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Abnormal Photoperiodic and Phototactic Reactions of the Beetle, *Epilachna vigintioctopunctata*, Reared on Sliced Potatoes

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Epilachna vigintioctopunctata could be reared on sliced potatoes for two generations, but adults obtained were smaller than the normal adults fed on the host foliage, and laid less number of eggs. These adults showed abnormal phototactic response, and some of them continued to oviposit when they were transfered into short-day photoperiod. Adults reared on sliced potatoes only after the adult emergence could respond to short-day photoperiod to stop their ovarian development, but they did not enter diapause. The inhibition of diapause incidence seemed to be due to the incompleteness of fat body development by the nutritional deficiency in potato tubers.

INTRODUCTION

Both quality and quantity of diets are the important factors affecting the photoperiodic response in insects (DANILEVSKY, 1957; BECK, 1968; SAUNDERS, 1976). However, only several examples have been reported on the alteration of the photoperiodic induction of diapause by the diets in phytophagous insects.

MIYAKE and HUJIWARA (1951) reported that the proportion of diapause incidence under short photoperiod in *Naranga aenescens* was influenced by the developmental stages of the host plant given as the diet. In the pink bollworm, *Pectinophora gossypiella*, oil contents in the artificial diet affected the diapause incidence: diets containing much oil produced high percentages of diapause larvae (BULL and ADKISSON, 1960; ADKISSON, 1961).

Investigating the effect of nutrition on the photoperiodic response seemed to bring a key to understand the mechanism of photoperiodic induction of insect diapause.

On this point of view, phytophagous ladybird beetles, *Epilachna vigintioctopunctata*, were reared on silced potatoes which contained more starch but less amounts of lipids and proteins than the host plant leaves, and their photoperiodic response was compared with that of beetles reared on the plant leaves.

MATERIALS AND METHODS

Stock cultures of the beetle were collected on Solanacean plants in a farm of our

laboratories in the middle of August in 1977.

In the first series of experiments, the insect was reared on sliced potatoes (variety: May queen) throughout its life cycle. Duration of larval and pupal development and pre-ovipositional period (period from adult emergence or photoperiodic transference to the day when cumulative number of egg masses exceeded 1/2 of the total number of females) were recorded and pupae were weighed.

Phototaxis of 3rd-4th day adults was tested individually in a simple black box ($38 \times 14 \times 10$ cm) having an orange light on one side.

Oviposition and feeding of adults were observed before and after the change of photoperiod from a long day to a short day.

The second generation was also reared on sliced potatoes.

In the second series of experiments, the adults which had been reared on the host foliage, *Solanum tuberosum* or *Solanum carolinense*, in their larval stages, were raised on sliced potatoes after the adult emergence in order to observe the photoperiodic reactions of these adults.

The diet was changed every other days.

Numbers of egg masses were counted and pre-ovipositional period and pre-diapause period (period from adult emergence to the day when all insects ceased feeding) were calculated.

All experiments were conducted under a long-day photoperiod (16 hr light-8 hr dark) or a short-day photoperiod (10 hr light-14 hr dark) at 24–27°C.

RESULTS

Rearing on sliced potatoes throughout life cycle

Larvae which hatched from an egg mass of *Epilachna* were divided in two groups, and one was reared on sliced potatoes and the other on the foliage of *Solanum carolinense*. One of the results was shown in Table 1. It can be seen that the duration of larval period elongated remarkably when reared on sliced potatoes and the weight of the pupae obtained was about one half of that on fresh leaves.

When rearing on sliced potatoes continued after the adult emergence, they were able to oviposit (4–31 eggs per batch, 15 on average), but significantly less than the normal adult, after the elongation of pre-ovipositional period.

Table 1. RESULTS OF REARING *Epilachna vigintioctopunctata* ON DIFFERENT FOODS

Food	No. of larvae inoculated	Larval instar period (days) 1st	2nd	3rd	4th	total	Pupal period (days)	% pupation	Pupal weight (mg) mean±S.D.	Pre-oviposi-tional period (days)
Sliced potato	22	4.8	4.0	6.1	7.9	22.8	4.0	50	14.6±2.9	18
Fresh leaf	22	2.9	2.5	2.1	5.0	12.5	4.2	100	27.0±3.2	11

Table 2. PUPAL WEIGHT OF SECOND GENERATION ON SLICED POTATOES

Food	No. of pupae examined	Pupal weight (mg) mean ± S.D.
Sliced potato	17	16.4 ± 3.0
Fresh leaf	40	28.7 ± 2.3

Larvae which hatched from these eggs appeared normal and could grow up to the adult on both sliced potatoes and fresh leaves (Table 2). Pupal weight of the second generation on sliced potatoes was almost equally reduced as seen in the first generation. Adults of the second generation also laid eggs, showing the possibility of continuous culturing this species on the sliced potatoes.

Phototaxis and photoperiodic reactions of the adults reared on sliced potatoes

Phototaxis of the adult beetles which had been reared on sliced potatoes in their larval and adult lives was compared with that of normal adults reared on fresh leaves. Individual recordings of ten beetles each were represented in Fig. 1.

All normal adults fed with the leaves orientated directly to the light source just when they were put on the centre of the black box in regardless with their head directions, and walked to the light source. In many cases, they flew up after some approach to the light and finally reached it.

On the contrary, only three out of the ten adults reared on sliced potatoes orientated to the light source directly. Five adults began to walk perpendicularly to the light source. After arriving at the side of the apparatus, they directed themselves to the light source and approached to the light showing thigmotactic movement. Two others showed no phototaxis, walking to the opposite direction from the light. But both could reach the light source showing the second (indirect) type of approach when they were subjected to the second trial.

Few of these adults reared on sliced potatoes took off through the observation.

Photoperiodic reaction of the ovipositing long-day adults was examined after the transference into short-day photoperiod (Fig. 2).

When the normal adults reared on the fresh leaves ovipositing under a long-day

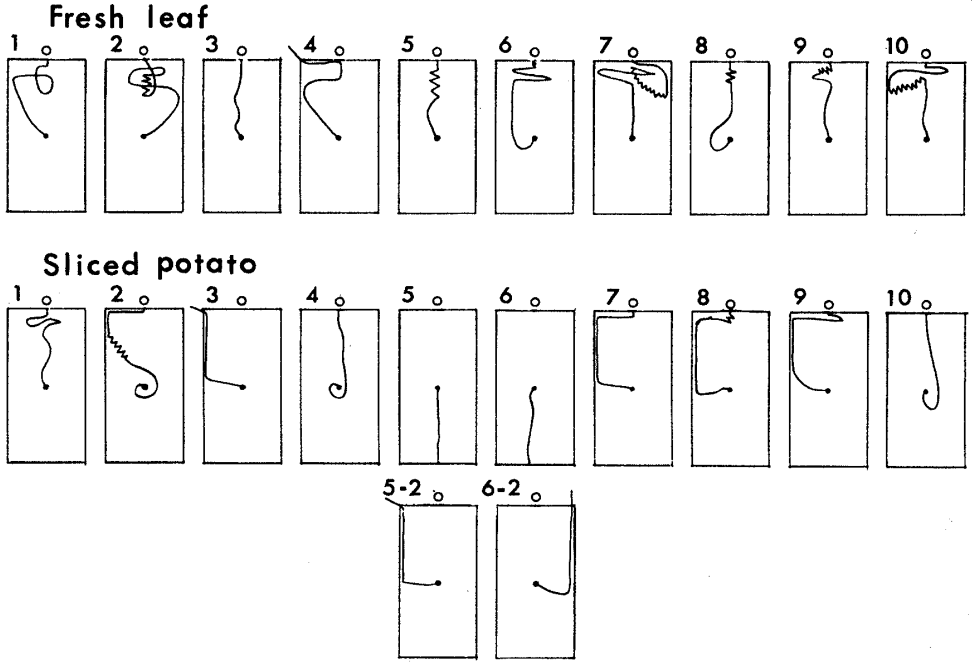

Fig. 1. Traces of phototactic behaviour in the 3-4 day old adults reared on sliced potatoes and on fresh leaves. ~, walking; MW, flight; ○, light source.

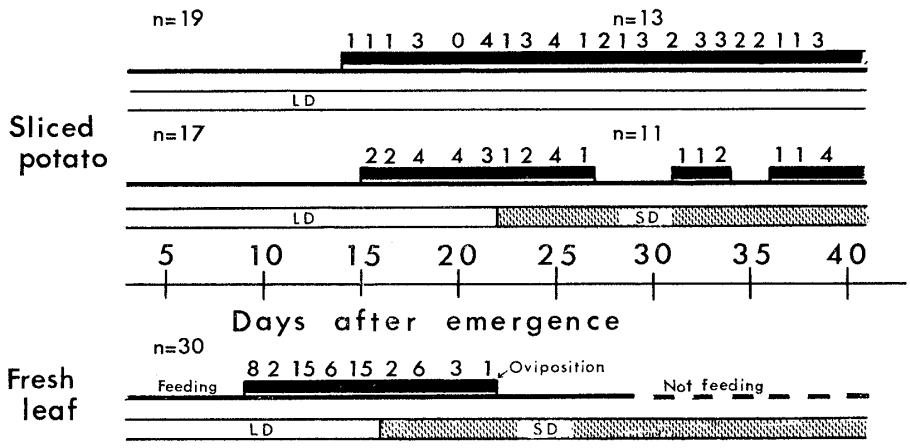

Fig. 2. Records of oviposition and feeding before and after the photoperiodic transfer in adults reared on two different foods throughout the life cycle. White bar, long day (LD); dotted bar, short day (SD); solid and broken lines, feeding and not feeding; black bar and number on it, oviposition and number of egg masses laid; n=, number of adults.

photoperiod were transfered to a short day, they stopped to oviposit in several days and ceased feeding in further 10 days.

On the other hand, adults reared on sliced potatoes continued to oviposit even after the transference from long-day to short-day conditions, except a few days intermission, and never ceased feeding.

Dissection of five females surviving at the end of the experiment under binocular

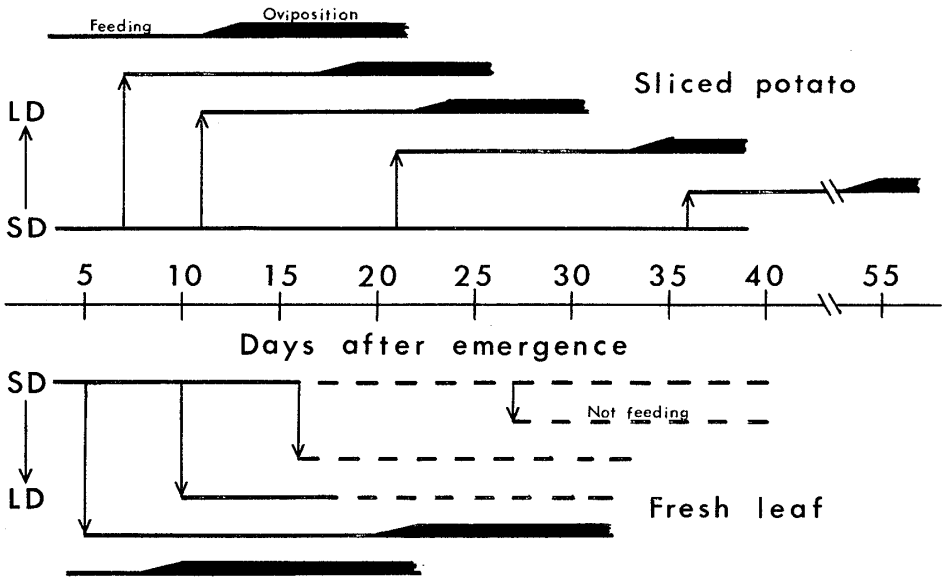

Fig. 3. Effect of the change of photoperiodic condition from short day to long day at various developmental days on the oviposition and feeding in the adults reared on sliced potatoes and fresh leaves. ↑, transference from short day (SD) to long day (LD); solid and broken lines, feeding and not feeding; black bar, oviposition.

showed that three out of them had matured eggs in ovaries and two others had degenerated ovarioles.

Effect of food in adult stage on the photoperiodic reaction

When the *Epilachna* beetles were fed on the fresh leaves of Solanacean host plants throughout the life cycle, they laid the first egg mass about 10 days after the emergence under a long-day photoperiod and continued to oviposit at intervals of two or three days, while they entered diapause without oviposition after 16 days of feeding under a short-day photoperiod (Fig. 3).

This type of response was not influenced by the photoperiodic conditions during the larval stages.

When transfered from a short-day to a long-day condition on the 5th day of adult stage, they reacted to the long day by the onset of oviposition after some elongated pre-ovipositional period. The transference after 10 days could not prevent the reaction to the earlier condition: they entered diapause even under a long day (Fig. 3).

When the adults reared on the fresh leaves in the larval stage were raised on sliced potatoes, reaction to the transference from a short day to a long day was somewhat different from that on the fresh leaves mentioned above (Fig. 3).

It took 13 days for these females to begin to oviposit under a long day and number of eggs in an egg mass was not inferior to that of the adult on the foliage. The adults did not oviposit but never ceased feeding for more than 35 days after the adult emergence under a short photoperiod.

Whenever the adults fed on sliced potatoes under a short day were transfered to a long day, they could react to long day by starting oviposition after 13 days of fe-

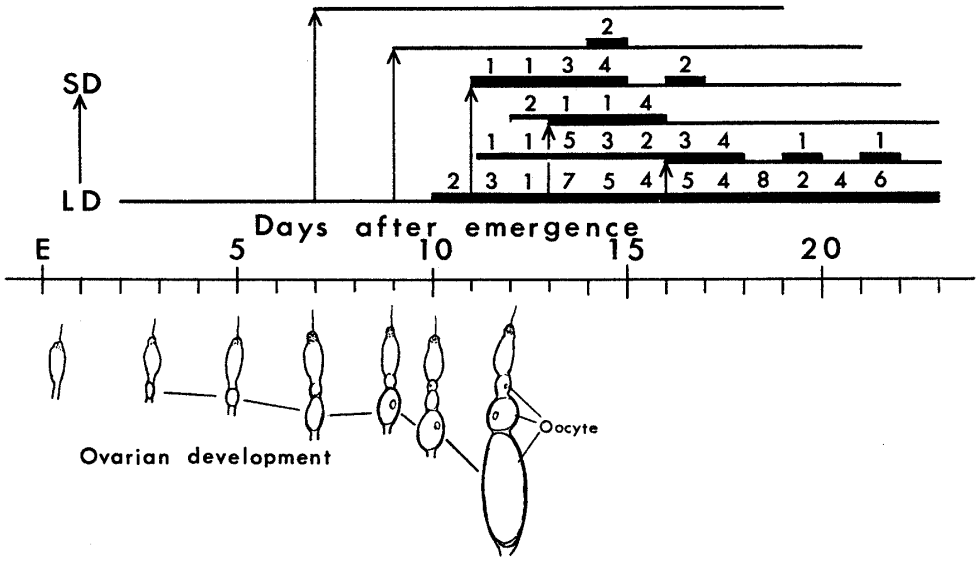

Fig. 4. Effect of the transference from long day (LD) to short day (SD) at various developmental days on the oviposition and feeding in the adults reared on sliced potatoes. Every experimental plot had 20 adults by 1 : 1 sex ratio. ↑, transference from LD to SD ; black bar and number on it, oviposition and number of egg masses laid. Below colum showed the development of oöcyte in the ovariole when the adults were reared on sliced potatoes under LD.

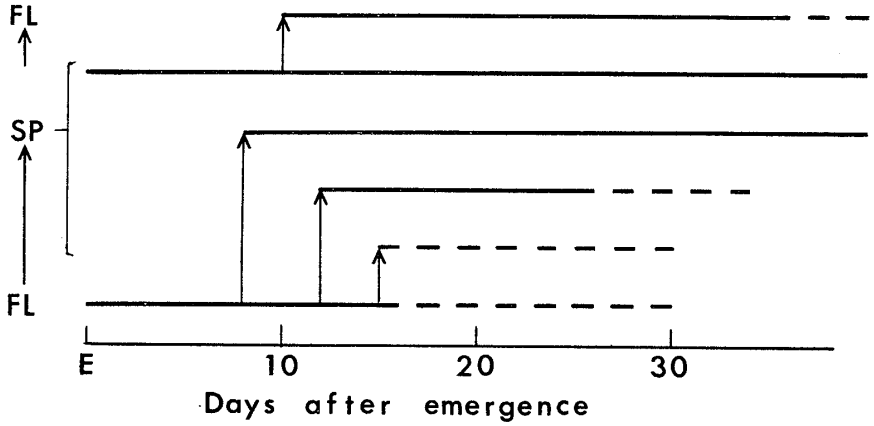

Fig. 5. Effect of the change of food on the entrance into diapause nuder short-day photoperiod. ↑, change of food ; FL, fresh leaves ; SP, sliced potatoes; solid and broken lines, feeding and not feeding.

eding from the transfer. Five days' delay in pre-ovipositional period was observed, only when the transference took place after 36 days of confinement under a short day.

Adults fed on sliced potatoes were reciprocally transfered from a long day to a short day at various stages of ovarian development (Fig. 4). They responded to a short day and stopped oviposition in several days after the transfer. They continued feeding and never entered diapause.

Effect of change of food on the photoperiodic reaction was observed under a short-day photoperiod (Fig. 5).

When the diet of adults was changed from fresh leaves to sliced potatoes on the 11th day of adult emergence, pre-diapause period elongated remarkably. The change on 9th day prevented the entrance into diapause.

When the diet was changed from sliced potatoes to fresh leaves, the beetles entered diapause after prolonged pre-diapause period.

DISCUSSION

When *Epilachna* was fed on sliced potatoes, it took twice longer duration of larval development than normal insects reared on fresh leaves of host plant, and only half-sized adult could be obtained. These results seemed to be due to the nutritional deficiency of the potato tuber. Potatoes contain much starch but small amounts of lipids and proteins, and only little carotenoids.

Continuous rearing of the insect on sliced potatoes will be successful when some addition of nutrients is made, because two generations could be maintained on only the sliced potatoes even though the adults were small in size.

Some abnormalities were observed in the phototaxis of the adults reared on sliced potatoes through their lives as expected from the carotenoid deficiency in the diet.

It was more interesting and suggestive that the change in photoperiodic reaction occurred in those adults reared on sliced potatoes only during adult stage as well as in those reared throughout life.

In the silkworm, *Bombyx mori*, carotenoid deficiency in the artificial diet also induced the phototactic abnormality (SHIMIZU et al., 1976) but no change occurred

in photoperiodic reaction (KATO, personal communication) nor in *Drosophila melanogaster* on carotenoids-free medium (ZIMMERMAN and GOLDSMITH, 1971; ZIMMERMAN and IVES, 1971).

Three kinds of abnormalities of photoperiodic reaction were observed in the present experiments:

First, apparently no photoperiodic response was shown after transfer from a long day to a short day in the adults reared on sliced potatoes for life. Some of the females continued to oviposit even under a short-day photoperiod. Second, the possibility of respose to the photoperiodic transfer was maintained for more than 35 days in the adults fed on sliced potatoes. Third, the adults fed on sliced potatoes in only adult stage failed to enter diapause under a short-day photoperiod though they stopped oviposition normally.

By the detailed survey of the experimental results, the adults seemed to keep the photoperiodic sensitivity in even the first case in spite of carotenoid deficiency, because they showed several days of cessation of oviposition normally reacting to the short-day photoperiod. Diapause incidence, to the contrary, inhibited in the adults which had been reared on the foliage in the larval stage and consequently possessed the functional photoreceptors as in the third case.

Carotenoids seemed to have no role in the photoperiodic sensitivity in this beetle as the two species mentioned above.

Similar decrease of diapause incidence by the nutritional defect has been reported in the pink bollworm, *Pectinophora gossypiella*, which did not enter diapause even under short-day conditions if they were fed on the wheat germ containing less fat than the host plant (BULL and ADKISSON, 1960; ADKISSON, 1961).

In the short-day adults of *Epilachna* reared on sliced potatoes after the adult emergence, fat body development was surpressed obviously in spite of the degeneration of ovaries, while the adults on the foliage developed their fat body markedly and their body cavities were filled with the fat body in a week following adult emergence (KONO, unpublished data).

Taking these anatomical features into account, it can be supposed that the photoperiod reception was normally done and endocrine organes were functioning corresponding to the short-day state in the adults fed on sliced potatoes as well as in the adults fed on the foliage, but fat bodies could not develop owing to the deficiency of the nutritional materials. Consequently, the endocrine activity did not fall into inactive state of diapause in the adults on sliced potatoes. Complete fat body development seemed to be neccesary for inactivation of endocrine system at the entrance of diapause in this species.

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