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WALKING AND NON-REPRODUCTIVE BEHAVIOUR IN TWO SPECIES OF *ENCARSIA* (HYMENOPTERA: APHELINIDAE) USED IN STUDIES ON BIOLOGICAL CONTROL OF THE WHITE PEACH SCALE (HOMOPTERA: DIASPIDIDAE)

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ABSTRACT

The parasitoids *Encarsia berlesei* (Howard) and *Encarsia diaspidicola* (Silvestri) are minute insects with a closely similar morphology. Identification of live parasitoids is needed for studies on biological control of *Pseudaulacaspis pentagona* (Targioni Tozzetti). The non-reproductive behaviour of both parasitoids was studied and described on peach trees and in the laboratory for the first time. Walking pace is a good diagnostic character for use with the naked eye. Workers not accustomed to observe live parasitoids can identify them at their habitat, by using a hand lens.

KEY WORDS: Aphelinidae, Diaspididae, *Pseudaulacaspis pentagona, Encarsia,* encounter behaviour, hopping behaviour, stopping behaviour, walking behaviour.

INTRODUCTION

The white peach scale, *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Diaspididae) (hereafter PP), has sporadic outbreaks in France, mainly on peach trees, sometimes on black mulberry and cherry trees, and in the cold parts of the country (mainly at Nancy and in the Paris region), on sheltered catalpa (Ch. Pinet, unpublished data). Integrated pest management of PP in peach orchards, in the Pyrenees-Orientales department, was difficult to implement, because when heavy infestations of the scale occurred, repeated summer sprays of chemicals had to be applied. Biological control of PP with two chalcids (Hymenoptera) was tested in a peach orchard, with releases of small numbers during spring and summer. Both chalcids, *Encarsia diaspidicola* (Silvestri) (Aphelinidae) and *Arrhenophagus chionaspidis* Aurivillius (Encyrtidae), are well-known endoparasites of PP in the tropics. The acclimatization of material introduced from the Reunion Island (Mascarene Islands) was attempted in some commercial orchards of Pyrenees-Orientales. In other regions, PP is controlled by *Encarsia berlesei* (Howard), established in France since 1919 (Poutiers, 1919).

Both species of *Encarsia,* namely *berlesei* and *diaspidicola,* are uniparental (thelytoky). Because of their minute size (0.4-0.7 mm) and close similarity, confusions occurred and controversy arose with regard to their effectiveness against PP in various countries (Sands et al., 1990).

MATERIALS AND METHODS

Difficulties in identifying live *Encarsia*

Several difficulties were encountered during our current work on biological control of PP. First, *E. berlesei* and *E. diaspidicola* were maintained in a small insectary. Rearing the two species without mixing them must be painstaking; mixing happened when routine recommendations were not carefully followed by the workers. A means to monitor cultures was to sample adults at random in cages, from time to time, and to verify morphological characters of dead insects. Their minute size prevented identification of live parasitoids with the naked eye in the cage or under a hand lens in a small container. They were moving frequently, and peculiarities of their habitus when alive are awaiting specific description to be used for identification with the naked eye or under a hand lens.

Secondly, biological control needed scale sampling and assessment of parasitism rates of each *Encarsia* species. Pieces of bark with settled scale stages were sampled on the trunk and the twigs of peach trees. Each bark sample was placed in an emergence box. The emerging adults were counted, to estimate the rate of parasitism of *E. berlesei* apart from that of *E. diaspidicola*. Quality of parasitoids used for the control of PP could be improved by introducing adults of field stocks in insectary cages.

In an attempt to elaborate practical means of species differentiation, we studied the non-reproductive behaviour of both *Encarsia* species.

Behaviour of *E. berlesei* on peach trees

Before starting the laboratory study, we tracked individual *E. berlesei* in a peach orchard with a low density of PP. Scales were scattered on the trunk and the twigs or aggregated at a rate of 10 to approximately 250 nymphs, male puparia and females. They frequently aggregated in small cracks of the bark or under minute bark peelings. More colonies settled on the lower side of the limbs. The size of colonies was from a few mm^2 to about 30 cm^2.

The path of moving *E. berlesei* (tree surface representing a 3-dimensional space) was observed under a hand lens. Behavioural events were not measurable under field conditions.

Materials and methods of the laboratory study

The choice of the experimental device and method was based on peach tree data obtained for *E. berlesei* and on preliminary trials in the laboratory on both this species and *E. diaspidicola*. In order to characterize ethological features for practical recognition, each parasitoid was kept under experimental conditions similar to those of mass rearing, except that it could not exhibit its reproductive behaviour. *E. berlesei* and *E. diaspidicola* were cultured under similar conditions. Hence they were tested with the same device and method.

Both species were observed in a clear polyethylene flat box (0.5 cm high, 5.6 cm long, 4.1 cm wide) under a binocular microscope with the lamp turned off. Behavioural events were easily observed through the transparent lid, with the box placed on a white background. Two symmetric holes (a 1-cm-diameter hole at the centre of each half of plastic wall) were made in the bottom of the box. Insects were introduced through one hole which was shut when the box lay on the white background. A piece of fine-mesh and white-coloured nylon cloth was carefully stuck with odourless glue over the second hole. Insects were collected (using an aspirator) from the breeding cages and introduced into the box by gently blowing air into the mouth aspirator. The function of the clothed hole was to avoid turbulence and over-pressure during this process. A minute drop of honey was placed on the internal surface of the nylon cloth, as food supply.

The age of the parasitoids was unknown. They were sampled at random in a breeding cage. Most of them were assumed to have experienced host handling and oviposition. PP was assumed to produce a kairomonal substance, as stated for some other diaspidid species (Quednau and Hübsche, 1964; Baker, 1976). If the kairomone was volatile, the cage atmosphere was assumed to be saturated with its odour. If the kairomone was not or only slightly volatile, the scale covering of the insects on scale-infested potato tubers used in *Encarsia* cultures were assumed to be impregnated with kairomone. Kairomone traces were tentatively simulated in a flat box: we delicately touched with a fine brush aggregates of PP settled on potato tubers, then it was brushed carefully against the inner side of the nylon cloth. During preliminary trials, a border effect was observed around the two holes in the plastic bottom of the device. However, parasitoids landed on the nylon cloth more often than on the white background at the location of the second hole. This would probably mean that the host-scale kairomone impregnated the nylon cloth more than other parts of the box.

Ten individuals of the same species were placed in the box, simulating the conditions of the mass culture where they frequently encounter. They were left for approximately 3 min in the dark, for a period of recovery before the behavioural events were scored under the binocular microscope. Activities of one individual were recorded continuously for 3 min. Then the box was cleared, cleaned and prepared for the next observation. Experiments included 25 replicates for each species, under the same laboratory conditions: room temperature 20.5 ± 1°C; all replicates were carried out in spring, on sunny days only (laboratory latitude is 43°38′ north); boxes were not exposed to direct radiation. Two scales graduated in 0.5 cm were drawn on the white background on which the box lay, as coordinate axes; one was along the length and another along the width of the box. Graduations were in the field of the binocular microscope. This double scale allowed to measure path and hop lengths within 5 mm. Duration of the main ethological events was timed by stopwatch within 1 sec; furtive acts could thus not be timed.

With this device, we were unable to distinguish quantitatively the part of the walk and hopping dynamics, which was due to random events and encounters between two individuals, from that attributable to the geometry of the closed area. Parasitoids were assumed to have an experience of angular parts of the box similar to those of the cage. The two species were in the same experimental conditions and their non-reproductive behaviours could be compared. Ethological features were comparable quantitatively even if qualitative differences were sufficient for species identification.

In this paper, the closed area is named 'arena' because both *Encarsia* species moved mainly on the bottom of the box, that is to say in a two-dimensional space.

RESULTS AND DISCUSSION

The qualitative data that we obtained were new for Diaspididae-parasitizing *Encarsia*.

Behavioural features observed under outdoor conditions

When not in the presence of scales, the antennae of parasitoids walking on bark were open, with flagella turned forward and being nearly straight.

When the parasitoid encountered an isolated host scale, it drummed the scale cover with its closely set antennae. It left faster when the insect beneath the scale cover was dead.

When it approached a crack in the bark or near a PP aggregate (even without a live host), it turned away from its track. This abrupt change of behaviour at the crack border or at the

scale-colony border was called "border effect." *E. berlesei* touched the border with the tips of its open antennae and moved along the border at the same time. Then it came down to the crack, even if only one live scale insect infested the spot. When it reached dead or live scale insects, the same antennal inspection occurred on the aggregate borders before it got on and drummed on the scale covers. A layer of remaining scale covers from the former generation often covered live individuals, which the aphelinid obviously detected with its antennae.

We thus distinguished between the part of the walk which was random and the part of the walk attributable to the border effect, which was a short-distance foraging towards a bark structure or a scale-insect aggregate.

The observation was often interrupted by an airstream which blew the insect out of the field of the hand lens. When a light puff of air occurred, the aphelinid could jump and opened its wings at the same time. As it was airborne, it could orientate its gliding flight, which always covered a relatively long distance (a few cm).

The parasitoid generally interrupted its walk randomly by short-distance hops (several mm). When it was disturbed, e.g. by an insect landing or moving in the vicinity of its track or by an air stream, its response was a long-distance leap (several cm long) with open wings. The posture was the same as during the gliding flight. With an airstream, a hopping act occurred probably before the gliding flight. Long-distance hopping was termed "flight hopping" in contrast to short-distance hopping which was termed "random hopping." Generally, *E. berlesei* performed a short-lasting stop before a random hop and its wings remained folded during the hopping act.

These qualitative results on peach trees are summarized in Table 1.

Behavioural features observed in the arena

Four categories of activities were recorded during 3 min in the 25 replicates of both species: walking, hopping, encounter between two individuals, and stopping. Behavioural features of the four categories are described in the note to Table 1. Walking was interrupted by a hopping, encounter or stopping act; then it was resumed.

Five peculiar features of walking (abbreviated in the note to Table 1: CS, CR, CZ, IZ, IZ*), three of hopping (RH, IH, FH), five of stopping (RE, LO, GR, TW, SW) and two kinds of encounters (EH, EA) were observed. Figures in Table 1 give the median values of the 25 replicates, the numbers of individuals in which the feature occurred and the range of variation of frequencies for each of the 15 features.

Frequencies of occurrence for CS (path of walking continuous, straight and slow) and CR (path of walking continuous, straight and rapid) in Table 1 did not need statistical analysis for comparison between the two species of *Encarsia*: obviously *E. berlesei* walked slower than *E. diaspidicola* (22 times against 3, and 4 against 22 for slow and rapid walks, respectively). Hence walking pace was a good characteristic for the practical identification of live insects with the naked eye. Certainly both species must be within reach for comparison. However, trained workers can use behavioural characteristics for recognition in the laboratory and in the field.

Angular paths (CZ, IZ) were identical in both *Encarsia* species, but the same difference of pace was visible with the naked eye; in each species, 6–7 and 10–11 individuals did not show the continuous and zigzag path (CZ) and the interrupted and zigzag path (IZ), respectively.

The data showed that 4 *berlesei* specimens were speedy and 3 *diaspidicola* ones were slow. Hence we suggest an additional diagnosis for such a doubtful situation.

Comparison of the frequencies of occurrence of each feature (other than CS and CR) in Table 1 (from CZ to SW) needed non-parametric analysis. The primary interest of the non-parametric

TABLE 1

Behavioural features (CS–SW) in the 4 categories of non–reproductive activities of *Encarsia berlesei* (Howard) and *E. diaspidicola* Silvestri

	Walking					Hopping			Encounter		Stopping				
Encarsia spp.	CS	CR	CZ	IZ	IZ*	RH	IH	FH	EH	EA	RE	LO	GR	VW	SW
berlesei (on peach trees)	25	0	23	20	0	2	0	17	0	0	1	12	9	0	0
berlesei	1	0	1	1	1	4	0	5	2	3	0	1	1	0	0
(in the arena)	22	4	19	14	2	21	11	23	22	10	8	15	20	7	9
	0–1	0–2	0–1	0–1	0–1	1–19	1–15	0–44	0–13	0–7	0–1	0–4	0–4	0–5	0–6
diaspidicola	0	1	1	1	0	1	0	3	0	1	0	1	1	0	0
(in the arena)	3	22	18	5	0	17	8	19	7	18	7	20	21	2	2
	0–1	0–1	0–1	0–1	–	0–10	0–6	0–34	0–2	0–7	0–1	0–5	0–3	0–2	0–1

Each of the 25 individuals was observed under a hand lens during 3–4 min for *E. berlesei* on peach trees and under a binocular microscope during 3 min in the arena for *E. berlesei* and *E. diaspidicola*. Figures in the 1st row are the number of individuals in which the feature occurred; in the 2nd and 5th rows, the median (the 13th order statistic of the 25); in the 3rd and the 6th rows, the number of individuals in which the feature occurred; in the 4th and 7th rows, the range of variation of the 25 frequencies of feature occurrence.

Explanation of abbreviations: (1) **Walking. CS**: continuous, straight and slow; **CR**: continuous, straight and rapid; **CZ**: angular (in zigzags), each new straight segment of path being at an angle of approximately 70° with previous one; **IZ**: interrupted by one or more short-lasting stops and in zigzags at an angle of 70° (the stop was too short to be timed with a stopwatch and was included in the walking time); **IZ***: during some stops of IZ path, the insect turned in circle around itself, at an angle of 360° toward the right or the left, then it continued on its path. (2) **Hopping. RH**: random hopping was a short-distance leaping (frequently about 5 mm long, otherwise less than 10 mm), with folded wings and a short-lasting stop of the insect at the point of jumping; stop lasted about 1 sec; **IH**: intermediate hopping was intermediary between a short- and a long-distance leaping (from 5 mm up to 15 mm long), with folded wings and without a stop. Two to six short-distance hops often followed one another. This feature was triggered by an encounter with a congener. Classifying events as IH was unsatisfactory because the experimental device did not allow observation of very fast postures. We felt, however, that they occurred. For instance, a stop lasting a fraction of a second could not be recorded by observation under a binocular microscope and would need analysis of a video-camera recording; **FH**: flight hopping, as described in the text on peach trees, was a long-distance jump (several cm long), with open wings. Its length was frequently 3.5 cm and reached 5 cm for a specimen of *E. diaspidicola*. (3) **Encounters. EH**: encounter with hopping happened when the antennae of the observed insect touched those of a congener; this triggered immediate hopping; **EA**: encounter with antennation (mutual drumming on antennae between two encountering insects) which lasted 1–2 sec before insects resumed walking. (4) **Stoppings. RE**: insect rested with antennae folded against head; **LO**: insect stopped on the lookout with antennae raised upwards; **GR**: insect stopped for grooming which involved successively different pairs of tarsi and tibial spurs for preening body and appendices; **VW**: insect stopped and immediately it raised and vibrated its wings; **SW**: insect stopped with antennae kept ahead and swung. Successively, it stood on its legs, stretched anterior legs, at the same time bending the posterior ones; then it inversed stretching and bending movements of legs. One or many swingings occurred which might last up to 6 sec.

procedure (individual data were not normally distributed) was centered on the location (median, which has replaced mean of normal distribution) of each population (i.e. population of 25 figures for one behavioural feature and each species). Following Tomassone et al. (1993), data referred to as paired replicates (25 pairs of replicates for each behavioural feature, i.e. 0 or 1 for CZ, IZ and IZ* in Table 1 and free-distributed figures for RH to SW) are representing pairs of *berlesei* and *diaspidicola* observations. Thus a suitable procedure was the distribution-free sign test of Fisher (Hollander and Wolfe, 1973). Calculations with a given level of significance were tedious and statistical analysis was not reported in the discussion or in a second table.

It was enough to say that *E. berlesei* was hopping more often than *E. diaspidicola*. Hopping, encounter and stopping did not provide a sound basis for distinguishing between species, as it became obvious by comparing data in Table 1. Walking pace was thus the single non-reproductive character of identification.

When some doubt persists as to species separation by the naked eye, salient morphological differences can be found, located at the posterior end of the body and easily seen on live insects under a hand lens:

1. The anterior wings are wider in *berlesei* than in *diaspidicola*.

2. At rest, the wings are folded on the abdomen but they overlap more completely in *berlesei* than in *diaspidicola*.

3. In *berlesei*, the margin of the anterior wings is broadly rounded apically and both wings form a rounded and continuous outline posteriorly. In *diaspidicola*, the margin of the anterior wing is narrowly rounded apically and both wings form a broken outline posteriorly, like a rounded indentation.

4. Through the translucent disc of the wings, the posterior end of the abdomen is easily seen. The folded wings are notably wider than the posterior end of the abdomen in *berlesei*; they hardly reach the width of the abdomen in *diaspidicola*.

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