Effect of food volume and photoperiod on initiation of diapause in the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae)

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Abstract

Trogoderma variabile overwinters as a fully grown larva in a state of developmental arrest called diapause. Diapausing larvae are less active than non-diapausing larvae, but they do emerge from refugia occasionally to feed. They are less susceptible to insecticidal treatments than are non-diapausing larvae. Understanding the mechanisms of diapause induction and termination may offer the potential to manipulate the environmental conditions to either prevent diapause or trigger its termination and therefore facilitate pest control. The induction of diapause is complex. Here we report on the effect of day length and volumes of food medium on the initiation of diapause and explain some of the irregular findings reported previously in the literature.

Introduction

Insects must cope with unfavourable periods associated with seasonal changes that are cyclical, persistent and geographically widespread. Most species have evolved the ability to recognise environmental cues that signal these cyclical changes and respond to them with a series of specific physiological, behavioural and morphological adaptations which comprise the diapause syndrome (Lees 1955; Tauber et al. 1986). Diapause is usually initiated and maintained when many environmental conditions are still favourable for growth and development, and tends to be triggered by seasonally changing variables such as photoperiod and temperature during a specific life stage of the insect. Insects in diapause have a much lower metabolic activity, increased resistance to environmental extremes and often show altered behaviour (Tauber et al. 1986). Bell (1994) has recently reviewed diapause in stored product insects.

Trogoderma variabile Ballion overwinters as a fully-grown diapausing larva. Diapause in *T. variabile* differs from classical diapause in that the insect can continue to feed and to moult. Instead of pupating at the normal time, the diapausing larva continues to grow to approximately twice the weight of its non-diapausing counterpart (Elbert 1979a) and remains as a larva for an extended period. In this state the larvae are more tolerant of phosphine (Banks and Cavanaugh 1985), low temperature and starvation (Elbert 1979a).

Under almost any situation, some larvae of a cohort will not pupate and will enter diapause. Diapause initiation in *T. variabile* is clearly complex, with a number of stimuli being implicated. Loschiavo (1960) found that daily disturbance of insects during development increased the proportion entering diapause compared withundisturbed controls. Larvae reared singly are much more likely to enter diapause than larvae reared in groups (Partida and Strong 1975; Elbert 1979a,b). One experiment by Elbert (1979b) showed that for larvae reared in groups in constant darkness, a higher proportion pupated when reared at 25 or 35 compared with 30°C. At 30°C and 50% r.h. less than 10% of larvae reared in groups entered diapause, irrespective of photoperiod. However, a high proportion of larvae reared singly entered diapause under conditions of constant darkness (88%) compared with a photoperiod of 8L:16D (47%) or 16L:8D (29%). Elbert (1979b) also showed that diapause was induced in fourth and fifth instar larvae. These results are consistent with induction of diapause in autumn when *T. variabile* larvae would experience short day length (or complete darkness in bulk grain within a store) and lower temperatures. Given that the original mode of life for *T. variabile* is to develop singly or in small groups in bee and wasp nests (Strong and Okumura 1966), it would seem that diapause is the normal condition and only extraordinary combinations of stimuli will prevent its induction.

One stimulus that has been implicated in diapause induction and has relevance to pest control is the volume of food available to the larvae. Burges (1961) compared the rate of diapause initiation in volumes of food ranging from 0.7 to 450 mL of food per container. In spite of having varying numbers of larvae per container, the general trend was for the rate of diapause to increase with decreasing volume of food. Elbert (1979b) and Loschiavo (1960) also contained data for proportion of isolated larvae pupating, in different volumes of food (1.5 and 8.5 mL, respectively), but these results were not consistent with those of Burges (1961). Therefore, the following experiment was designed to investigate the effect of food volume on diapause induction in *T. variabile* larvae. The experiment was done with isolated larvae in either constant darkness or with a photoperiod of 13L:11D (longest summer day in southern New South Wales, Australia). In order to distinguish the importance of the spatial dimension (volume) of the food medium from the nutritional requirements (amount of food that is good to eat), volumes of food medium were created by mixing food with cellulose powder, that the insects would ingest but which would have no nutritional value. Experiments were run at 30–32°C and 50–60% r.h., so as to be least conducive to diapause induction and maximally comparable to the data of other workers.

Methods

Stock cultures were maintained using a method that allowed successful culturing of large numbers of larvae that pupated at a very high rate (i.e. did not diapause) and resulted in consistently rapid development of large adults. The method was to add about 5–10 males and 50 females to a 650 mL jar containing 200 g of rearing medium. This medium (SWO) comprised 1 part finely ground Savoiardi sponge biscuits, 7 parts kibbled wheat and 2 parts ground rolled oats, at an overall moisture

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content of 10%. The culture jars were kept in a totally dark incubator at 30–32°C at 50–60% r.h. and were seldom disturbed. The time from addition of adults to emergence of the next generation of adults was 5–6 weeks.

First instar larvae were used to start the experiments on diapause induction. These were obtained by collecting eggs and allowing them to hatch on black filter paper in a petri dish. Individual larvae were easily seen and could then be transferred individually using a very fine brush.

The experiment was designed to investigate the effect of the spatial dimension (volume) of the food medium compared with the quantity of food required for adequate development on the rate of diapause induction. This was done by creating different volumes of medium, by mixing food (SWO) and non-nutritive cellulose powder in different proportions. Thus, the experimental media were prepared as 100% SWO or 1/2, 1/4, 1/8, or 1/16 SWO and the rest cellulose powder (Table 1). The volumes used were 1, 2, 4, 8 and 16 mL. A number of larvae were reared exclusively on cellulose powder, to confirm the non-nutritional quality of this filler material.

Three sizes of glass vials were used in the experiment depending on the volume of medium required: 150/20, 160/20, 75/15 mm height/diameter. Fluon (polytetrafluoroethylene) was applied internally to the top 1–2 cm of each vial to prevent escape of larvae and fine metal mesh lids were used to close each vial. Vials were set up in series with each volume in each series having 10 replicates on each occasion. The weight of medium necessary to reach the required volume was determined and weighed for each vial. The medium was then added to each vial and fluffed up or tamped down to achieve the required volume. Thus, each vial in each series contained both the same weight and the same volume of medium.

All vials were incubated at 30–32°C and 50–60% r.h. Seven weeks later the proportion that pupated was determined for each combination. Any larva that had not pupated by then was deemed to be in diapause, following Burges (1961).

The complete experiment with differing proportions of food and cellulose powder was set up on six occasions with 10 vials per combination (except on one occasion when there were only 5 per combination) and incubated in constant darkness. One complete experiment was set up and incubated at 13 hours light and 11 hours dark. On another 7 occasions, the 100% SWO portion only of the experiment was prepared and run at 13L:11D, all with 10 vials per combination.

Results

Following the convention of earlier workers, results are presented as percent pupation rather than diapause, emphasising the normal nature of diapause and the specific stimuli required to induce pupation.

All larvae held exclusively on cellulose powder died very quickly, confirming the non-nutritive nature of the filler.

Table 2 shows the results of using different proportions of food and the total volume of medium on the proportion of insects that pupated under conditions of constant darkness. There was a trend towards increasing pupation with increasing volume of medium, irrespective of the proportion of food in the medium.

Figure 1 shows the effect of different volumes of food medium on the proportion of larvae pupating under conditions of long days (13L:11D). In contrast to the situation in the dark, there is a much higher rate of pupation under long days, although very small volumes inhibit pupation under both regimes.

Discussion

For insects reared singly under constant dark conditions, the spatial dimension (volume) of the food medium was more important than food quality in diapause initiation. Addition of cellulose powder to a small amount of food made the resulting medium equivalent to a much larger amount of food in its effects on pupation (Table 2). This result also showed that the food quality per se is not very important, and it is reasonable to compare results of different workers that fed the larvae on different diets. These results confirm the work of Elbert (1979b) on the effect of photoperiod on diapause induction.

These results also allow us to understand the results of previous work. Figure 2 compares the data from different sources on diapause induction in isolated larvae reared at

Table 2. Effect of volume of medium on proportion of *Trogoderma variabile* pupating at 30–32°C, 50–60% r.h. in constant darkness, with standard errors (SE). The medium was composed of varying proportions of food (SWO) and cellulose powder (a non-nutritive filler).

Proportion SWO		Total volume of medium (mL) 1	2	4	8	16
1	proportion	0.06	0.14	0.14	0.16	0.27
	SE	0.03	0.04	0.05	0.09	0.15
1/2	proportion		0.08	0.21	0.27	0.34
	SE		0.06	0.11	0.08	0.06
1/4	proportion			0.14	0.20	0.34
	SE			0.06	0.06	0.06
1/8	proportion				0.20	0.44
	SE				0.08	0.05
1/16	proportion					0.50
	SE					0.14

Table 1. Experimental design for the effect of different food volumes on diapause induction in *Trogoderma variabile*. For each final volume, the constituent volumes of food (SWO) and non-nutritive cellulose powder (cell) are given, with their corresponding weights in brackets. Because of the packing effect with larger volumes of food, the weights of food used were not simple multiples of the weight of 1 mL. The cellulose powder was much less dense than the SWO.

Proportion SWO	Total volume of medium in mL (g) 1		2		4		8		16	
	SWO	cell	SWO	cell	SWO	cell	SWO	cell	SWO	cell
1	1 (0.49)	0	2 (1.12)	0	4 (2.44)	0	8 (5.27)	0	16 (10.07)	0
1/2			1 (0.9)	1 (0.25)	2 (1.12)	2 (0.37)	4 (2.45)	4 (0.80)	8 (5.27)	8 (1.52)
1/4					1 (0.49)	3 (0.58)	2 (1.12)	6 (1.14)	4 (2.45)	12 (2.28)
1/8							1 (0.49)	7 (1.40)	2 (1.12)	14 (2.66)
1/16									1 (0.49)	15 (2.85)

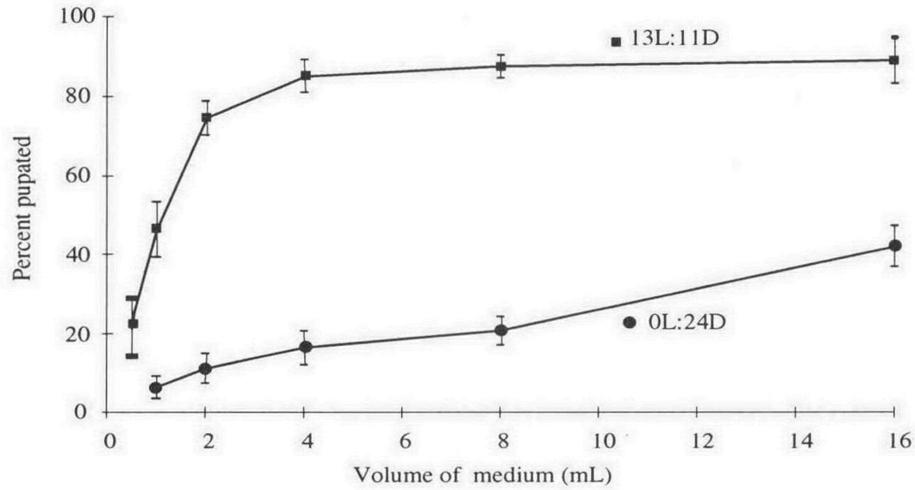

Fig. 1. Effect of volume of medium on pupation of *Trogoderma variabile* reared at 30–32°C, 50–60% r.h. under conditions of constant darkness or a 13L:11D photoperiod. The error bars represent ±1 standard error. The line for constant darkness combines all the data of Table 2. ■ 13L:11D, ● 0L:24D.

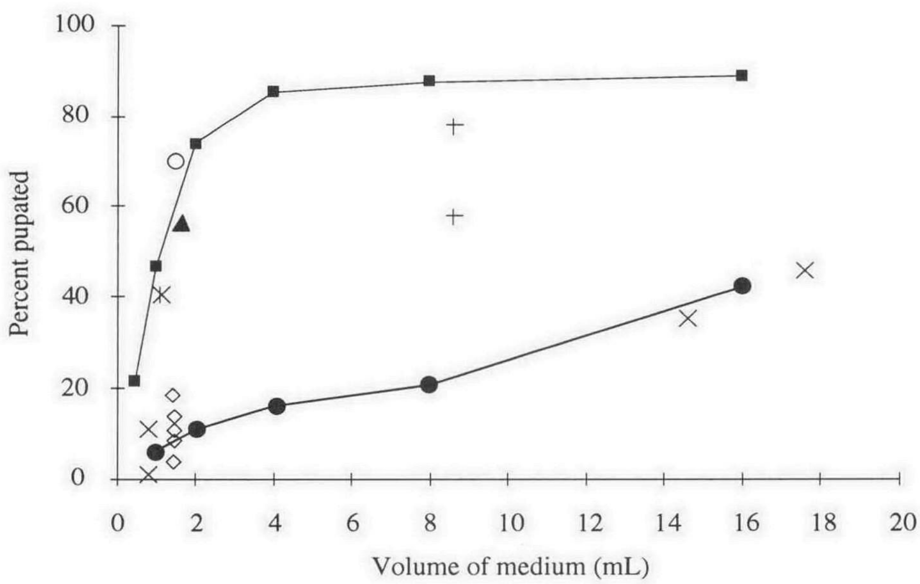

Fig. 2. Comparison of data from different workers on the effect of volume of medium on pupation of *Trogoderma variabile*. All data refer to conditions of 30–32•C and 50–75% r.h. ■ Long day 13L:11D, ● Constant dark 0L:24D, ◇ Elbert (1979b) 0L:24D, ▲ Elbert (1979b) 8L:16D, O Elbert (1979b) 16L:8D, × Burges (1961), + Loschiavo (1960), * Partida and Strong (1975).

30–32°C and 50–75 % r.h. The data are from the current study, from Elbert (1979b) for conditions of constant darkness, short day and long day, and from Partida and Strong (1975), Burges (1961) and Loschiavo (1960) none of whom specified the light regime. The various data of Elbert agree well with the data collected in this study for complete darkness, short and long days. Partida and Strong (1975) used 0.6 g of ground rolled oats for each larva (approximately 1 mL), and the data fall close to the line for long days. The data of Burges fall along the line for complete darkness. Although there was no direct reference to photoperiod in Loschiavo (1960), the way the data fall between the dark and light data suggests that the insects were exposed to a daily photoperiod of less than 13 hours light.

The induction of diapause is triggered by several factors including photoperiod, food volume, temperature and density of larvae, which can act alone or in combination. The larvae enter diapause in late summer and autumn when the photoperiod is shortening, and the temperature is falling. Good storage practice always emphasises hygiene, drastically reducing volumes of residues and numbers of larvae, both of which will act to promote diapause. Therefore, it seems unlikely that we can operationally manipulate environmental conditions to prevent induction of the tolerant diapausing stage. The most practical approach for control of this pest will be to ensure that all control techniques are adequate to control the more tolerant diapausing larva.

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