Effect of Applied Dose of Insecticide on Penetration into Lepidopterous Larvae¹

Kimihiko SATO and Jun-ichi FUKAMI²

*Faculty of Agriculture, Tokyo University of
Agriculture and Technology, Fuchu,
Tokyo 183, Japan*

²*The Institute of Physical and Chemical Research,
Wako, Saitama 351-01, Japan*

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Amounts of carbaryl, fenitrothion and diazinon found in developing larvae of *Spodoptera litura* and *Galleria mellonella* 2 hr after topical application increase with larger larvae and have similar levels at a certain larval weight (SATO and FUKAMI, 1984).

To determine the effect of applied dose changes of insecticide to a certain weight insect and its penetration into the insect body, various doses of

¹⁴C-labelled insecticides were applied topically to a certain weight larva. Internal radioactivity, nonpenetration, etc. were then determined.

Two–5 larvae of *S. litura* (fresh weight: 250 ± 5 mg) reared aseptically on an artificial diet (HATTORI and ATSUSAWA, 1980) were treated topically on a dorsal part with 0.5–32 μ l of an acetone solution containing 4 μ g/ μ l of ¹⁴C-carbaryl, 2.45 μ g/ μ l of ¹⁴C-fenitrothion and 4.44 μ g/ μ l of ¹⁴C-diazinon (SATO and FUKAMI, 1984) using a microsyringe MS-10 (Terumo Co., Tokyo, Japan). Larvae of *G. mellonella* (fresh weight: 100 ± 3 mg) reared on an artificial diet (MITSURU et al., 1973) were also treated with 0.25–20 μ l of an acetone solution respectively containing each of three insecticides. Two or 3 replicates were made at 25°C. Treated larvae were kept in a petri dish (diameter: 6 cm) under the same conditions.

Four hr after application the treated larvae were rinsed with 1–2 ml acetonitrile to remove radioactivity remaining on the body surface. For determination of the internal radioactivity, the rinsed larvae were put into small filter cups and were

Table 1. Amounts of radioactivity found in each fraction 4 hr after topical application of ¹⁴C-carbaryl (2.0–64 μ g), ¹⁴C-fenitrothion (2.45–78.4 μ g) and ¹⁴C-diazinon (2.22–71 μ g) on *Spodoptera litura* larvae^a

Insecticide	Applied dose (μ g)	Unpenetrated (μ g)	Internal (μ g)	Excreted (μ g)	Container (μ g)
Carbaryl	2.0	0.05	1.43	0.14	0.15
	4.0	0.08	2.81	0.15	0.31
	8.0	0.10	5.32	0.32	0.69
	16	0.35	7.44	1.70	1.82
	32	0.55	10.1	1.95	4.53
	64	1.78	19.4	7.50	5.90
Fenitrothion	2.45	0.35	1.36	0.11	0.28
	4.9	1.10	2.51	0.15	0.71
	9.8	1.21	4.43	0.91	0.96
	19.6	1.42	8.17	2.23	1.18
	39.2	1.84	14.2	2.59	1.31
	78.4	2.11	26.3	8.55	2.14
Diazinon	2.22	0.32	1.31	0.09	0.20
	4.44	0.58	2.17	0.64	0.42
	8.88	1.91	3.67	1.22	0.92
	17.8	3.73	6.18	2.53	3.95
	35.5	7.82	10.7	4.83	8.81
	71	15.2	20.7	7.65	18.7

^a Fresh larval weight: 250 ± 5 mg.

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Table 2. Amounts of radioactivity found in each fraction 4 hr after topical application of ^{14}C -carbaryl (2.0–48 μg), ^{14}C -fenitrothion (1.72–49 μg) and ^{14}C -diazinon (1.8–44.4 μg) on *Galleria mellonella* larvae^a

Insecticide	Applied dose (μg)	Unpenetrated (μg)	Internal (μg)	Excreted (μg)	Container (μg)
Carbaryl	2.0	0.76	0.92	0.05	0.16
	6.0	2.40	2.52	0.10	0.63
	12	5.10	4.39	0.25	1.56
	24	7.48	6.17	0.45	7.79
	48	11.95	9.22	1.53	14.2
Fenitrothion	1.72	0.82	0.31	0.06	0.36
	2.94	1.29	0.35	0.16	1.04
	9.80	5.02	1.06	0.56	2.82
	17.2	8.81	1.99	1.19	4.58
	49	11.46	5.48	2.32	21.2
Diazinon	1.8	0.88	0.14	0.13	0.42
	3.6	1.65	0.22	0.15	0.92
	5.6	2.28	0.31	0.17	1.51
	22.2	8.70	1.24	0.69	6.10
	44.4	16.87	2.42	1.42	12.7

^a Fresh larval weight: 100 ± 3 mg.

Fig. 1. Percentage of internal radioactivity found in 250 ± 5 mg larvae of *Spodoptera litura* 4 hr after topical application of various doses of ^{14}C -carbaryl (○), -fenitrothion (▲) and -diazinon (×).

Fig. 2. Percentage of internal radioactivity found in 100 ± 3 mg larvae of *Galleria mellonella* 4 hr after topical application of various doses of ^{14}C -carbaryl (○), -fenitrothion (▲) and -diazinon (×).

combusted using a sample oxidizer (Packard Tri-Carb, Model B306). Determination of the radioactivity in each fraction was made by a Packard liquid scintillation spectrometer 460 CD (SATO and FUKAMI, 1984).

Average amounts of radioactivity found in each fraction 4 hr after topical application of various

doses of ^{14}C -carbaryl, -fenitrothion and -diazinon are shown in Tables 1 and 2. Disappearance of radioactivity from the surface (unpenetrated) of *S. litura* was faster than from *G. mellonella* with all doses of insecticide applied. Penetration rates of the insecticides to *G. mellonella* were in the order of carbaryl, fenitrothion and diazinon in all cases,

but the order in *S. litura* differed depending on the dose level of each insecticide.

The percentage of internal radioactivity calculated on Tables 1 and 2, found in larvae 4 hr after application of various doses are shown in Figs. 1 and 2. Those of carbaryl, fenitrothion and diazinon in *S. litura* decreased with larger applied doses, and the gap in carbaryl (71.5% at 2.0 μg to 30% at 64 μg) was especially wide. As LD_{99} of the three insecticides to *S. litura* in this experiment was 30–40 $\mu\text{g}/\text{larva}$ of each insecticide, 30–40% of applied dose would penetrate the larval body during 4 hr (Fig. 1.) On *G. mellonella*, the percentage of carbaryl decreased greatly from 46% at 2.0 μg to 19% at 48 μg . Penetration of fenitrothion and diazinon into *G. mellonella* was poor, and the levels remained respectively at 11–12% and 5–6% even at increased doses (Fig. 2). This fact suggests that high applied dose has little effect on penetration into the larval body. Because the partition

of an insecticide to internal lipid and water would have a limit or equilibrium, a high level dose will not penetrate into the internal side in proportion to the dose.

Therefore, in controlling an insect application of a high insecticidal dose is unnecessary. Only a dose which will penetrate the insect body and reach a target site need be used. It is essential for us to decide the most effective dose so that insect pests may be controlled while, at the same time, environmental safety is preserved.

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Development of Male Sex Cells in Transplanted Testes of the Swallowtail, *Papilio xuthus* L. (Lepidoptera: Papilionidae)¹

Hideharu NUMATA² and Toshitaka HIDAKA

Department of Zoology, Faculty of Science, Kyoto University, Sakyo, Kyoto 606, Japan

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In the swallowtail, *Papilio xuthus* L., some spherical spermatids degenerate in the fifth (final) instar larvae destined to enter pupal diapause (diapause-type larvae) and all the spermatids and sperms degenerate in the diapausing pupae (NUMATA and HIDAKA, 1980). After diapause termination, sperms develop *de novo* from the spermatogonia and spermatocytes which are saved from degeneration (NUMATA and HIDAKA, 1981).

NISHITSUTSUJI-Uwo (1961) carried out trans-

plantation of the testes of fourth or fifth instar larvae between diapause and non-diapause types of *P. xuthus* and concluded that the humoral conditions in the latter are more effective for promoting spermatogenesis than those in the former. However, the transplanted testes contained many degenerated sex cells regardless of the type of host larvae. She ascribed this degeneration to a mechanical shock at operation.

NUMATA and HIDAKA (1980) emphasized the role of the degeneration of male sex cells in the diapause program of *P. xuthus*. In the present study, therefore, testis transplantation was carried out again in order to clarify the cause of this degeneration in diapause-type larvae and diapausing pupae.

The experiment was performed at $25 \pm 1.5^\circ\text{C}$. Larvae of *P. xuthus* were reared on an artificial diet (KAMANO, 1965: slightly modified) under a diapause-preventing photoperiod of 16L–8D or a diapause-inducing photoperiod of 12L–12D (HIDAKA and HIRAI, 1970). The pupae of both groups were kept under 16L–8D.

Non-diapause-type larvae on the third day of

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² Present address: *Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi, Osaka 558, Japan*