* with its antennae); 2. in the greater number of cases the female reinforces this external signal by rubbing its abdomen against the CPB egg on which it has oviposited (marking); 3. even when the parasitized egg is not marked, simple oviposition puncturing is usually sufficient to leave a signal indicating that the egg has been parasitized; 4) a female is probably capable of detecting that the egg has already been parasitized also by introducing its ovipositor in the host egg; 5) when the external parasitized egg signal is concealed by overturning the eggs, in most cases the female oviposits again in the same eggs it had already parasitized shortly before on the other side, which suggests that the internal signal alone is generally insufficient to avoid superparasitization; 6) even when the female is capable of recognizing the parasitized egg, it may nevertheless superparasitize it but usually only after having unsuccessfully searched for other unparasitized hosts; 7) if the female, as sometimes occurs, performs more than one oviposition behavior on the same CPB egg, this is due to the fact that its first attempts to oviposit have been unsuccessful.

Comportamento e superparassitizzazione di *Edovum puttleri* Grissell (Hym. Eulophidae), parassitoide oofago di *Leptinotarsa decemlineata* (Say) (Col. Chrysomelidae).

RIASSUNTO

Con la presente ricerca si sono studiati alcuni aspetti del comportamento dell'Imenottero Eulo-

fide *Edovum puttleri* Grissell, con particolare riferimento alla superparassitizzazione, cioè alla deposizione di più uova del parassitoide all'interno di un solo uovo ospite. Per questo scopo sono state compiute osservazioni mantenendo un numero ridotto di uova ospiti in presenza di una o più femmine di *Edovum*.

Gli esperimenti sono stati condotti esponendo a gruppetti di 4 femmine 8 uova di CPB. A seconda del tipo di prova, il tempo di soggiorno del parassitoide con l'uovo ospite è stato di 2 o di 24 o di 48 ore; l'attività delle femmine è stata direttamente osservata al binoculare o tramite videocamera. Le uova ospiti, alla fine delle osservazioni, potevano essere aperte per verificare l'eventuale presenza in esse di uova e/o larve di *Edovum*, oppure venivano lasciate sviluppare per registrare le successive nascite. Dall'insieme delle prove si è riscontrato che: 1) la superparassitizzazione è frequente; un singolo uovo di *Leptinotarsa* può essere parassitizzato anche fino a cinque volte; 2) dall'uovo superparassitizzato non emerge mai più di un *Edovum*; 3) nei casi di sovraffollamento mantenuto per tempi relativamente lunghi (24 o 48 ore) si è notato che, mentre la percentuale di parassitizzazione è elevata, quella di sfarfallamento dell'*Edovum* è invece bassa e tanto minore quanto più si prolunga il tempo di soggiorno delle femmine con le uova ospiti. Ciò perché queste ultime ricevono un numero di punture elevato e quindi letale anche allo sviluppo del parassitoide; 4) assieme alle uova superparassitizzate rimangono spesso anche uova in cui non è stato ovideposto; 5) l'inibizione dello sviluppo della *Leptinotarsa* è però quasi totale anche nelle uova non parassitizzate, sempre in conseguenza delle punture inferte dal parassitoide; 6) più femmine assieme si disturbano reciprocamente per cui spesso svolgono un'attività ridotta ed irregolare sulle uova di CPB, in modo che diverse di queste ultime finiscono per essere ripetutamente punte ma non parassitizzate mentre altre vengono superparassitizzate.

In altre prove ciascun gruppo di 4 femmine è rimasto assieme ad un numero di ospiti più elevato (24 uova) per il tempo di soggiorno di 24 ore e le uova di CPB venivano lasciate sviluppare. È risultata ancora la quasi totale inattivazione dello sviluppo della *Leptinotarsa*, ma la percentuale di sfarfallamento dell'*Edovum* è molto più elevata rispetto ai casi precedenti. Ne consegue che, ai fini dell'allevamento del parassitoide e forse anche per il suo impiego in campo, il rapporto più conveniente tra uova ospiti e femmine di *Edovum* è probabilmente intorno a quello di una femmina per 6-8 uova ospiti giornaliere.

In un altro esperimento si è studiata l'attività di singole femmine su gruppetti di 4 uova ospiti. Le uova venivano disposte in modo che il parassitoide potesse visitarle solo da una parte e successivamente venivano capovolte affinché fossero visitate sul lato opposto. È risultato che: 1) la femmina distingue le uova già parassitizzate da quelle indenni e ciò si spiega ritenendo che venga rilasciato sulla superficie dell'uovo ospite un segnale chimico che poi la femmina di *Edovum* è in grado di percepire con le antenne; 2) nella maggioranza dei casi la femmina rafforza tale segnale esterno strofinando l'addome (marcatura) contro l'uovo di CPB in cui ha appena ovideposto; 3) anche quando la marcatura dell'uovo parassitizzato non viene eseguita la semplice puntura di ovideposizione è di solito sufficiente a lasciare il segnale di uovo parassitizzato; 4) è probabile che la femmina possa percepire che un uovo è già stato parassitizzato pure in seguito all'introduzione in esso dell'ovopositore; 5) si è però constatato che se il segnale esterno di uovo parassitizzato viene nascosto, come avveniva in seguito al capovolgimento delle uova, la femmina il più delle volte torna a ovideporre anche nelle uova che aveva, poco tempo prima e dall'altro lato, già parassitizzato; e che quindi il solo segnale interno, in assenza cioè di quello esterno, non è di solito sufficiente ad evitare la superparassitizzazione; 6) anche quando la femmina è in grado di riconoscere le uova parassitizzate, a volte può pure superparassitizzare, ma di solito solo dopo avere cercato invano altri ospiti indenni; 7) se la femmina, come qualche volta succede, effettua più comportamenti di ovideposizione senza cambiare uovo di CPB, è perché coi primi di questi tentativi non è riuscita a deporre.

Key words: *Edovum*, Eulophidae, Egg parasitoid behavior, Superparasitism.

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