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QUANTITATIVE DETERMINATION OF THE MAIN COMPONENT OF THE PHEROMONE OF THE BEET ARMY WORM MOTH AS A FUNCTION OF THE TIME OF DAY INFLUENCE OF A BRAIN EXTRACT ON THE INDUCTION OF THE PHEROMONE

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The majority of insects are active during specifically determined times of the day, producing and emitting a sex pheromone. The transmission of a physiological and environmental signal for the production of the sex pheromone is effected by the endocrine system. The behavioral technique of biotesting used in the majority of investigations does not provide the possibility of determining the influence of hormones on the synthesis and emission of the components of sex pheromones.

The direct chemical determination of the pheromone titer shows that the production of pheromones takes place under hormonal control in *Heliothis zea* [1], *Chilo suppressalis* [2], *Heliothis armigera* and *Spodoptera littoralis* [3], and, possibly,

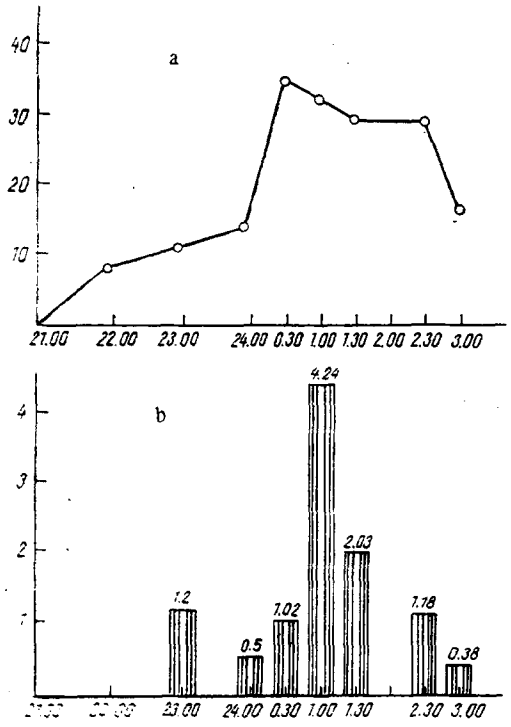

Fig. 1. Dynamics of the secretion of the main component of the pheromone of female beet army worm moths.

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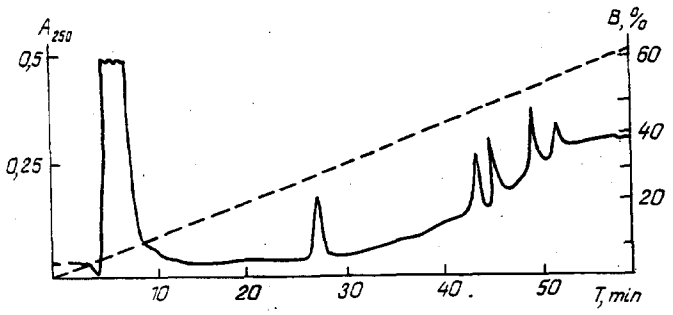

Fig. 2. High-performance liquid chromatography of an extract of beet army worm moth heads.

in *Platynota stultana* [4]. We have investigated the pheromone of female beet army worm moths of the Central Asian population *Spodoptera exiqua.*

The activity of the females was observed under weak red light. Four groups wth 10 females in each, were observed at 15-minute intervals, and the numbers of active females with projecting glands (9th segment of the abdomen) was recorded.

With acute squeezing of the abdomen, its 9th segment was removed and was extracted with 0. 1 ml of ethyl acetate containing 4.33 μg of tridecyl acetate as internal standard. Gas-liquid chromatography was conducted on a Hewlett-Packard instrument with an OV-1 column at a rate of flow of the csarrier gas (helium) of 43 ml/min, the temperature of the evaporator and detector being 230°C and that of the thermostat 160°C.

The activity of the production of the pheromone in female beet army worm moths was determined from 9. 00 p.m. to 4.00 a.m. on the following day, i.e. for 7 h. The amount of the main component of the beet army worm pheromone — tetradeca-*cis*-9,*trans*-12-dienyl acetate secreted by the females was determined by gas-liquid chromatography, the maximum peak being observed at 1.00 a.m. (Fig. 1). On considering the numbers of active females as percentages, the maximum is found at 0.30 a.m., and a plateau characterizing the most active time of day for the female moths coincides with maximum level of secretion of the main component (Fig. 1).

During the secretion of the pheromone, the female moths adopted a pose visually differing slightly from the rest pose. The abdomen was raised, its tip was lightly bent, and its distal segments projected upwards and swayed slighly from side to side, while the tip pulsated.

The duration of such behavior was considerable - of the order of 10 min in the active period of the day.

We investigated the influence of the brain hormone on the production and emission of the pheromone. For this purpose, the heads of 2- to 3-day female and male beet army worm moths (11 individuals in each case) were homogenized and were then extracted with double-distilled water (1 ml). After the head tissue had been precipitated by centrifugation (10 min at 8000 rpm), the supernatant was treated with 1 μl of a 20% solution of phenylmethanesulfonyl fluoride (PMSF) to inhibit proteolytic enzymes (the presence of which is possible). The extract obtained was analyzed by reversed-phase HPLC (Nucleosil RP-300-7 column, 4.6 × 150 mm, Macherey-Nagel, FRG) in a concentration gradient of acetonitrile.

This showed that 95% of the substances present in the extract (fraction 1) issued from the column with the solvent front, i.e., they consisted of a mixture of low-molecular-mass organic compounds. In addition to this, 5 fractions (fractions 2-6) containing compounds of protein nature were isolated from the extract. It must be mentioned that the times of retention on the column of the fractions obtained from the extracts of the heads of the females and the males were the same, which shows the identity of these components. At the same time, the amount of protein components in relation to that of the low-molecular-mass substances in the extracts of the heads of the males was only half that in the extracts of the heads of the females.

The solution to be tested (20 μl) was injected into each female through the intersegmental membrane with a Hamilton syringe. The injection was made in the period of a distinct fall in activity between 3.00 and 4.00 a.m. This led to a restoration of the secretion of the hormone to a level no different from the normal secretion of the females — 3.84 ng per female. No appreciable difference was found between the homogenates from the females and the males, which agrees with literature information [3, 5]. After injection, a marked change in the behavior of the females was observed; they moved actively and then adopted the receptive pose, their wings and abdomens trembled, and an extension of the last segment was observed. Thus, it is obvious that a brain factor controls the secretion of the pheromone in female beet army worm moths.

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