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Induction of Ovarian Development by Juvenile Hormone and Pyrethroids in *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae)

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Adults of *Henosepilachna vigintioctopunctata* enter diapause under short-day photoperiods, while they become reproductive under long-day conditions. When JH-I or JHA (ZR-515) was applied on the beetle under diapause-inducing conditions, the ovaries began to develop, but the last stage of yolk deposition and the chorion formation did not occur even at high dosages. Number of oöcytes in a vitellarium of the ovarioles of treated animals tended to increase to 4 whereas normal ovarioles contained 3 oöcytes at most. Of the insecticides tested, pyrethroids such as cypermethrin and flucythrinate showed stimulating effect on the ovarian development, and partly induced oöcyte maturation and oviposition.

These results suggested that two endocrine factors, JH and neurosecretory factor, were engaged in the control of ovarian development and that secretion of both factors was stimulated by the pyrethroid application.

INTRODUCTION

Adults of the 28-spotted lady beetle, *Henosepilachna vigintioctopunctata*, fall into diapause under short-day conditions (MIYAKE and TAMURA, 1943; YASUE and KAWADA, 1964; KONO, 1979, 1986). In a Kyoto population, critical day-length for diapause induction was 13 hr 50 min (25°C), and the ovaries of all females developed under photophases longer than 15 hr (KONO, 1986). When adults were reared on host plant (potato) foliage under short day-length, they ceased feeding and entered into diapause without vitellogenesis on about the 17th day of adulthood. Diapause of this species was marked by only 5 cycles of short-day following the adult emergence, and therefore the diapause incidence could not be prevented by long-day exposure after the 6th day of adulthood (KONO, 1986).

By anatomical and histological observations, activities of both corpus allatum and neurosecretory cells in the pars intercerebralis of the beetle were shown to be reduced from the prediapause period (KONO, 1980), indicating the low level of endocrine activities in the brain corpus allatum system. A juvenile hormone analogue (JHA), ZR-515, topically applied to the beetles under short-day condition elicited vitellogenesis but failed to complete oöcyte maturation (KONO, 1980), while in many diapausing coleopteran insects JHAs have been shown to induce both these actions (BOWERS and BLICKENSTOFF, 1966; CONNIN et al., 1967; HODEK et al., 1973; MOHAMED ALI, 1979; ASHIDA, 1980). Other factors are therefore assumed to be necessary for the completion

of vitellogenesis in *Henosepilachna*. Similar results have been reported in the Colorado potato beetle, *Leptinotarsa decemlineata* in which JH application induced vitellogenesis and oviposition in allatectomized long-day females, but did not in non-operated or allatectomized short-day females (DE LOOF and DE WILDE, 1970). Furthermore, a neurosecretory factor originating in the brain was suggested to stimulate the vitellogenesis along with JH (DE LOOF and DE WILDE, 1970).

In the present paper, JH and JHA application on the short-day *Henosepilachna* beetle was carried out under various photoperiodic regimes to ascertain the role of JH and to elucidate the photoperiodic regulation of the endocrine system for the ovarian development of the beetle. Also, the effects of neuroactive insecticides on this process were tested either with JHA or alone.

MATERIALS AND METHODS

Insects. A stock culture of *H. vigintioctopunctata* was maintained on host plant foliage in our laboratory and newly emerged adults were selected from this culture. For experiments, 5 females and 3 males were confined in a petri dish (9 cm in diameter) and were fed on potato slices unless especially noted. The potato slices were changed every three days. Maintenance of stock culture and all experiments were conducted under controlled temperature ($25\pm1°C$) and photoperiods (light intensity was 150–200 lux).

Chemicals. JH-I (Sigma Chemical Co.) and ZR-515 (Zoecon Co.) were used as JH and JHA, respectively. γ-BHC, DDT and fenitrothion were purchased from Wako Chemical Industries, Ltd. Permethrin and cypermethrin were synthesized and purified in our laboratories. Other insecticides used were industrial ingredients of more than 95% purity.

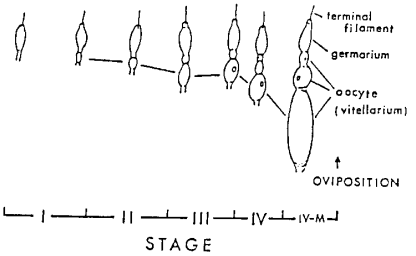

Fig. 1. Five stages of ovarian development in the adult *Henosepilachna*. Yolk deposition was most prominently advanced in stage IV, and completed in stage IV-M with chorion formation.

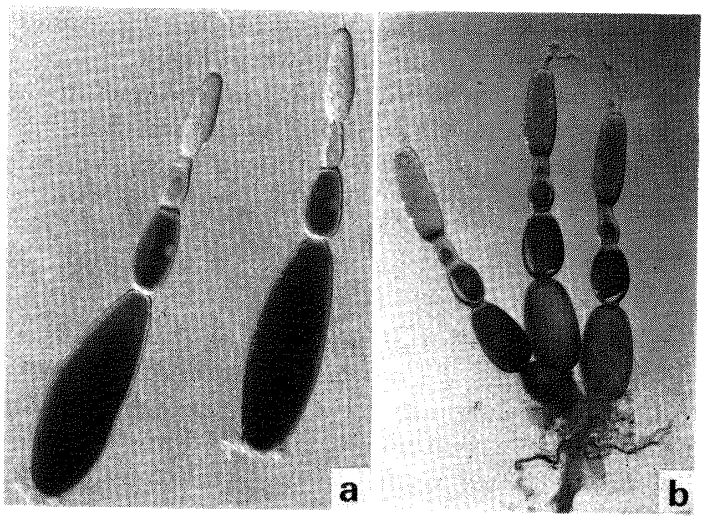

Fig. 2. Photograph of normal matured ovarioles of *Henosepilachna* beetle having 3 oöcytes (a), and abnormal ovarioles composed of 4 oöcytes in a vitellarium obtained by the application of JHA (b).

Table 1. Effects of JH-I and JHA (ZR-515) on the ovarian development of *Henosepilachna*

Compound	Dosage μg/insect	No. of adults	Stage of ovarian development				
			I	II	III	IV (IV-4)	IV-M
			Per cent individuals				
JH-1	0.05	21	42.9	38.1	19.0	0 (0)	0
	0.5	21	9.5	28.6	61.9	0 (0)	0
	5.0	19	0	36.8	63.2	0 (0)	0
	0.5×5	24	0	0	0	95.8 (58.3)	4.2
ZR-515	0.05	18	0	33.3	61.1	5.6 (0)	0
	0.5	21	0	0	42.9	52.4 (4.8)	4.8
	5.0	22	0	0	18.2	77.3 (31.8)	4.5
	50.0	18	0	0	0	100.0 (44.4)	0
Control	—	61	81.5	15.1	3.7	0 (0)	0

The chemicals were applied to adults 2 days after emergence kept under 10L–14D at 25°C on sliced potato, and the grade of ovarian development was checked 13 days after the treatment, in the manner depicted in Fig. 1. Numbers in parentheses are percents of individuals with 4 oöcytes in a vitellarium. 0.5×5: 0.5 μg was applied every other day for a total of 5 times starting from 2 days after emergence.

Application of chemicals. One microliter of acetone solution containing a known dosage of a chemical was topically applied on the dorsal thorax of each adult. Control insects were each applied with 1 μl of acetone only.

Assessment of ovarian development. Adults applied with chemicals were reared on potato slices unless noted.

The females were dissected 10–13 days after the first application of chemicals, unless otherwise noted, to assess the degree of ovarian development, which was graded into five stages (I, II, III, IV, and IV-M), depending primarily on growth of the primary oöcyte in the vitellarium as shown in Fig. 1.

Normally, each vitellarium has 3 oöcytes at the most, but the number tended to increase to 4 when JH or JHA was applied (Fig. 2). Therefore, the number of oöcytes was also checked when they attained stage IV, and individuals having 4 oöcytes were indicated by the symbol 'IV-4.'

RESULTS

Dose response reaction to JH and JHA

When adults of *Henosepilachna* were reared under a short-day condition (10L–14D, 25°C) from adult emergence, vitellogenesis did not begin in females, as shown in Table 1. Under the same condition, 0.05 μg JH-I applied on each adult two days after emergence slightly stimulated vitellogenesis, and 0.5 μg and 5 μg elicited vitellogenesis reaching stage III in more than 60% of the females, but failed to develop the ovaries to stage IV. When 0.5 μg JH-I application per insect was repeated every other day from the 2nd day of adulthood for a total of five times the ovaries of all females developed to stage IV, but none of them completed vitellogenesis.

ZR-515 showed a stronger effect than JH-I (Table 1). Single application of 0.05 μg/insect resulted in more than 60% of stage III vitellogenesis. With 0.5 μg/insect

Table 2. Effect of JHA (ZR-515) application on ovarian development of *Henosepilachna* adults under different photoperiodic regimes

Photoperiodic conditions	No. of adults	Time of examination	Stage of ovarian development				
			I	II	III	IV (IV-4)	IV-M
			Per cent individuals				
10SD	10	10	0	0	0	100 (40.0)	0
10SD–6SD	10	16	0	0	0	100 (70.0)	0
10SD–6LD	9	16	0	0	0	100 (88.9)	0
10SD–10LD	10	20	0	0	0	100 (70.0)	0

0.5 μg/insect ZR-515 was applied topically every other day for a total of 5 times during 10 cycles of short-day photoperiod (SD, 10L–14D). The insects were kept under 6 or 10 cycles of short-day (SD) or long-day (LD, 16L–8D) photoperiod. Other details as in Table 1.

Table 3. Effect of JHA (ZR-515) application on the ovarian development of *Henosepilachna* adults under critical photoperiod (14L–10D) or constant darkness (DD)

Photoperiod		No. of adults	Stage of ovarian development				
			I	II	III	IV (IV-4)	IV-M
			Per cent individuals				
14L–10D-1	T	23	0	0	0	34.8 (21.7)	65.2
	C	10	20.0	10.0	10.0	0 (0)	60.0
14L–10D-2	T	18	0	0	0	33.3 (14.3)	66.7
	C	14	21.4	21.4	0	0 (0)	57.1
DD	T	24	0	0	0	87.5 (8.3)	12.5
	C	24	83.3	8.3	8.3	0 (0)	0

5 μg/insect ZR-515 was applied topically on the 2nd day of adulthood (T). C: control. Other details as in Table 1.

ovaries of over 50% of the females developed to stage IV. With 5 and 50 μg/insect of ZR-515, 77.3% and 100% of stage IV ovaries were obtained respectively, but the late yolk deposition and chorion formation specific to stage IV-M were rarely induced. In the latter two cases, the number of oöcytes in a vitellarium abnormally increased to 4 in many whereas in normal ovaries a maximum of 3 oöcytes were observed (see also Fig. 2).

JHA application and photoperiodic sensitivity

When the adults were reared on host plant foliage, they lost photoperiodic sensitivity after the 6th day of adulthood (Kono, 1982). Therefore, in order to learn the influence of JHA application on the sensitivity to photoperiod, 10 cycles of short-day (SD) during which 0.5 μg/insect JHA was applied every other day for five times, were followed by different lengths of long-day photoperiod (LD) or SD, as shown in Table 2. The vitellogenesis proceeded to stage IV on the 10th day with JHA under SD condition. Four out of 10 females had 4 oöcytes and none of them had maturated eggs. Vitellogenesis stages did not proceed thereafter, but 6 days later under SD, the ratio of females having 4 oöcytes increased to 70%.

Even when the females treated were transferred to LD regime for 6 or 10 days, no egg maturation was observed, as though they had been kept in SD condition. Thus, long-day photoperiod did not affect the ovarian development. This result implied that photoperiodic sensitivity was not restored even when vitellogenesis was initiated by JHA application.

JHA application under critical day-length

Under a critical photoperiod (14L–10D) or continuous darkness, parts of the beetles produced matured eggs, although half or more of them failed in ovarian development (KONO, 1986). If the physiological state of these individuals was intermediate between diapause and non-diapause, JHA application to them would be expected to have more obvious influence than on short-day females. As shown in Table 3, the oöcytes of about 60% of females matured without JHA application under the critical day-length. Unexpectedly, even when 5 μg/insect JHA was applied to the adults, the ratio of females with matured oöcytes did not increase, but oöcytes of other females developed up to stage IV, whereas the ovaries of 40% of the control females remained in undeveloped stages of I–III.

Under continuous darkness, most ovaries of control females stopped developing and only 8.3% of them developed to stage III, while in treated animals, most ovaries developed to stage IV and 12.5% of them attained the mature stage (IV-M). Under critical daylength and continuous darkness, JHA accelerated ovarian development as under SD conditions but could not stimulate the final stage of oöcyte maturation.

Effects of insecticides on ovarian development

The previous results suggested that JH or JHA stimulated vitellogenesis, but could not evoke the complete ovarian development. Therefore, in this experiment, sublethal dosages of nerve stimulating insecticides were applied together with JHA to determine the effects on egg maturation. The results are summarized in Table 4, with LD_{50} values of the chemicals to the beetles.

No stimulating effect was found by organochlorine insecticides, BHC and DDT; organophosphorous insecticides, TIA-230 and fenitrothion; a carbamate, carbaryl; a monoamine inhibitor, chlordimeforme; or an acetylcholine receptor's blocker, TI-78. On the other hand, effects were found by the pyrethroid insecticides, flucythrinate, permethrin and cypermethrin. About half of the females completed egg maturation even with 0.05 μg/insect of these pyrethroids. Most of them with matured eggs laid eggs during the period of the experiment for 10–13 days.

With a sublethal dosage of pyrethroids, most of the adults showed poisoning symptoms: hyperactivity followed by quiescence, and some of them died before the end of the experiment.

Secondly, the effects of pyrethroids on vitellogenesis without JHA were tested (Table 5). Pyrethroids alone also showed stimulating effects to vitellogenesis, but induced oöcyte maturation in far lower percentages of females than when applied with JHA. Cypermethrin and flucythrin showed almost the same effects: 0.05 μg/insect of cypermethrin caused about half of the females to proceed to stage III of vetellogenesis and one third to stage IV, but none to stage IV-M. Pyrethrin also showed stimulating effects.

Table 4. Effects of sublethal doses of insecticides applied with ZR-515 (5 μg/insect) on the ovarian development of *Henosepilachna* adults

Insecticide	Dosage μg/insect	No. of adults alive (dead)	Stage of ovarian development					LD_{50} μg/insect
			I	II	III	IV (IV-4)	IV-M	
			Per cent individuals					
BHC	2.0	20 (0)	0	0	0	100.0 (85.0)	0	4.30
DDT	0.2	22 (3)	0	0	9.1	81.8 (0)	9.1	0.67
Chlordimeform	20.0	20 (0)	0	0	0	100.0 (75.0)	0	>20
TIA-230	0.05	17 (3)	0	0	0	100.0 (52.9)	0	0.211
Fenitrothion	0.05	19 (6)	0	0	0	100.0 (26.3)	0	0.193
Carbaryl	0.05	36 (14)	0	0	2.7	88.9 (22.2)	11.1	0.139
TI-78	0.2	7 (18)	0	14.3	14.3	71.4 (14.3)	0	0.381
Fulcythrinate	0.02	15 (10)	0	0	0	80.0 (—)	20.0	0.334
	0.05	20 (30)	0	0	0	50.0 (20.0)	50.0	
Permethrin	0.02	19 (6)	0	0	0	68.4 (—)	31.6	0.151
	0.05	20 (5)	0	0	0	50.0 (—)	50.0	
Cypermethrin	0.05	16 (9)	0	0	0	56.3 (—)	43.8	0.126
ZR-515 alone	5.0	78 (2)	0	0	1.3	92.3 (32.1)	6.4	—
Control	—	30 (0)	86.7	13.3	0	0	0	—

Number of oöcytes was not checked (—). Data of LD_{50} were on adults of both sexes. Other details as in Table 1.

Table 5. Effect of sublethal dose of pyrethroids on the ovarian development of *Henosepilachna* adults

Treatment	Dosage μg/insect	No. of adults alive (dead)	Stage of ovarian development				
			I	II	III	IV	IV-M
			Per cent individuals				
Permethrin	0.02	24 (1)	45.8	41.7	8.3	0	4.2
	0.05	24 (1)	50.0	37.5	12.5	0	0
Cypermethrin	0.02	19 (6)	15.8	47.4	31.6	5.3	0
	0.05	11 (14)	0	18.2	45.5	36.4	0
Flucythrinate	0.02	20 (5)	15.0	40.0	40.0	5.0	0
Pyrethrin	0.1	19 (6)	21.1	42.1	26.3	5.3	5.3
Acetone	1.0	20 (5)	65.0	25.0	0	5.0	5.0
Control	—	22 (3)	54.5	31.8	0	0	13.6

Ovarian development was checked 10 days after treatment. Other details as in Table 1.

Application of cypermethrin to adults of different ages

Adults were fed foliage and applied with cypermethrin 3 days after their emergence when they still maintained their photoperiodic sensitivity, 10 and 15 days after emergence when they had lost sensitivity, and 20 days after emergence when they were in diapause state.

As shown in Table 6, the application more or less enhanced vitellogenesis in most of the females and induced some of them to lay eggs, despite the different adult ages treated. Ovaries developed faster when reared on foliage than on sliced potato.

Table 6. Effect of cypermethrin applied to *Henosepilachna* adults of different ages under short-day photoperiod

Application (age of adults)		No. of adults alive (dead)	Stage of ovarian development				
			I	II	III	IV	IV-M
			Per cent individuals				
3	T	10 (15)	20.0	30.0	20.0	10.0	20.0
	C	20 (5)	55.0	40.0	5.0	0	0
10	T	24 (1)	20.8	20.8	41.7	8.3	8.3
	C	6 (4)	66.7	33.3	0	0	0
15	T	31 (9)	3.2	41.9	41.9	6.5	6.5
	C	22 (3)	36.4	45.5	18.2	0	0
20	T	11 (4)	0	18.2	45.5	18.2	18.2
	C	8 (2)	50.0	12.5	25.0	12.5	0

Adults reared on tomato leaves. T: 0.05 μg/insect of cypermethrin was applied. C: 1 μl/insect of acetone only was applied. Other details as in Table 1.

DISCUSSION

It has been reported that JH or JHA can elicit ovarian development in diapausing coleopteran insects (BOWERS and BLICKENSTOFF, 1966; CONNIN et al., 1967; HODEK et al., 1973; MOHAMED ALI, 1979; ASHIDA, 1980). In most of these species, egg maturation and oviposition were induced by JH application. In the alfalfa beetle, degree of vitellogenesis depended on the dosage and the chemical structure of JHA (MOHAMED Ali, 1979), while in *Henosepilachna* adults, as elucidated in the present study, vitellogenesis was enhanced in dose-dependent manners by JH-I and JHA (ZR-515), but the maturation of oöcytes, i.e., completion of yolk deposition and chorion formation could not be induced even by an abnormally high dose of JHA (50 μg/insect) as an ordinary dose. Furthermore, high doses of JHA or repeated applications of JH-I or JHA induced an abnormal feature of ovarioles composed of 4 oöcytes in a vitellarium which was never observed in control insects. These results imply that another factor in addition to JH is necessary for normal ovarian development in this beetle.

Involvement of two endocrine factors, JH and a neurosecretory factor from the brain during vitellogenesis has been shown in several insect species: Colorado beetles (DE WILDE and DE BOER, 1961, 1969; DE LOOF and DE WILDE, 1970), *Locusta migratoria* (GIRARDIE, 1966) and *Tenebrio moritor* (MORDUE, 1965; LAVERDURE, 1972). A neurosecretory factor stimulates the previtellogenesis in the latter species. In the Colorado beetle, JH application induced vitellogenin production to some extent but failed to complete the ovarian development under short-day photoperiods (DE LOOF and DE WILDE, 1970). The same regulation mechanism seems to function in *Henosepilachna* beetles.

In short-day *Henosepilachna* beetles, inactive histological appearances were observed in neurosecretory cells of the pars intercerebralis, accompanied by a smaller corpus allatum, indicating a reduced activity of this organ (KONO, 1980, 1982). It is well known in many insects that the corpus allatum needs an allatrophic hormone, a neurosecretory material from the brain for the release of JH (RAABE, 1982). The neurosecretion from the brain, therefore, has two different roles in the vitellogenesis of these insects, allatrophy and stimulation of egg maturation.

As to the influence of insecticides on the release of neurosecretory substances in insects, there have been several reports on a hyperglycemic hormone by DDT in *Periplaneta americana* (GRANETT and LEELING, 1972) and by γ-BHC in *Schistocerca gregaria* (SAMARANAYAKA, 1974), an adipokinetic hormone by γ-BHC in *Schistocerca* (SAMARANAYAKA, 1974), by phoxim in *Schistocerca* (SAMARANAYAKA, 1977) and by bioresmethrin in *Locusta* (SINGH and ORCHARD, 1983), and a diuretic hormone by TEPP, Zectran and DDT in *Rhodnius prolixus* (MADDRELL and REYNOLDS, 1972). These effects were in good accordance with the action of insecticides: permethrin, DDT, and carbaryl (ORCHARD and OSBORNE, 1979), decamethrin, bioresmethrin and permethrin (ORCHARD, 1980) and bioresmethrin (SINGH and ORCHARD, 1983) on the electrical activity of neurosecretory cells of insects. Among these insecticides, the pyrethroid, permethrin showed much higher activity than DDT and carbaryl in increasing the frequency of the spontaneously generated action potential of *Carausius* neurosecretory neurons (ORCHARD and OSBORNE, 1979). This result suggests that the stimulating action of pyrethroids can be manifested by a dose lower than a lethal level *in vivo*.

With the application of pyrethroids even at sublethal doses, the *Henosepilachna* beetle showed hyperactive symptoms for several hours. At the same time, neurosecretory neurons including possibly those for allatrophic hormone and the second factor were supposed to be excited and to release the hormones, which might work to complete ovarian development, as shown in the present study.

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