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SOME PRELIMINARY OBSERVATIONS ON THE INFLUENCE OF FAECAL
LIPIDS ON THE INDUCTION OF LARVAL DIAPAUSE IN AN INSECT

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LARVAE of the khapra beetle, Trogoderma granarium Everts (Dermestidae, Col.) is a serious pest of stored grains, especially wheat. In fairly crowded populations of Trogoderma, at 30°C, a certain percentage of the last instar larvae enter into a state of developmental arrest which has been characterized as a weak facultative diapause.^{1,2} Extensive studies by Burges³ have established that the factors which influence the inception of diapause in this insect are low temperatures, very low as well as very high humidities and high population densities. In an analysis of the mode of action of high population densities, Burges³ found that the presence of large amounts of faecal pellets in the culture medium as well as the feeding of a mixture of whole and damaged grains from old cultures could bring about diapause. Thus at 30°C and 70 per cent r.h., diapause could be induced even in larvae reared singly in excess fresh food by mixing faecal pellets with the food which suggested that the diapause is initiated by something transmitted from the faecal pellets to the food.³ Stanic et al.⁴ repeated the experiments with faecal pellets and found that the presence of a high proportion of faecal pellets in the food caused some larvae to enter into diapause even at 33°C. They also showed that the admixture, upto 75 per cent, of fine

inert materials into the diet did not induce diapause and suggested that the induction of diapause in the presence of faecal matter in the food is attributable to the specific effect of some metabolic end-products present in the same rather than to dilution of the available food.

While temperature and photoperiods are known to control the diapause of many species, Trogoderma granarium is the only species in which the faecal pellets have been shown to play a role in the initiation of diapause. It is therefore of great interest to know how the faecal matter brings about this effect. Since Karlson and Schmialek⁵ had discovered the presence of principles possessing juvenile hormone activity in the faeces of Tenebrio larvae, which have been identified as farnesol and its aldehyde, farnesal⁶ it was thought interesting to examine whether the possible presence of similar substances also in the faecal pellets of Trogoderma larvae may play a role in the induction of diapause in this insect. There is increasing evidence that the immediate cause of natural diapause is probably a lack of moulting hormone attributable to lack of activation of the prothoracic gland by the brain hormone.⁷ On the other hand, many workers have suggested a role for the juvenile hormone in the control of larval, pupal and adult diapause (see reviews by Gilbert⁸ and Wigglesworth⁷). The possibility that the diapause of Trogoderma larvae may be induced by some juvenile hormone like principle in the excreta seemed particularly attractive because of the following reasons. Diapausing Trogoderma larvae are known to moult and feed intermittently⁹ unlike most other diapausing insects. Since the process of moulting is initiated by the secretion of the thoracic gland (moulting hormone) which is activated normally by the brain hormone⁷ it may be

assumed that the brain and throacic gland of the diapausing Trogoderma larvae are not inactive. It is tempting therefore to compare such larvae to the supernumerary larvae produced artificially by implantation of the corpora allata such as in Rhodnius and other insects⁷ or by contact with agents possessing high juvenile hormone activity as in Pyrrhocoris¹⁰ and the cabbage aphid Brevicoryne brassicae.¹¹ Some apparent diapause effects in some of the artificial adultoid larvae of Pyrrhocoris produced by application of dihydrochloride of methyl farnesoate has also been noted by Zdarek and Slama.¹⁰ The above observations suggested that the presence of some juvenile hormone like principle in the faecal pellets of Trogoderma larvae may perhaps induce diapause either by a contact effect or by active or accidental ingestion of the faecal pellets that accumulate in crowded cultures. Since extraction with ether is widely used as a method for the isolation of juvenile hormone the faecal pellets were extracted with ether and the effect of the addition of the lipid free (ether extracted) pellets and the extracted lipids to the food on the incidence of diapause in T. granarium was investigated.

Material and Methods

The experiments were carried out at $30 \pm 1^\circ\text{C}$ and 70 per cent r.h., with larvae not more than one day old derived from cultures of T. granarium maintained at $35 \pm 1^\circ\text{C}$. Constant temperatures were maintained in incubators and the humidity was controlled by placing the culture tubes in a desiccator over appropriate saturated salt solution.¹² The experimental cultures were maintained in small specimen tubes (4.5 x 1 cm).

Faecal pellets were collected from cultures of larvae maintained on whole wheat at 35°C by passing them through appropriate

sieves. To this were liberated a large number of second or third instar larvae which depleted the contaminating food particles within a few days. The faecal pellets thus freed of food particles were used for the experiments. Lipids from the faecal pellets were removed by extraction with 20 volumes of diethyl ether at room temperature for 12 hr. The faecal pellets were washed twice with another 10 volumes of the solvent. The combined ether extract was evaporated to dryness at 60°C and the lipids were weighed and redissolved in ether. For incorporation of lipids into the food, the requisite amount of wheat flour was added, the contents mixed thoroughly while drying, and the last traces of ether evaporated off at 60°C.

Pupation and mortality were recorded at the end of 25 days and every 5 days thereafter until the 50th day. Larvae which failed to pupate within 40 days are regarded as diapause larvae.^{3,2} All the larvae were found to be fully grown by 40 days and between the 40th and 50th day only a negligible number of them entered pupation. Most of the mortality occurred before the 25th day. At the end of 50 days the larvae were transferred to 37°C and it was found that most of them underwent pupation within the next 10 days, thereby confirming that the larvae were in a diapause state.

Results and Discussion

In one set of experiments (Table 1), the larvae were reared singly and each larva was provided with 100 mg of wheat flour which is about 10 times its approximate food requirement (Burges³ had shown that a single larva would require only 9 mg of food to complete the larval development). In the control group the larvae were left with wheat flour alone while the experimental cultures contained 100 mg of wheat flour mixed with 100 mg of either whole

TABLE 1

Incidence of Diapause in Cultures of Trogoderma maintained at a total Food Concentration of 100 mg Wheat Flour/Larva with and without added Faecal Constituents.

Percentage and nature of faecal constituents added to food	Total No. of larvae employed	Mortality (No.)	Number of Pupae after days					Percentage diapause
			35	40	45	50	55	
Nil (Control)	100	5	90	95	95	95	95	Nil
50% lipid free (ether extracted) faecal pellets	100	7	78	91	92	92	92	2
50% whole faecal pellets	100	4	76	91	93	93	93	5

faecal pellets or faecal pellets from which the ether soluble lipids were removed. It may be seen (Table 1) that no diapause larva was encountered in the control group and that only a small percentage entered diapause in both the other groups.

In another set of experiments (Table 2), the concentration of faecal pellets was maintained the same but the food available per larva was reduced to about 33 mg by placing 3 larvae in each tube. In these cultures 9.3 per cent of the larvae in the control group, 49.7 per cent in the group with added ether extracted faecal pellets and 83.0 per cent in the group with added whole faecal pellets entered diapause.

The above experiments show that the addition of faecal pellets at a concentration of 50 per cent of the total diet substrate had no significant effect in inducing diapause when the food available per larva was 100 mg and that the effect of added

TABLE 2

Incidence of Diapause in Cultures of Trogoderma maintained at a Food Concentration of 100 mg Wheat Flour/3 Larvae* with and without added Faecal Constituents

Percentage and nature of faecal constituents added to food	Total No. of larvae employed	Mortality (No.)	Number of Pupae after days					Percentage diapause
			25	30	35	40	50	
Nil (Control)	150	7	49	126	129	129	131	9.3
50% lipid free (ether extracted) faecal pellets	300	22	76	105	127	129	133	49.7
50% whole faecal pellets	300	17	11	21	32	34	36	83.0

* Each culture had a total food concentration of 33.3 mg flour/larva in all the groups. However, the quantity of food per larva was not uniform for all the larvae during the entire experimental period due to mortality and slight variation in the larval period of individual insects.

faecal pellets began to manifest itself when the food was only about 30 mg. It may be noted that the food available per larva in both the experiments was considerably more than its normal requirement. It appears that when the quantity of food was comparatively less more of the larvae might have fed occasionally on the faecal pellets which might have caused them to enter into diapause. Stanic *et al.*⁴ showed that when the food is mixed with fine inert materials upto a concentration of 75 per cent, all the larvae completed development within the normal period, which led these authors to suggest that the larvae may feed selectively on the

food when extraneous inert materials such as cellulose powder or saw-dust is mixed with the food, the possibility that they may feed on the faecal pellets cannot be ruled out. The intake of faecal pellets may be more when the food is comparatively limited.

It may also be seen from Table 2 that the removal of ether soluble lipids from the faecal pellets did reduce the percentage of larvae entering diapause though it did not prevent diapause totally.

In a third set of experiments the larvae were reared singly with 30 mg of wheat flour with and without added faecal constituents as shown in Table 3. It may be seen that a significant

TABLE 3

Incidence of Diapause in Cultures of Trogoderma maintained at a total Food Concentration of 30 mg Wheat Flour/Larva with and without added Faecal Constituents

Percentage and nature of faecal constituents added to food	Total No. of larvae employed	Mortality (No.)	Number of Pupae after days						Percentage diapause
			25	30	35	40	45	50	
Nil (Control)	50	2	1	19	45	47	47	47	2
75% lipid free (ether extracted) faecal pellets	100	3	Nil	4	30	39	41	43	58
75% whole faecal pellets	100	4	Nil	5	14	19	19	19	77
2.5% faecal lipids (ether extract)	50	3	Nil	9	30	32	33	33	30
5.0% faecal lipids (ether extract)	50	4	Nil	-	24	25	26	26	42

percentage of the larvae entered diapause in cultures in which the faecal pellets were added. In this experiment the faecal pellets were added at a concentration of 75 per cent of the total diet substrate as against 50 per cent in the previous experiment (the quantity of food per larva remaining more or less the same); however, this did not result in a consistent increase in the percentage of diapause. That the percentage of larvae entering diapause could be reduced by the removal of ether soluble lipids from the faecal pellets is again demonstrated by the present set of experiments. It is also significant that a certain percentage of diapause could be induced by addition of the extracted lipids into the food.

The data presented in Tables 1, 2 & 3 also show that the larval period of some of the non-diapause larvae was slightly longer in those reared in the presence of added faecal constituents than in those reared on wheat flour alone.

Since the juvenile hormone is extractable with ether¹³ it should be expected that ether extracted faecal pellets, as against whole faecal pellets, would be totally ineffective in inducing diapause if the diapause is caused by juvenile hormone like principles present in the faecal pellets. The present experiments showed that a certain percentage of diapause could be induced even by faecal pellets from which the ether soluble materials were removed. It may be noted that a certain percentage of diapause could also be induced by the ether extract. These observations point to the fact that the diapause inducing factor could be removed partially by ether extraction. The nature of this factor and its similarity to juvenile hormone remains uncertain at present. However, it is evident that the diapause inducing com-

ponent of the faecal pellets is either a lipid component or a contaminant of the lipid extract. It may be mentioned here that some studies which are in progress show that the addition, at 2.5 per cent level, of a chloroform-methanol extract of the faecal pellets to the food causes retardation of the growth of larvae. It could well be that an optimum concentration might allow normal growth of the larvae but inhibit pupation i.e. induce diapause. Extensive studies are in progress to isolate and characterize the biologically active principle present in the faecal lipid extracts.

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