beats/min); 2. the Ca^{++} concentration (in the narrow range that can be studied); 3. the pH of the bathing medium (7.0–7.8, buffered with bicarbonate, bicarbonate-HEPES, or phosphate); and 4. temperature (30° or 37 °C). Furthermore, the sensitivity of hearts from embryos obtained from eggs laid by hens at the beginning and end of their productive period did not differ. The contractility of preparations stabilized in a phosphate buffered medium aerated with 100% O_2 deteriorated if the medium was then bubbled with air, indicating that the preparations do not suffer from O_2 toxicity under our routine conditions of study. Therefore, it seems unlikely that methodological differences are the basis for the conflicting results that have been obtained in different laboratoires, or within our own laboratory. It would seem more probable that the different results that have been reported by our own and other laboratories are due to true differences in the preparations studied.

Influence of larval diapause on pheromone communication in the Khapra beetle, *Trogoderma granarium* Everts

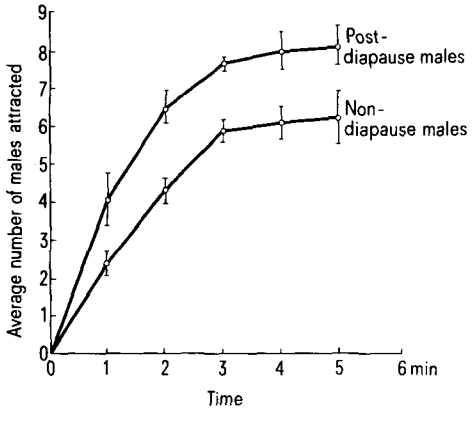
C. Adeesan, A. J. Tamhankar and G. W. Rahalkar

Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085 (India), 10 February 1976

Summary. In *Trogoderma granarium*, induction of larval diapause by sub-optimal temperature enhanced the efficiency of pheromone perception by adult males. Such diapause also altered the pattern of pheromone production by females.

In pheromone communication among insects, release of the chemical stimulus, as well as the response of the target insect, is influenced by a variety of factors[1]. In our earlier studies, we had observed that in *T. granarium* the female secretes a pheromone which attracts the male, and that factors such as male age, prior mating and presence of females affect the male's response to the pheromone[2]. The larva of this beetle undergoes a facultative diapause under suboptimal temperatures[3]. Such diapausing larvae feed intermittently, resulting in the accumulation of fat, glycogen and protein[3,4]. Further, post-diapause females have been shown to have higher fecundity[5]. The present study relates to the influence of this type of diapause on the adult behaviour of *T. granarium* in terms of pheromone production by females and the responsiveness of males to the pheromone.

Materials and methods. The insects used in this study were obtained from a stock culture maintained for several generations at 36 ± 1 °C on broken wheat. For inducing diapause, 18–20-day-old larvae from the stock culture were released on fresh medium and maintained at 25 ± 1 °C. After a period of 4 months, the larvae were returned to 36 °C to break the diapause. On pupation, males and females were separated and kept for adult emergence.



Response of post-diapause males to female sex pheromone.

Since the behavioural response of an insect to sex pheromone bears a direct relationship to the pheromone content over a range of concentrations, the level of response of males was considered a reliable indicator of the quantity of pheromone secreted in unit time[6,7]. For collection of pheromone, 3 batches of freshly eclosed females from both post-diapause and normal lots were released separately in petri dishes lined with absorbent paper. The papers were removed at pre-determined intervals and fresh papers were provided till the insects started dying in any of the replicates. Pheromone from each such paper was extracted separately in known volume of diethyl ether. Each extract was bioassayed in an olfactometer using 6–7-day-old virgin males obtained from the stock culture. For comparing the level of responsiveness of post-diapause and normal males to the female pheromone, 6–7-day-old males from these 2 categories were assayed with a known concentration of stock pheromone extract. The number of males attracted to the pheromone source was scored every minute for 5 min. All assays were repeated 10 times with 10 fresh males per assay. The olfactometer and the method of assay employed in these studies were the same as described by Adeesan et al.[2].

Results and discussion. The pattern of pheromone secretion, by both post-diapause and normal females, in relation to their age was significantly different (table). In the case of normal females, pheromone secretion was highest during the first 7 days after eclosion and subsequently the secretory activity diminished. By about the 12th day, the females started dying. However, in post-diapause females maximal secretory activity was observed up to 14 days post emergence. Even after this period, the females continued to secrete the pheromone up to 18 days, when they started dying.

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Effect of larval diapause on sex pheromone secretion by female *T. granarium*

Female age (days)	No. of males responding[a] to pheromone extract from post-diapause females I	II	III	normal females I	II	III
1–2	8.7	6.0	8.3	9.0	8.7	8.8
3–4	8.2	7.2	7.0	8.7	7.6	8.5
5–7	8.2	8.4	8.5	7.6	8.0	8.4
8– 9	8.8	8.2	9.4	6.7	6.0	5.7
10–11	8.1	7.9	7.8	4.8	6.6	6.2
12–14	8.0	8.4	8.7	–	–	–
15–16	6.6	5.8	5.9	–	–	–
17–18	5.2	5.1	5.9	–	–	–

[a] Each figure represents mean of 10 assays. I, II, III: Pheromone extracted from 3 batches of 50 females each.

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The degree of response of post-diapause males was also significantly higher than that of normal males (figure). The enhanced responsiveness of diapause males was evident even within the first minute of observation; and this difference was discernible throughout the period of assay. It is therefore apparent that the diapause males are able to detect the presence of pheromone more quickly. It may be due to the enhanced agility of the post-diapause males as a result of increased energy reserves. Also the possibility of lowered threshold for pheromone perception cannot be ruled out. This, and the fact that such diapause permits enhanced nutrient reserves, tends to suggest that these insects may have greater mating vigour than normal ones. Enhanced mating vigour as a result of diapause has been observed in alfalfa weevil[9].

The results presented here clearly indicate that induction of larval diapause by sub-optimal temperature, enhances the efficiency of pheromone communication between the sexes. In *T. granarium* diapause is also induced by factors like crowding, presence of faecal matter in the medium, and sub-optimal nutrition[10,11]. However, it is not known whether larval diapause induced by these factors will lead to enhanced pheromone secretion and male response.

Effects of high hydrostatic pressures on the movements of Na^+, K^+ and Cl^- in isolated eel gills

A. Péqueux and R. Gilles[1]

Laboratory of Animal Physiology, 22, quai Van Beneden, B–4020 Liège (Belgium), 19 May 1976

Summary. Hydrostatic pressure applied to isolated eel gills induces changes in the tissue Na^+, K^+ and Cl^- contents. It also inhibits the activity of the ($Na^+ + K^+$) ATPase. Results are discussed in terms of an effect of pressure on the Na^+ and Cl^- pumps and on the passive permeability processes.

When isolated gills from sea water eels are incubated in oxygenated sea water, a constant level of NaCl is maintained against the diffusion gradient[2–5]. This seems to result from an active NaCl extrusion coupled with K^+ entry. The maintenance of this constant ionic level indeed is very sensitive to the presence of oxygen in the medium and to the action of specific inhibitors like ouabain and 2–4(α)-dinitrophenol[4–6]. Nevertheless, the connections between different transport processes, as well as the real nature of the active mechanisms, are still unknown.

Most of the theories on ionic passive and active transfers involve the existence of chemical reactions, ionization processes, binding to carriers or enzymes. These chemical processes may be associated with volume changes and may therefore be sensitive to hydrostatic pressure[7]. Hence high pressure could be utilized as an analytical tool to approach the study of the structure of the cell membrane in relation to function. From this point of view, pressure has already been shown to act selectively on passive ionic permeability and to inhibit the Na^+ active transport of the isolated frog skin[8–10]. Such results led us to study the effects of high hydrostatic pressure on the movements of Na^+, K^+ and Cl^- in isolated gills of the eel.

Material and methods. Experiments have been performed on isolated gills from silver eels Anguilla anguilla L. adapted to sea water (521 mEq/l Na^+, 19 mEq/l K^+, 610 mEq/l Cl^-, pH 7.8, t° 15 °C). The isolated tissues have been submitted for 1 h to hydrostatic pressures in an apparatus designed to avoid the presence of any gas phase which has been described elsewhere[8]. In the present study, the volume of preoxygenated saline used in the experimental set-up was 50 ml. Dissection of the gills as well as incubation conditions have been described previously[5].

At the end of the experiments, gill filaments are cut off the incubated gills; they are blotted on filter paper, weighed and dried at constant weight in an oven at 110 °C for dry weight measurements. The gill filaments are then digested for 48 h in 4 ml HNO_3 0.1 N. Tissue ionic concentrations have been calculated from the measurements of the ions content in the digestion medium. Na^+ and K^+ determinations were done by flame photometry. Cl^- content was estimated with a Buchler-Cotlove chloridometer. The results, calculated in μEq of ion per g tissue dry weight, have been expressed in this report as ratio of the

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