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PREVENTION AND ARTIFICIAL INDUCTION OF IMAGINAL DIAPAUSE IN *COCCINELLA SEPTEMPUNCTATA* L. (COL.: COCCINELLIDAE)

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Excess food, long photoperiod, and high temperature prevents diapause in *Coccinella septempunctata* L. after the beetles had been reared through several generations under these conditions. These is an interaction between photoperiod and temperature in induction of diapause, photoperiod being the more important factor. A long photoperiod inhibits diapause even at a low temperature. The proportion of diapausing females in later generations varies inversely with the temperature if the photoperiod is short. Which factor induces diapause in adults of the first generation is not yet known.

The physiological condition of beetles artificially induced to enter diapause is apparently similar to that of beetles in diapause in the field.

Diapause in *C. septempunctata* occurs at a time when environmental factors apparently still favour development and activity. Beetles in diapause have a large fat body and ovaries with not yet differentiated germaria (HODEK & ČERKASOV, in litt.).

The second phase of imaginal dormancy is a quiescent period during which the resumption of activity and development can be evoked by favourable conditions (HODEK, 1960).

DOBRZHANSKY (1922) states that the incidence of diapause in coccinellids is not affected by environmental conditions. JAKHONTOV (1940) reared coccinellids under controlled conditions, which unfortunately he fails to describe, and he succeeded only in increasing slightly the number of generations occurring in the field. Therefore the prevention and induction of diapause in coccinellids under controlled conditions merits further investigation.

MATERIALS AND METHODS

Beetles collected in the field after they emerged from hibernation (Generation 0) were reared in the laboratory under crowded conditions in cylindrical glass or plexite cages of various sizes, each covered with a silon lid. One or twice daily, plant material infested with aphids was placed on a piece of zigzag folded filter paper in each cage. Eggs which were laid by the coccinellids on this filter paper were also collected daily. Larvae emerging from these eggs were transferred to another cage and in this way the generations were kept separate. Some larvae and adults were kept singly in 11 cm × 14 cm vials covered with silon and containing a piece of filter paper. The number of eggs laid and the mortality in each cage were recorded daily.

The proportion of active beetles in each generation was determined by dissecting the adult females. Twenty-day-old females with ovaries consisting of undifferentiated germaria only were recorded as being in diapause. No apparent difference is found between testes of active and diapausing males.

Cultures were started in five successive seasons, the first in 1956, the fifth in 1960. At the end of each season, after about ten generations were bred the cultures were brought to an end by increasing mortality (see also Table VIII).

The adults used to start the first culture were kept under nearly natural conditions of temperature and daylength. Cages containing the second and later generations of the first culture, and all generations of the other cultures, were kept in a constant temperature room maintained at between 22° C and 28° C, and under artificial fluorescent lighting kept on for a period of 16 hours at first, and after October 1958, 18 hours a day. The first three generations of the first culture were fed on the aphids, *Aphis fabae* Scop., *Uromelan aeneus* H.R.L., *Anoecia corni* F., and *Pergandeida craccae* L., collected in the field. Later generations of the first culture, and the larvae and adults of the other four cultures, were fed on *Pergandeida craccivora* Koch, the parthenogenetic viviparous generations of which were reared on *Vicia faba* L. under the same conditions of daylength and temperature as the coccinellids.

The number of generations which appeared in the cultures was slightly lower than that which is theoretically possible. In the first culture 9 generations completed their development in a year; in the second, only 8; in the third, 9 generations were reared in 11 months; and in the fourth, the theoretical number was obtained: one generation a month.

In the first culture a constant excess of food was maintained by providing aphids twice a day. Fewer aphids were provided to the second and third cultures, where intermittant shortages of food occurred. In the fourth culture food was rather more plentiful.

The first experiment on induction of diapause was carried out in a light proof cage kept in a room maintained at 23° C. The cage was internally illuminated by a small tungsten filament lamp. Heat from the lamp raised the temperature within the cage to 28° C. Incubators, similarly illuminated, and kept in a cellar room where the temperature varied from 10° C in winter to 15—17° C in summer, were used for the other experiments. Heat from the lamps did not raise the temperature in the air-jacketed incubators, but various controlled ranges of temperature could be obtained in the water-jacketed incubators by using lamps of different wattages.

The percentage glucose content of adult beetles was determined by the anthrone method (CARROL, LONGLEY & ROE, 1956) after hydrolysis of desiccated specimens with potassium hydroxide and precipitation with alcohol (WALAAS & WALAAS, 1950). The intensity of colour was measured on a Lange's colorimeter. By multiplying by the coefficient 0.9 the glucose values were then converted into glycogen values. The percentage fat content was determined gravimetrically after extraction of the homogenised coccinellids with alcohol-ether and purification with ether. A more detailed description of the method will be given elsewhere (HODEK & ČERKASOV, in litt.).

The temperature ranges used in the experiments are given in the shortened

forms, 19—22.5° C (D)/22—25° C (L). In the course of the experiment the temperature may increase gradually from the lower to the greater value (→), or alternate in the given range (—). The temperature range differed in the dark (D) and light periods (L).

RESULTS

Situation under field conditions

Early in July in 1959 and 1960, 202 adult *C. septempunctata* females, collected in sugar-beet fields in Central Bohemia, were dissected. Only newly emerged adults with orange elytra were taken, so that adults which had already overwintered were excluded.

TABLE I

Dissection of females collected in the field

Instar collected	date collected	date dissected	number dissected	percentage diapausing females*
prepupae or pupae	19.6.59	13.7.59	19	90.5
adults	6.7.59	16.7.59	59	93.2
adults	17.7.59	18.7.59	79	92.4
adults	13.—15.7.60	21.7.60	45	84.4

*) females with ovaries consisting of undifferentiated germaria only.

The beetles were collected when day length was longest and in both years it was warm and there was an abundance of aphids in the sugar-beet fields. Nevertheless, in all the samples collected the majority of the females (84.4—93.2%) had ovaries with undifferentiated germaria only (Table I). Some of the beetles were kept for ten days in warm field conditions but the ovaries remained unchanged.

TABLE II

The proportion of diapausing females in each generation of culture I of C. septempunctata *(1956)*

generation	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	3. 7.— ?	?—54	22	86.4
2	10. 8— ?	?—99	11	63.6
3	30. 9.— 7.10.	38—45	9	11.1
4	6.11.—10.11.	36—40	9	0
5	9.12.— ?	?—44	17	0
6	21. 1.—23. 1.	58—60	10	0
7	3. 3.—11. 3.	50—54	10	0
10	25. 7.—21. 8.	28—55	14	0
12	27.10.—11.11.	28—43	10	10

* See note under Table I

Continuous cultures

In the first culture the proportion of diapausing females gradually decreased over the first three generations and in the following six generations no diapausing females were recorded (Table II). A certain variable proportion of diapausing beetles was recorded in the later generations of the second, third and fourth cultures (Tables III, IV & V). The differences in the availability of food between the cultures may be responsible for this difference between cultures. Aphids were supplied in constant excess to the ladybirds in the first culture whereas in all the other cultures fewer aphids were provided.

TABLE III

The proportion of diapausing females in each generation of culture II of C. septempunctata *(1957)*

generation	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	12. 7.—12. 8.57	37—68	27	63.0
3	26.10.—13.11.57	42—60	14	14.3
4	3.12.—12.12.57	37—46	19	15.8
5	20. 2.— 7. 3.58	46—61	30	6.7
6	15. 4.—27. 4.58	38—50	18	16.7
8	18. 6.—30. 6.58	56—68	11	45.4
9 A	30. 7.—24. 8.58	22—47	29	10.3
B		36—61	29	10.3

* See note Table I

TABLE IV

The proportion of diapausing females in each generation of culture III of C. septempunctata *(1958)*

generation	replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1		25. 6.— 9. 7.58		18	66.7
2	A	7. 8.—29. 8.58	17—39	10	20.0
2	B	7. 8.— 4. 9.58	35—63	14	50.0
2				24	37.5
3		11. 9.— 9.10.58	27—49	11	18.2
4	A	20.10.— 8.11.58	33—52	23	47.8
4	B	1.11.—19.11.58	40—58	16	12.5
4				39	33.3
5		24.12.—30.12.58	30—36	16	6.3
6	A	20. 1.—25. 1.59	40—45	19	31.6
6	B	26. 1.—29. 1.59	36—39	16	6.3
6	C	29. 1.—11. 2.59	23—36	21	4.8
6				56	14.3
7		23. 2.— 1. 3.59	33—39	13	7.7
8	A	31. 3.—13. 4.59	42—55	11	9.1
8	B	17. 4.—20. 4.59	37—40	13	7.7
8				24	8.3
9	A	22. 4.— 4. 5.59	25—37	9	11.1
9	B	13. 5.—17. 5.59	22—26	12	0
9				21	4.8

* See note Table I

TABLE V

The proportion of diapausing females in each generation of culture IV of C. septempunctata *(1959)*

generation	replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1	A	16. 6.—18.6.	23—25	12	33.3
	B	20. 6.—24.6.	17—21	10	30.0
				22	31.8
2	A	14. 7.—19.7.	43—49	4	0
	B	22. 7.—24.7.	39—41	2	0
	C	26. 7.—29.7.	34—37	5	20.0
				11	9.1
5	A	26.11.—29.11.	21—24	11	9.1
	C	4.12.—10.12.	27—33	10	0
	D	8.12.—11.12.	27—30	11	0
	E	11.12.—17.12.	20—26	5	0
	F	16.12.—23.12.	14—21	7	14.3
	G	22.12.—26.12.	11—15	6	0
				50	4,0
6	A	1.12.— 9.12.	10—19	11	9.1
	B	23. 1.—30.1.	18—25	11	0
				22	4.5
8	A	29. 1.— 2.2.	37—41	6	16.6
	B	8. 2.—16.2.	23—31	7	14.3
	D	26. 2.— 1.3.	15—19	23	0
				36	5.5
9		22. 2.— 1.3.	15—23	10	10.0

* See note Table I

A relatively high percentage of diapausing females occurred in the first generation of all four cultures, and also in the first generation reared in 1960 (Table VI & VII).

TABLE VI

The proportion of diapausing females in the first generation of culture Va of C. septempunctata *(1960)*

replicate	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
A	7.3.— 8.3.	30—31	13	46.2
B	8.3—10.3.	28—30	13	100.0
C	10.3.—12.3.	26—28	18	88.9
D	11.3.—14.3.	28—31	7	85.7
F	13.3.—14.3.	28—29	13	84.6
G	15.3.—16.3.	31—32	10	70.0
H	17.3.—19.3.	28—30	14	92.9
I	18.3.—19.3.	28—29	20	85.0
J	19.3—20.3.	32—33	21	76.2
K	25.3.—27.3.	25—27	8	75.0
			137	81.0

* See note Table I

TABLE VII

The proportion of diapausing females recorded in the first generation of C. septempunctata *at different temperature ranges and with 16—19 hours of light each day*

year	cultures	replicate	temperature	date of emergence of adults	age of adults at dissection (days)	number dissected	percentage diapausing females*
1956	I			3.7.— ?	?—54	22	86.4
1957	II		ca 25° C const.	12.7 12.8.	37—68	27	63.0
1958	III		(with slight	25.6.— 9.7.		18	66.7
1959	IV		changes)	16.6.—24.6.	17—25	22	31.8
1960	V a**			7.3.—27.3.	25—33	137	81.0
1960	V b	1 A	D : 22 → 25/L : 28 → 31° C	27.6.— 1.7.	24—28	13	38.5
		1 B		1.7.— 4.7.	21—24	14	50.0
		1 C		3.7.— 6.7.	22—25	12	66.6
						39	51.3
		2 A	D : 19 → 22.5/L : 22 → 25° C	10.7.—12.7.	20—22	16	0
		2 B		16.7.—17.7.	15—16	17	29.4
						33	15.2
		3 A	D : 25 → 26/L : 27 → 29.5° C	17.7—22.7.	17—22	18	72.2
		3 B		23.7.—27.7.	12—16	13	53.8
						31	64.5

* See note under Table I
** See also Table VI.

In the first culture a high mortality occurred in the 11th and 12th generations, especially in the 12th. Also a striking decrease in fecundity of the female beetles and longevity of the males was observed when adults from the second culture were reared singly in vials from the third to the sixth generation (Table VIII).

TABLE VIII

Fecundity and longevity in generations three, four and six in the second culture of C. septempunctata

generation	No. of days on which oviposition occurred	average longevity (days)		number	
		females	males	females	males
3	20.6	59.4	71.3	26	27
4	13.3	62.9	60.5	29	30
6	7.7	47.6	46.9	23	30

Although no precise general conclusions can be drawn from a comparison of the proportion of diapausing females in the different experiments, as not all conditions were constant, e.g., the number of specimens used to start a culture, locality from which the beetles were collected, age of females at the time of oviposition and time of dissection, number of specimens bred and dissected, nevertheless certain facts emerge:

1. Diapausing females are recorded in the first generation of all the cultures even under conditions otherwise favourable for the prevention of diapause.
2. In the later generations of the first culture kept under optimal conditions of photoperiod, temperature and food supply diapause does not occur, but any deviation from the optimal conditions, as in cultures 2, 3 and 4, results in some beetles entering diapause.
3. After about 10 generations under constant conditions, the vitality of the insects decreases and there is high mortality.
4. Under controlled conditions it is possible to maintain a laboratory culture of ladybirds producing 10—12 generations in a year providing the culture is supplemented every spring by the addition of beetles collected in the field after they have emerged from hibernation. The only difficulty in maintaining a culture is procuring a plentiful supply of aphids especially during the unfavourable part of the year. Perhaps an artificial diet, using dried aphids could be used for this purpose (SZUMKOWSKI, 1952; SMIRNOFF, 1958).

Artificial Induction of Diapause

Throughout their larval and early imaginal development a number of individuals of *C. septempunctata*, taken from the later generations of the cultures, were subjected to various combinations of photoperiod and temperature ranges. The results are recorded in Table IX. Temperature obviously influences the proportion of diapausing females at a given photoperiod.

At relatively low temperatures a short photoperiod induces a high proportion of females to enter diapause. High temperatures, even when combined with the shortest photoperiod used, markedly inhibit the induction of diapause. A long

TABLE IX

The proportion of females of C. septempunctata *entering diapause under different conditions of temperature and daylength*

source of material		age of larvae at beginning of experiment (days)	temperature range (° C)	photoperiod (hours per day)	age of adults at dissection (days)	number dissected	diapausing females %
culture	generation						
I.	7.	3	17—18(D)/20—22(L)	12	42—51	15	93.9
I.	7.	pupae	17—18(D)/20—22(L)	12	56—62	15	86.6
I.	9.	5—8	17—18(D)/20—21(L)	12	24—41	30	86.7
I.	9.	5—7	22 (21.5—22.5)	12	33—35	10	70
II.	4.	4—5	22 (21 —22.5)	8	24—34	42	59.5
I.	9.	3—7	25 (24.5—25.5)	12	30—33	40	32.5
I.	6.	1	24—25(D)/27—28(L)	8	24—26	20	10
III.	4. 5.	2—3	18 (17.5—18.5)	19	26—43	24	12.5
II.	4.	6—7	18—19(D)/20—21(L)	16	35—46	6	0

photoperiod inhibits diapause at all temperatures used, even at such a low temperature that larval development scarcely proceeded.

In experiment I/7 where pupae instead of young larvae were transferred from the culture to experimental conditions, no difference was found between the adults obtained from these pupae and the adults from larvae kept under similar experimental conditions from an early age.

Preliminary investigations were made of the effect of physical factors on the termination of artificially induced diapause. By keeping females in diapause under conditions of 12 hours of light per day at high temperatures, they gradually came out of diapause. After 68—70 days 64% had emerged from diapause. In experiment I/9b none remained in diapause after 80—97 days, irrespective of the experimental temperature used (Table X). Thus artificially induced diapause does not seem to need low temperature for its termination.

TABLE X

Results of keeping diapausing female beetles of C. septempunctata *for varying periods at different temperatures (12 hr. photoperiod)*

	Experiment I/9 a		Experiment I/9 b		
time of treatment in days*	33—35(I)	68—70(I)	24—41(II)	59—76(II) + 21(IV)	24—41(II) + 35(III) + 21(IV)
number of females dissected	10	39	30	7	11
per cent of diapausing females	70	36	86.7	0	0

*Temperature
I . . . . . 22° C const.
II . . . . . 17—18° C (D)/20—21° C (L)
III . . . . . 4—5° C const.
IV . . . . . 25° const.

Comparison of adults naturally and artificially induced to enter diapause

Dissections revealed no difference in the ovaries or fat body between adults in diapause either collected in the field or reared in the laboratory.

Chemical analysis was carried out on adult males and females in diapause collected in early October from a hibernating site (Raná-hill, near Louny, Northern Bohemia) and on diapausing and normally active females reared in the laboratory. Laboratory reared females were first dissected to ensure that they were in diapause, or active, and were then analysed together with the alcohol in which they were dissected.

The proportion of glycogen is practically the same in beetles naturally and artificially induced to enter diapause. The fat content is one fifth lower in the laboratory reared beetles (Table XI). Active females contain half the quantity of glycogen and less than half (43.9%) the quantity of fat that females in diapause contain. Hence the physiological state of the adults in diapause, whether taken in the field or reared in the laboratory, can be considered equivalent in this respect.

TABLE XI

The proportion of fat and glycogen in diapausing and active adults of C. septempunctata

	proportion of fat (mg of lipids in mg of fresh weight)		number of specimens	proportion of glycogen (γ of glycogen in mg of fresh weight)		number of specimens
	♀♀	♂♂		♀♀	♂♂	
Adults naturally induced to enter diapause	0.216	0.190	7	8.64	9.16	3
	0.223	0.219	7	6.00	7.00	3
	0.204	0.212	7	8.40	10.80	3
	0.177	0.219	7	7.05	8.76	3
	0.205	0.210	28	7.55	8.94	12
Adults artificially induced to enter diapause	0.144		7	8.00		2
	1.184		6	6.74		2
	0.164		13	7.36		4
Active adults (reared in laboratory)	0.073		9	3.78		2
	0.070		8	3.60		2
	0.072		17	3.69		4

DISCUSSION

Several normally univoltine temperate zone insects, such as *Stenocranus minutus* Fabr. (MÜLLER, 1957), are potentially polyvoltine; i.e. they continue to reproduce as long as environmental conditions remain favourable. In populations of *Coccinella septempunctata* in Czechoslovakia, the situation is not so clear.

DOBRZHANSKY (1922) believes that induction of diapause in *Coccinella septempunctata* L. and other coccinellids is quite independent of environmental conditions and is directed by internal stimuli. But the present results indicate an evident dependence of the induction of diapause on environmental conditions. The precise relationship between environment and the induction of diapause is, however, not fully understood.

Even when the first generation obtained from overwintering adults of *C. septempunctata* has developed under apparently favourable conditions both in the field as well as in laboratory cultures, a very high proportion of the females entered diapause. It is not clear whether the determination of diapause in this generation is entirely endogenous, or whether only the reaction to environmental factors differs.

However, the possibility is not excluded that Central Europe represents a transitional area climatically (BODENHEIMER & VERMES, 1957) between the regions settled with univoltine and polyvoltine populations of *C. septempunctata.* The hereditary tendency to diapause would have been gradually excluded in the first generations of the culture.

Of other insects undergoing imaginal diapause, *Leptinotarsa decemlineata* Say

has also been reared continuously over several generations. A certain proportion of the females in *L. decemlineata* enter diapause in each generation, partly due to the density of beetles in the rearing cages (DE WILDE & STEGWEE, 1958). The proportion entering diapause decreased from approximately 30% when they were reared in groups of 30—50, to less than 10% when reared singly. Thus, diapause in *C. septempunctata* could also have resulted from similar sub-optimal rearing conditions.

Imaginal diapause in *C. septempunctata* synchronises its life history with the seasonal change in environmental conditions. This results in the coccinellid being active when the environment is most favourable to it.

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ZUSAMMENFASSUNG

DIE VERHINDERUNG UND KÜNSTLICHE INDUKTION DER DIAPAUSE BEI COCCINELLA SEPTEMPUNCTATA *L. (COL.: COCCINELLIDAE)*

Die Aufzucht des Marienkäfers *Coccinella septempunctata* L. unter optimalen Laborbedingungen (Langtag, erhöhte Temperatur, Futterüberfluss) bewirkte eine stufenweise Unterdrückung der Diapause. Demzufolge konnten dann diapausefreie Generationen in lückenloser Folge herangezogen werden. Wenn es aber zu irgendeiner Abweichung von den optimalen Bedingungen kommt (z.B. vom Futterüberfluss), entwickelt sich auch in den nachfolgenden Generationen ein gewisser Anteil inaktiver Käfer.

Nach einer mehr als einjährigen Zucht (etwa in der elften oder zwölften Generation) wurde eine erhöhte Mortalität beobachtet. Die Zuchten würden deshalb in jedem Frühjahr durch im Freien gesammelten Imagines erneuert.

Versuche mit künstlich aufgezogenen Marienkäfern der späteren Generationen zeigten, dass bei der Diapause-Auslösung die Tageslänge eine entscheidende Rolle spielt. Diesem Befund zufolge verhindern Langtagbedingungen die Auslösung der Diapause sogar bei relativ niedrigen Temperaturen. Durch Kurztagbedingungen wird die Diapause ausgelöst, aber der Anteil der diapausierenden Weibchen steht in direkter Abhängigkeit von der Temperatur, indem dieser bei niedriger Temperatur erhöht, bei höherer Temperatur herabgesetzt ist.

Auf Grund vergleichender Sektionen und insbesondere biochemischer Analysen kann man vermuten, dass die physiologischen Verhältnisse bei den künstlich diapausierenden Marienkäfern denen der natürlich überwinternden ähneln.

Es fällt auf, dass im Freien bei dem überwiegenden Teile der ersten Generation die Diapause unter allen Bedingungen ausgelöst wird, die sich experimentell als optimal diapausehemmend erwiesen haben. Analoge Resultate haben wir auch in unseren Labor-Versuchen bei den ersten Generationen erhalten. Es ist bisher nicht klar, ob dieser Unterschied durch variable Sensibilität zu den Aussenbedingungen oder eher durch innere genetische Unterschiedlichkeit verursacht ist. Bei der letztgenannten Möglichkeit könnte eine Erklärung darin gesucht werden, dass man für die zentraleuropäischen Populationen von *Coccinella septempunctata* L. eine heterogene Zusammensetzung annimmt, die zum überwiegenden Teil von Monovoltinen und nur zu einem kleineren von Polyvoltinen gebildet wird.

Es zeigt sich, dass man durch präzise experimentelle Zuchten monovoltiner Insekten des gemässigten Klimas unter eindeutig optimalen Bedingungen latente Voltinismus-Tendenzen — wie z.B. potentiellen Polyvoltinismus, obligatorischen Monovoltinismus (verschiedenartig fixiert), bzw. ein Gemisch beider Typen —, welche in der Natur durch die Uniformität des Monovoltinismus verdeckt sind, aufklären könnte.

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