Note

Electrophysiological Response of the Tobacco Cutworm, *Spodoptera litura* (F.), to Antifeeding Compounds*

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The electrode was inserted in the hypopharynx of the tobacco cutworm larva and the antifeeding compounds were topically applied at the various mouth parts by means of the microsyringe. The larval hypopharynx and maxillary palps were the most sensitive targets to chlordimeform and clerodin, respectively. The results led to the conclusion that the proposed primary sites of action of clerodin are the maxillary palps. On the other hand, primary site of action of chlordimeform is the hypopharynx.

Receptors of several specialized feeding deterrent have been described in lepidopterous larvae. Concerning gustation, a series of experiments was reported on responses of various receptors in the larvae of the silkworm, *Bombyx mori*, to carbohydrates and sap of some plant leaves.¹⁻³⁾ Recently, the relationship between chemoreceptor sensitivities towards natural feeding inhibitors and food preference in various chewing insects was reported.⁴⁻⁸⁾

A lot of reports were published on sensory coding from various receptors located on the antennae of insects. Electrophysiological observations on the silkworm larvae to some chemicals⁹⁾ and bombykol,¹⁰⁾ on the rice stem borer larvae and the fruitpiercing moth to food attractants,^{11,12)} and on the tobacco hornworm to various plant extracts¹³⁾ were reported.

Schoonhoven¹⁴⁾ emphasized perception of antifeedants at the sensory level may involve different mechanisms. Feeding deterrents may stimulate specialized receptors or may modify the activity of receptors tuned to other compounds and thus alter the sensory code.

Chlordimeform and clerodin caused behavioural changes after their topical application to certain organs¹⁵⁾ and affected the rates of movements of not only the treated targets but also the neighboring head appendages.¹⁶⁾ The electrophysiological study was thought to be useful for better understanding of the mode of action of these antifeedants.

The 4 hr starved 5th instar larvae of the tobacco cutworm, *Spodoptera litura*, were used. Chlordimeform (8000 ppm) and clerodin (125 ppm) were prepared in the same solvent mixture as used in the previous experiment.¹⁵⁾

The larvae were fixed, ventral side (sternite) upwards, on a piece of plastic foam plate with three loops of steel around the neck, thorax and the posterior abdominal segments.

Recording was accomplished by means of a stainless steel electrode (5 μ m diameter, J-3002 MT, Giken Co.). The electrode was inserted in the proximal part of the hypopharynx by means of a micromanipulator.

* Mode of Action of Antifeeding Compounds in the Larvae of the Tobacco Cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) (Part 6)
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This method permitted simultaneous recordings of muscle potentials and nerve impulses. The impulses were conducted proximally toward the central nervous system, and also distally toward the hypopharynx tip (indicated by the arrows in Figs. 1 and 2). The effectiveness of the treatments was based on the number of impulses. The test compounds were applied topically to various mouth parts and antennae by means of a fine glass capil-

lary adapted to the microsyringe attached to a manual microapplicator. To compare sensitivities of different applications, injection was conducted in the pleura of the first abdominal segment of the same animal. Each larva was received first $0.00466 \mu\text{l}$ of the solvent and then the same volume of anti-feedant solution. Each application was made after the response in the previous trial had disappeared. Each material was applied to

Fig. 1 Spike records in oscillographs of the electrophysiological responses occurring at the central part of the hypopharynx after application of chlordimeform on various parts of the head appendages.

- a: Control.
 - b: 1 min after treatment on the two pairs of sensilla styloconica.
 - c: 5 min after treatment on the two pairs of sensilla styloconica.
 - d: 1 min after treatment on the antennae.
 - e: 5 min after treatment on the antennae.
 - f: 1 min after treatment on the top of both maxillary palps.
 - g: 5 min after treatment on the top of both maxillary palps.
 - h: 1 min after treatment on the hypopharynx.
 - i: 5 min after treatment on the hypopharynx.
 - j: 10 min after treatment on the hypopharynx.
 - k: 15 min after treatment on the hypopharynx.
 - l: 1 min after treatment of injection.
 - m: 15 min after treatment of injection.
- Arrows indicate spikes of the neurone.

at least ten animals. The experiments were performed at environmental conditions as constant. The indifferent electrode (platinum wire) was inserted into the insect body cavity of thorax. Responses from the hypopharynx were amplified by a microelectrode amplifier (MEZ-8201) and a biophysical preamplifier (AVB-2B). The oscilloscope (VC-8) to which attached recording camera (PC-2B) was attached was used. All apparatus were products of Nihon Kohden Co.

The effect of chlordimeform in 8000 ppm showed very weak initial effects on the sensi-

tivities when applied to two pairs of sensilla styloconica (Fig. 1 b and c). The weak response of the larval antennae treated with chlordimeform solution (Fig. 1 d and e) supports the explanation reported by Dethier and Schoonhoven¹⁷⁾ that the magnitude of responses reflects the differences in sensitivity among sensory cells. It also supports the data depicted by Shimizu *et al.*,¹⁸⁾ that the application of chlordimeform hydrochloride on the larval antennae caused weak repetitive burst in mandibular movements. On the other hand, clerodrin (125 ppm) showed no effect when

Fig. 2 Spike records in oscillographs of the electrophysiological responses occurring at the central part of the hypopharynx after application of clerodrin on various parts of the head appendages.

- a: Control.
- b: 1 min after treatment on the two pairs of sensilla styloconica.
- c: 5 min after treatment on the two pairs of sensilla styloconica.
- d: 1 min after treatment on the antennae.
- e: 5 min after treatment on the antennae.
- f: 1 min after treatment on the hypopharynx.
- g: 5 min after treatment on the hypopharynx.
- h: 1 min after treatment on the top of both maxillary palps.
- i: 5 min after treatment on the top of both maxillary palps.
- j: 1 min after treatment of injection.
- k: 5 min after treatment of injection.

Arrows indicate spikes of the neurone.

applied on larval antennae (Fig. 2 d and e). Clerodin evoked a very strong initial effect when treated on to the pairs of sensilla styloconica (Fig. 2 b). This strong stimulating effect decreased about 5 min after the application (Fig. 2 c). This rapid short response may suggest that these organ may not contain deterrent receptors¹⁴⁾ or may be based on the explanation of Ma.⁶⁾

The violent response to chlordimeform was shown by the larvae whose hypopharynx were treated (Fig. 1 h-k). Three probable explanations can be given for this evoked action potential. The first one is the sensitivity of the pair of epipharyngeal sensilla coeloconica to the volatile vapor of chlordimeform. The second one is the sensitivity of the spines that richly cover the hypopharynx itself. The third is a direct biochemical reaction with secreted saliva.

A treatment of clerodin on two maxillary palps evoked a clear excitatory effect soon after the application (Fig. 2 h), and this effect also continued clearly until 5 min elapsed after the application (Fig. 2 i). This effect clearly confirms the interference of any gustatory receptor cell of any one of the sensilla basiconica located on maxillary palps.

The same sensitivities and responses were clearly noticed in the previous study concerning the rate of movement of different treated targets and other related neighboring head appendages.¹⁶⁾

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要 約

摂食阻害物質に対するハスモンヨトウの電気生理学的反応*

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ハスモンヨトウ幼虫の下咽頭に微細電極を挿入し、ミクロシリンジで口器各部に摂食阻害物質を局所処理した。幼虫の下咽頭がクロルジメホルムに対して最も感受性が高く、クレロディンに対しては小腮鬚が最も感受性が高かった。クレロディンの作用点は小腮鬚で、クロルジメホルムのそれは下咽頭であろうと考えられる。

* ハスモンヨトウ幼虫における摂食阻害物質の作用機構 (第6報)