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Food as a Cue Factor Controlling Adult Diapause in the Lady Beetle *Harmonia sedecimnotata* (Coleoptera, Coccinellidae)

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Abstract—The lack of aphids in the diet seems to be the only external factor that induces diapause in the laboratory strain of *Harmonia sedecimnotata* originating from southeastern China (Guanchzhou environs). This diapause may last for months, but it can be terminated fast at any time provided the aphid diet is resumed. However, the rate of reactivation indicated by the time when oviposition is resumed, depends on the amount aphids supplied. Appropriate experiments made it possible to clearly distinguish the common metabolic role and the signal action of this specific food. The latter function is most likely perceived via chemoreception. The investigated reaction to specific food is as strong and precise as the well known insect response to photoperiod.

This experimental investigation was motivated by previously published assumptions (Zaslavskii, 1996a, 1996b) that, possibly being manifestations of one and the same physiological mechanism, environmental reactions determining the seasonality of insect development can be considered members of a common series. If this is the case, then, firstly, reactions to photoperiod, temperature, food, and intrapopulational interactions are initially equivalent in the sense that each of them may become dominant, secondary or silent at all in the course of the life cycle evolution. Secondly, it must be admitted that each of them must be investigated in the same way as the most fundamentally investigated photoperiodic response. In the first place this means that the actual inducing factor ("inductive event") and, at least, general features of the operation of receptive mechanism (qualitative or quantitative reception etc.) are to be revealed for reactions to temperature, food, or other factors.

The reaction to food, widespread in insects and most accessible to laboratory investigations was chosen as an example of a poorly studied reaction determining presence or absence of diapause. The tropical lady beetle *Harmonia sedecimnotata* (Fabr.) was found to be a convenient object for investigations of the food reaction. In this species, only the lack of aphids in the diet induces a diapause which may last for months, but quickly ceases as soon as the contact with aphids is resumed. We failed to reveal any reaction to photoperiod in this species.

The present paper is mostly concerned with just the initial stage of this reaction, i.e. the acting factor and perception of it. There is no question as to whether or not the specific food, aphids, is a cue factor. It is, because its presence or absence switches development from one of the alternative ways to another. The point to be elucidated is the essence of this signal. In which way does the primary and obvious role of the food as food proper (metabolic role) correlate with its signal role? Does an insect percept as a signal the fact of presence or absence of satiation by itself, or there is some specific sensor mechanism for cue effect of food, perceiving some of its specific characteristics, not necessarily associated with satiation or hunger?

MATERIAL AND METHODS

A laboratory strain of *H. sedecimnotata* from southeastern China was used in the study. This species is wide spread in Pakistan, India, Vietnam, China, the Philippines, and Malaysia (Bielawski, 1959; Hoang, 1983). The original material was collected in fields in the environs of Guanchzhou on hemp infested with the aphid *Phorodon cannabis* Pass. and on corn infested with the aphid *Rhopalosiphum maydis* Fitch. on June 12, 1990, during mass emergence of beetles from pupae.

In laboratory, the peach aphid (*Myzus persicae* Sulz.) bred on horse beans (*Vicia faba* L.) served as a food source. The material used in quantitative experiments with reactivation was prepared according to the following scheme. Young beetles matured while feeding on aphids, and laid eggs for 15–20 days more. Thereafter they were fed with 10% sugar solution only to induce the diapause. All experiments started after

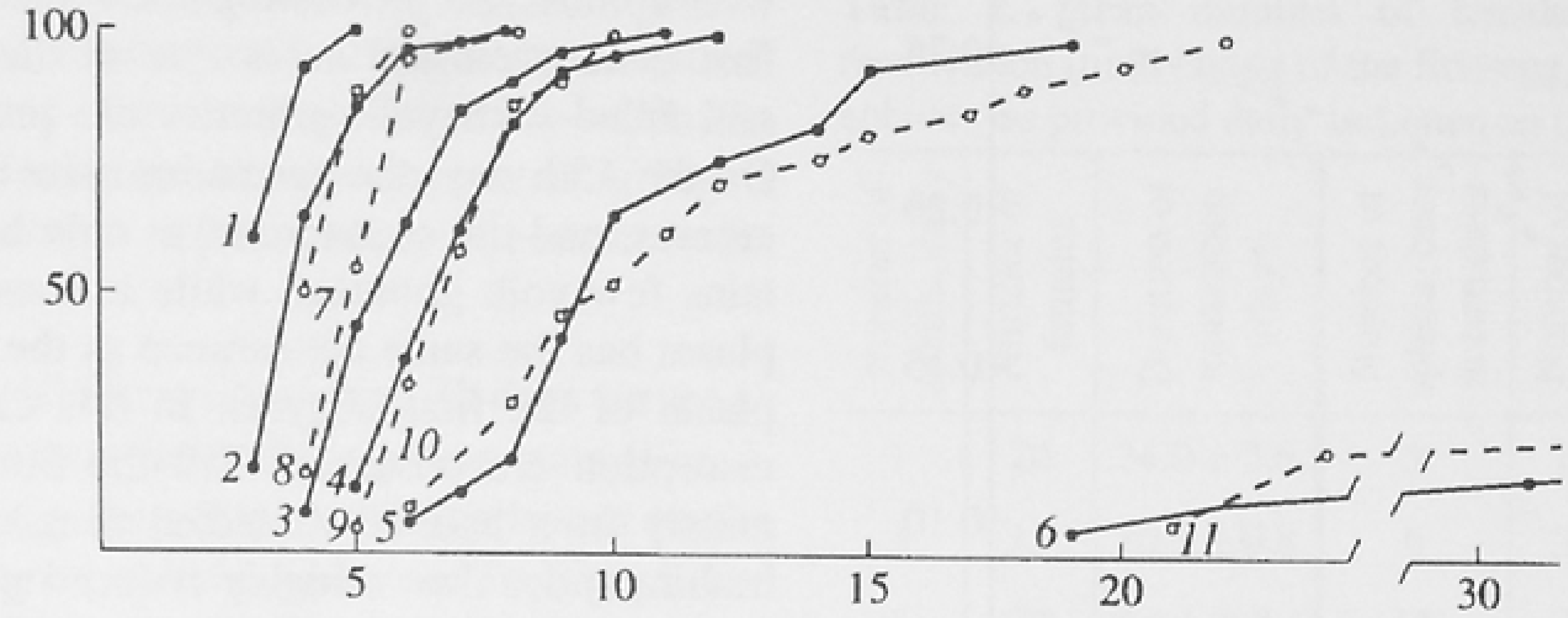

Fig. 1. Influence of the number of aphids in daily ration on the dynamics of reactivation (until laying a first egg) in diapausing females. *Solid lines*—daily feeding with aphids: (*1*) 50 aphids, (*2*) 25, (*3*) 12, (*4*) 6, (*5*) 3, (*6*) 1. *Dotted lines*—feeding once in two days: (*7*) 50 aphids, (*8*) 25, (*9*) 12, (*10*) 6, (*11*) 3 aphids. *Vertical axis*—number of females that have started oviposition (%); *horizontal axis*—time till laying a first egg (days).

30 days of diapause. Females were placed individually in Petri dishes 9 cm in diameter and, with carbohydrate feeding preserved, were fed with a certain number of aphids (in different variants, 1, 3, 6, 12, 25, and 50 aphids per day and also 3, 6, 12, 25, and 50 aphids once in two days). Laid eggs were counted daily.

To determine whether females are able to react to presence of aphids at a short distance without a possibility to touch them, a pot with beans and aphids sitting thereon was isolated by a double layer of kapron gauze cloth. This pot was placed at the bottom of a 3-liter glass cylinder covered with cheese-cloth. Beetles prepared for the experiment according to the foregoing scheme were placed in the cylinder and supplied with carbohydrate food. In different variants, 25, 50, 500 and 1000 aphid nymphs were placed on plants in these cages. Aphids were replaced daily to maintain a given quantity. On the 5th, 10th, and 15th days of keeping in the cylinder, females were dissected to determine the state of their ovaries.

RESULTS

Induction of Food Diapause and Its Features

Giving a permanent opportunity to feed on aphids, i.e., to use food necessary for the larval development, *H. sedecimnotata* beetles, having emerged from pupae, become mature and start intensive oviposition. At 25°C the oviposition lasts continuously for 3–4 months, gradually decreasing in intensity until total termination.

However, when beetles in full oviposition are deprived of aphids, they lay all the remaining mature egg during 2–3 days and then completely terminate reproduction and aggregate in folds of paper and other recesses, manifesting the typical behavior of diapausing coccinellids. When offered additional carbohydrate food (sugar syrup), beetles well survive over 30–40 days despite the constant temperature of 25°C and, moreover, are able to restart oviposition fast when feeding with aphids is resumed. Thus, the typical behavior and the capacity for long-term survival and reactivation prove that termination of contact with aphids induces imaginal diapause in *H. sedecimnotata*, all other conditions being the same.

However, the imaginal diapause is primarily a reproductive diapause. That is why its most direct manifestations are termination of vitellogenesis and oocyte growth and such changes in ovaries that to a certain degree revert them to a state in which they had been before the beginning of maturation.

In *H. sedecimnotata*, each ovary consists of 28 ovarioles connected with paired oviducts. A characteristic feature of this species is that each ovariole contains at any moment only 2 follicles. In this feature our object differs from the previously studied *Chilocorus bipustulatus* L. and *Coccinella septempunctata* L. (Vagina, 1974), in which the vitellarium is a chain of 4–5 follicles. In *H. sedecimnotata*, the first follicle contains either a young oocyte of the 1st generation with transparent cytoplasm, or a previtellogenic oocyte of the 1st generation with mat-white cytoplasm, slightly enlarged owing to deposition of a "white yolk." Oocytes of the 2nd generation in the second follicle may be on various growth stages, they are yellowish or yellow through deposition of yolk granules.

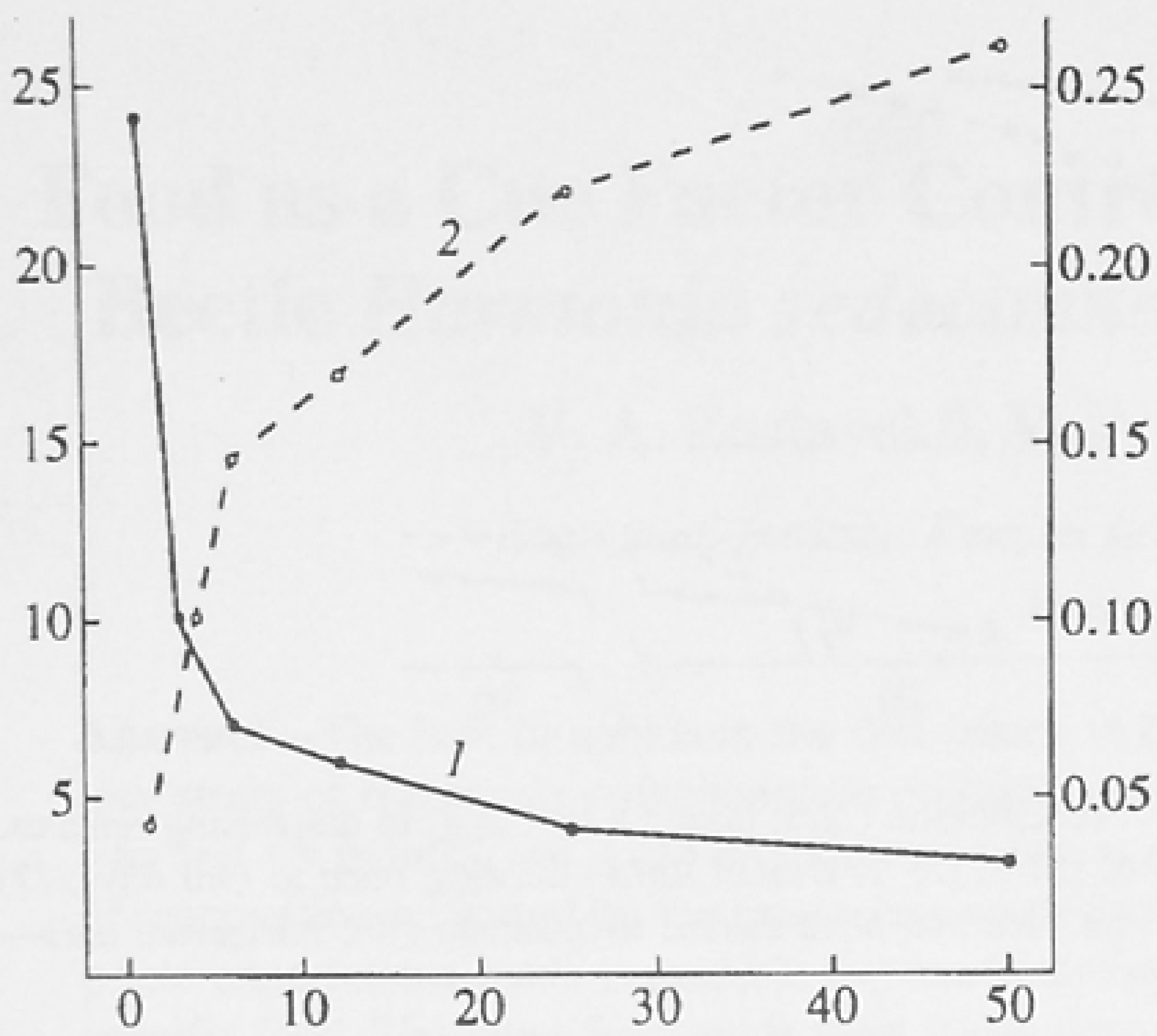

Fig. 2. Influence of the number of aphids in daily ration on the mean duration (T) and rate ($1/T$) of female maturation in reactivation (until laying a first egg). *Vertical axes*: *left*—duration (days), *right*—rate; *horizontal axis*—number of aphids per female per day.

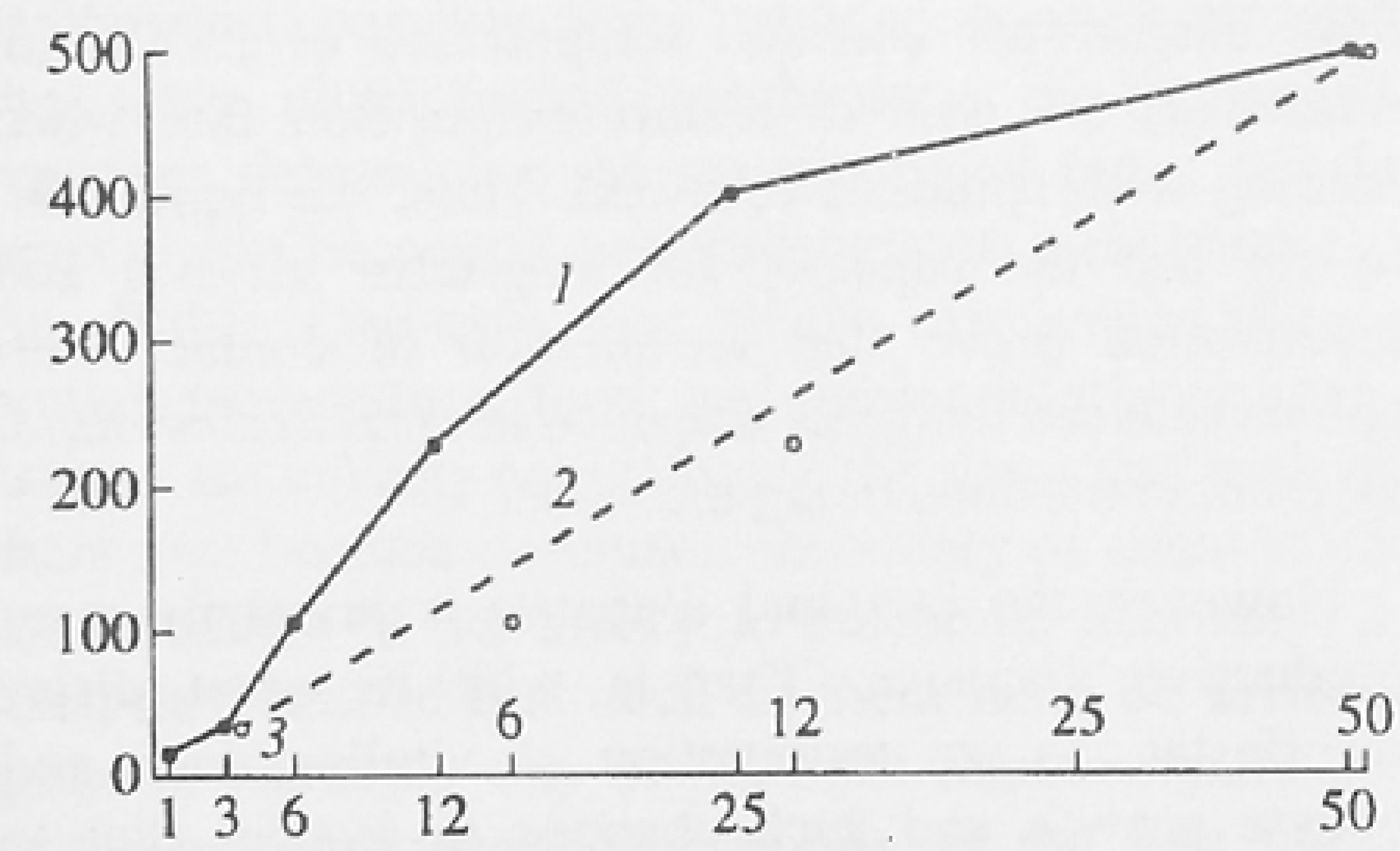

Fig. 3. Influence of the number of aphids in daily ration on the mean total fecundity during 30 days after reactivation with the same number of aphids plotted: (*1*) on natural scale, (2) on logarithmic scale. *Vertical axis*: number of laid eggs; *horizontal axis*: *lower scale*—number of aphids per one female per day, *upper scale*—same on the logarithmic scale.

After eclosion of females from pupae, ovarioles are represented only by germariums. Three days later, oocytes of the first generation are already present, and germariums are substantially enlarged. By the 5th day new oocytes of the 1st generation are already separated, while in older oocytes production of yolk granules in the cytoplasm is observed. On the 10th day in part of females ovaries already contain mature chorionated eggs; first oviposition is recorded on the same day.

The above-mentioned pattern of how this active state is interrupted and diapause formed is essentially supplemented by the results obtained in dissection of females. On the 7th day after termination of feeding with aphids, the previtellogenic ("mat") oocyte of the first generation and an oocyte of the 2nd generation still filled with yolk granules are present in ovaries. On the 15th day, the germarium size significantly decreases, and the second oocyte only occasionally contains few yolk granules, while in most cases its cytoplasm has the same appearance as the mat-white cytoplasm of the first oocytes. In this case, no signs of resorption are observed. Till the 30th day, approximately in a half of dissected females ovarioles are nothing more than a highly reduced germarium in the form of a thin distally dilated tube and a pedicle with a yellow body, an indication of previous ovulation. In the rest of females, at the same general state of ovarioles, germarium is followed by a small transparent oocyte of the 1st generation.

It is this state occurring after 30 days of the absence of aphids in the ration that was used as a starting position in all the following experiments on reactivation.

Reactivation of Diapausing Females

When feeding with aphids is resumed and they are abundant, females that have diapaused for one month reactivate and very fast, in 3–5 days, start intensive oviposition (Fig. 1, *1*).

The following experiments were aimed to obtain at least preliminary quantitative information about how both the reactivation itself and the further state of females depend on the number of consumed aphids. In different variants, each female was supplied with 1 to 50 aphids per day (see "Material and Methods"). Daily counts gave curves describing the dynamics of reproduction resumption (Fig. 1), the mean duration of maturation for each experimental group (Fig. 2, Table 1), and the mean total fecundity during 30 days of oviposition (Fig. 3, Table 2). The same indices were also obtained in a special experiment when beetles were supplied with the same mean number aphids per day, but only once in two days (Tables 1 and 2).

Before starting this series of experiments, we assumed that reducing the number of aphids in the daily ration would reveal the threshold quantity, below which feeding with aphids is no longer an activating factor. However, an unexpected and surprising fact was observed: at the given initial state of females, feeding even with one aphid per day causes full maturation in part of females, and three aphids per day are enough for maturation, although significantly delayed, of 100% females (Fig. 1). Recall that in the total absence of aphids diapause may last for one more month.

Figure 1 demonstrates a gradually lagging onset of maturation and its constantly increasing duration. Generalized indices of maturation dynamics—duration and mean rate of maturation—are presented in Fig. 2 and Table 1 in relation to the number of contacts with aphids.

The mean overall fecundity during 30 days serves as a quantitative characteristic of the active state (Fig. 3, Table 2). It is easily seen that two dependences on the number of consumed aphids, presented in Figs. 2 and 3, are obviously different.

An interesting feature was revealed in experiments when aphids were offered not daily, but once in two days. At equal mean numbers of aphids per day, indices of mean duration of maturation and mean fecundity were practically the same (Tables 1, 2).

In spite of an as yet low number of experimental points, the difference in shape between curves in Figs. 2 and 3 is hardly occasional and, consequently, their interpretations must be different. The fecundity logarithmically depends on the number of consumed aphids. Obviously, it is a "dose-effect" dependence, as supported by its linearity with "dose" plotted on the logarithmic scale (Fig. 3). By contrast, curves illustrating the influence of the number of aphids on the duration and rate of maturation (Fig. 2) are characterized by a slope change (break) once the daily number of aphids exceeds 6. With this information in hand, one may suppose that the curve segments corresponding to numbers of aphids from 1 to 6 and from 6 to 50 may reflect different aspects of the influence of this specific food on the reactivation and maturation.

Thus, daily consumption of even a single aphid stimulates diapause termination and onset of oviposition in part of females, while total absence of aphids induces and maintains diapause. A situation which is intermediate between "yes" and "no" can be conceived, when aphids are present nearby but are beyond the reach of beetles.

In a corresponding experiment, diapausing females were kept in a cage together with aphids, but were separated by an air-permeable barrier (see "Material and Methods"). The results—a slight impetus to reactivation—are presented in Table 3. So, one can suppose existence of a volatile factor and a distant stimulation of maturation.

Table 1. Mean duration of female maturation upon reactivation (until laying of the first egg) when feeding with aphids was provided daily and once on two days

Number of aphids, daily	Number of females	Duration of maturation (days)	Number of aphids, once in two days	Number of females	Duration of maturation (days)
1	20	24.0 ± 3.6	3	16	30.6 ± 9.4
3	15	10.3 ± 0.8	6	20	11.6 ± 1.0
6	23	7.2 ± 0.34	12	21	7.3 ± 0.2
12	17	6.1 ± 0.25	25	20	5.4 ± 0.2
25	14	4.4 ± 0.12	50	7	4.4 ± 0.2
50	46	3.5 ± 0.2	–	–	–

Table 2. Mean total fecundity during 30 day after reactivation (25°C) with aphids provided daily and once on two days

Number of aphids, daily	Number of females	Mean fecundity	Number of aphids, once in two days	Number of females	Mean fecundity
1	20	12.0 ± 2.3	3	16	8.0 ± 2.0
3	15	31.0 ± 5.0	6	20	32.2 ± 4.5
6	23	106.0 ± 6.4	12	21	105.0 ± 5.0
12	17	234.0 ± 16.0	25	20	205.0 ± 14.4
25	14	400.0 ± 24.6	50	7	369.0 ± 44.6
50	46	504.0 ± 26.6	–	–	–

Table 3. Results of dissection of diapausing females in reactivation by the smell of aphids (without contact)

Day of dissection	number of aphids							
	25		50		500		1000	
	Number of dissected females with different states of ovaries							
	act.	diap.	act.	diap.	act.	diap.	act.	diap.
5	0	15	0	15	6	8	5	9
10	0	15	0	15	3	11	9	6
15	0	15	0	20	0	14	6	11

Note: act—two developing oocytes were found in ovaries, diap—only germarium and (in part of females) one small oocyte of the 1st generation with transparent cytoplasm were found in ovaries.

DISCUSSION

The object of this study was a fortunate choice. In contrast to *C. septempunctata*, in which both the photoperiodic reaction and the food reaction are present and interact with each other (Savoiskaya, 1960; Hodek, 1973, 1986; Sem'yanov, 1986; Zaslavskii and Vagina, 1996), in *H. sedecimnotata*, only the second of these was found. This comparison confirms once more the supposition that any of physiological reactions controlling the seasonal development, secondary, or silent at all, may become dominant in the evolution of life cycles.

The distinct, not complicated by influence of other factors, food reaction facilitates its special investigation. The main target of the present study was to come close to an understanding of the course of this reaction and, in the first place, of its initial stages. This problem was the objective of experiments on limited feeding with a specific food—aphids.

Initially, it was assumed that a threshold quantity would be revealed in going from 1 to 50 aphids, below which reactivation no longer occurs. However, it was found that, at least in the given initial state of the material used in this study (see above), no threshold exists, because the stimulating influence starts with a single aphid was provided and then gradually increases.

This brings up the key question of the present study: what aspect of feeding on aphids is a signal that switches beetles from diapause to active state? One can imagine two kinds of the cue effect of food. In one case, this is the quantity of food in itself, i.e. the degree of female's satiation. In other case, signal by itself may be devoid of nutritional value, being perceived by a certain sensory system.

An example of a direct influence of satiation is easily observable in Fig. 3 and Table 2 in the form of a relationship between the number of consumed aphids and that of laid eggs. The relationship is of the usual "dose-effect" type, characterizes the state of already reactivated females, is unrelated to the reaction which determines activation, and is not a cue reaction.

A second correlation between the number of aphids and reactivation (Fig. 2), of special interest for us, was revealed in the same experiments with the same material, but it is obviously different from the preceding one. This suggests that not the degree of satiation, but another cue emanated by aphids induces the active state of females. This is indirectly confirmed by acceleration of their maturation and consequently a sharp increase of the signal at the quantities of aphids (1–3) with negligible nutritional value. A direct evidence in favor of the non-nutritional character of the signal perceived in contact with aphids is furnished by the first results of experiments which revealed weak but clear reaction to some volatile factor emitted by aphids. Data of this kind point to the truly cue character of the reaction to food and indicate the possibility of its association with chemoreception.

Returning to the curves in Fig. 2, we see that their shape clearly suggests that they are formed by two different sets of points, reflecting two different dependences on the number of aphids. Possibly, the first of these is the dependence of the induction force (steep parts of the curves), and the second, the dependence of the rate of oocyte growth (gently sloping parts).

Such interpretation of the curves in Fig. 2 is based on the following. One female can consume ca. 50 aphids per day. However, a sharp increase in the rate of reactivation and maturation is observed in the range from 1 to 6 aphids per day, i.e. at the poorest ration (Fig. 2, Table 1). In particular, a significant difference in time of oviposition onset is obvious (Fig. 1). In contrast to the range from 1 to 6, increasing the ration from 6 to 50 aphids causes only a slight decrease of the mean duration of maturation (Fig. 2, Table 1), and the time of oviposition onset is shifted by two days only (Fig. 1). It is possible that, with even a small number of aphids (ca. 6 per day) consumed, the overall induction effect reaches a value when the period required to develop a hormonal impetus towards oogenesis is reduced to its natural limit. That is why further increase in the number of aphids does not influence the rate of reactivation in itself, and only slightly accelerates the growth of oocytes.

What is the inductive event in the reaction of *H. sedecimnotata* to food determining the diapause? Having considered all the above data, we conclude that this is contact with prey, its capture, and perception of stimuli emitted by prey. The following—satiation and assimilation of the prey—produces the result illustrated in Fig. 3.

In conclusion, we are forced to accept the fact that the data presented here raise problems rather than provide answers. However, some of important features of the reaction to food and comparison of this reaction with the photoperiodic reaction can be already outlined.

(1) In photoperiodism, the long or short days are perceived by the mechanism of "photoperiodic clock" and, by definition, create a single impulse per day. In the reaction to food of the predatory ladybird, the inducing factor acts most probably via chemoreception and is of multiple nature.

(2) The strength of photoperiodic induction gradually changes, depending on the length of day and night (Zaslavskii, 1984). In the food reaction studied, the strength of induction (per day) also changes gradually, depending on the number of contacts with aphids. Thus, in both reactions, the inducing factor is perceived quantitatively.

(3) Quantitative perception is associated with the common feature of photoperiodic and food reactions, i.e. with the existence of a distinct threshold value of the overall effect of induction, to be achieved for the final effect of the reaction to occur (e.g. for termination of diapause). The existence of such a value follows from the negative correlation between the period of action and the strength of a factor (Saunders, 1976; Zaslavskii, 1984, 1995). This is particularly obvious in the food reaction of *H. sedecimnotata*, because in this case the strength of induction is arbitrarily assigned by an experimenter, instead of being indirectly estimated in special experiments.

(4) Unexpectedly, it was found that the exactness of the reaction to the number of aphids is comparable with the precision of the photoperiodic reaction. This was demonstrated by an experiment when aphids were provided once in two days, rather than daily. At equal mean daily amounts of consumed aphids, the results, i.e. duration of maturation, were the same (Table 1).

Thus, in spite of the necessity for further investigations, the first results suggest that the food reaction is a natural component of a series that includes reactions to photoperiod and temperature (Zaslavskii, 1984) and that is why all these reactions should be studied and interpreted using the same paradigm. Recall that just this was the hypothesis to be tested in the present study.

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