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Biological activity of flucycloxuron, a novel benzoylphenylurea derivative, on *Tenebrio molitor*: comparison with diflubenzuron and triflumuron

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Abstract. Flucycloxuron, a novel benzoylphenylurea (BPU) derivative, exhibited insecticidal activity when injected into newly ecdysed pupae of *Tenebrio molitor*. Mortality occurs because of defective adult ecdysis. Treatment caused a reduction in both cuticle thickness and incorporation of ^{14}C-labelled precursor into chitin, although it had no significant effect on the protein synthesis. The potencies of other BPU compounds as inhibitors of chitin biosynthesis have been examined and results showed that diflubenzuron was less effective than either flucycloxuron or triflumuron.
Key words. Insecticides; chitin; proteins; cuticle; *Tenebrio molitor*.

The benzophenylureas (BPUs) prevent molting in insects and some other arthropods by interfering with cuticle deposition, and are considered as inhibitors of chitin biosynthesis[1–3]. Diflubenzuron (DFB), the most investigated benzoylphenylurea derivative, was found to be effective on *Tenebrio molitor* pupae[4] and several biochemical effects have been described[5–9]. Since the introduction of DFB, a number of other benzoylphenylurea derivatives have been developed. Flucycloxuron (FCX) is a new BPU controlling mites and insects[10] by interference with chitin synthesis[11]. The aim of the present investigation was a) to test the effectiveness of FCX by injection into *T. molitor* pupae, and b) to examine its effects on the cuticule by using radiolabelled precursors of chitin and protein.

Materials and methods

Insects. Tenebrio molitor pupae were collected from a stock colony reared on wheat flour, at 27 °C and 70% relative humidity, in continuous darkness. Pupae were staged according to their age in days from pupal ecdysis. Animals used in this study weighed from 120 to 140 mg at pupal ecdysis.

Treatment. Three BPUs were used in this study: flucycloxuron (FCX) and diflubenzuron (DFB) were both kindly provided by Philips Duphar B.V. (The Netherlands) while triflumuron (TFM) was supplied by Bayer AG. (Germany). All compounds were injected into newly ecdysed pupae. Each compound was diluted in distilled water with 10% acetone and injected (3 µl per insect) into the body with a Hamilton syringe between sternites 3 and 4. The same procedure was repeated with solvent alone for controls.

Insecticidal bioassay. The insecticidal assay was performed with FCX. The experiment was conducted with 3 replicates each of 10–60 pupae. Dosages ranging between 0.1 and 2 µg were tested. The percentage mortality obtained was corrected[12] and toxicity data were analysed by probit analysis[13].

Histology. Three days after treatment, sample pupae from control and FCX-treated series were dissected and fixed in Bouin's liquid. Sections (5 µm) were stained with azan stain[14]. The thickness of the cuticles was measured on 5 or 6 different pupae per series and then averaged.

N-acetyl-D-(1-^{14}C)glucosamine incorporation into chitin. Chitin biosynthesis was estimated in vivo by a method adapted from Grosscurt et al.[11]. Every day, during pupal-adult development, insects were injected with 0.2 µCi of N-acetyl-D-(1-^{14}C)glucosamine (=NAG) (54 mCi/mM, Amersham) and incubated for 2 h at 27 °C. The abdominal sternites were then taken out and after lipid elimination (chloroform-ether) and hydrolysis (NaOH 2N at 100 °C for 2 h) the cuticle obtained was solubilized overnight (NCS). After neutralization (HCl 5N) and addition of scintillation liquid (ACS), the radioactivity was measured in a Beckman LS 7000 counter.

Tritiated leucine incorporation into proteins. One-day-old pupae from control and treated series were injected with 3µ Ci L-(4,5–^{3}H)leucine (140 Ci/mM, Amersham) and incubated for 2 h at 27 °C. Cuticle proteins were extracted[15] from the abdominal sternal integument. After trichloroacetic acid precipitation and centrifugation (2000 G for 10 min), the resulting pellet was washed with ethanol, then with ethanol-ether (3:1), solubilized in NCS (Amersham), and neutralized with HCl before addition of scintillation liquid. Radioactivity was measured as described above.

Results

Morphological effects. Treatment of newly ecdysed pupae of *T. molitor* with various doses of FCX induced

Table 1. Insecticidal activity of flucycloxuron applied by injection into newly ecdysed pupae of *T. molitor*

Doses (µg per pupa)	Mortality (%)*
0.1	2.8[a]
0.2	3.3[a]
0.25	20.5[b]
0.3	29.4[b]
0.4	46.7[b]
0.5	73.1[c]
0.75	89.7[d]
1	96.3[d]
2	100.0[d]

*Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. Comparative toxicity of some benzoylphenylurea derivatives injected into newly ecdysed pupae of *T. molitor*.

Insecticides	ID^{*}_{50}	FL^{**}_{95}		Slope
		Minimum	Maximum	
Flucycloxuron	0.37	0.32	0.42	1.65
Diflubenzuron	0.98	0.69	1.39	3.37
Triflumuron	0.85	0.63	1.13	2.60

Data are expressed as µg per pupa.
*50% inhibition dose. **95% fiducial limit.

morphogenetic changes. Two abnormal types were observed: in the first, the insects failed to ecdyse and died inside the old exuviae, while in the second, the insects ecdysed partially and only the abdominal part remained inside the pupal cuticle.

Insecticidal activity. FCX injected into newly ecdysed pupae exhibited insecticidal activity with a dose-dependent relationship. Mortality occurred because of defective ecdysis. The results of the insecticidal test are summarized in tables 1 and 2. The 50% inhibition dose (ID50) was 0.37 µg (limit values: 0.32–0.42).

Table 3. Effect of flucycloxuron, injected into newly ecdysed pupae of *T. molitor*, on the thickness of the pupal post-ecdysial cuticle measured at the apolysis ($m \pm s$, $n = 5-6$).

Doses (µg per pupa)	Cuticle thickness (µm)*
0	14.6 ± 0.5^{a}
0.5	13.6 ± 3.0^{ab}
1	10.7 ± 1.1^{b}
2	6.1 ± 0.5^{c}

*Values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Measurement of cuticle thickness. The histological study of sternal cuticles showed that FCX treatment affected the cuticular secretion. In controls, the thickness of pupal post-ecdysial cuticle increased until apolysis (day 3) to reach a maximum of about 14 µm. The pre-ecdysial adult cuticle was secreted from day 4 after pupal edysis. FCX injected into newly ecdysed pupae reduced the cuticle thickness and slightly delayed the apolysis. Depending on the dose, FCX caused a 7–60% decrease in the thickness of the pupal post-ecdysial cuticle measured at apolysis compared to controls (table 3). In the treated series the apolysis occurred at day 4.

Effect on N-(1-^{14}C)acetylglucosamine incorporation into chitin. The in vivo incorporation of NAG into the sternal abdominal cuticle was determined at various times during pupal-adult development. In controls, incorporation of NAG was significant during the secretion, of

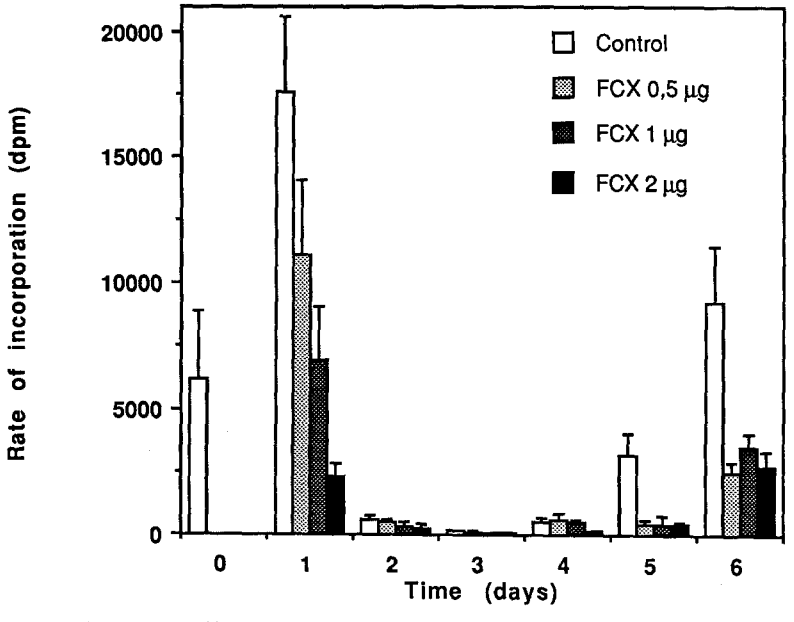

Figure 1. The in vivo incorporation of N-(1-^{14}C)acetylglucosamine (dpm/2h/explant) into the sternal abdominal cuticle during the pupal development of *T. molitor* in control and flucycloxurontreated series. Treatment was by injection into newly ecdysed pupae ($m \pm s$, $n = 3-6$).

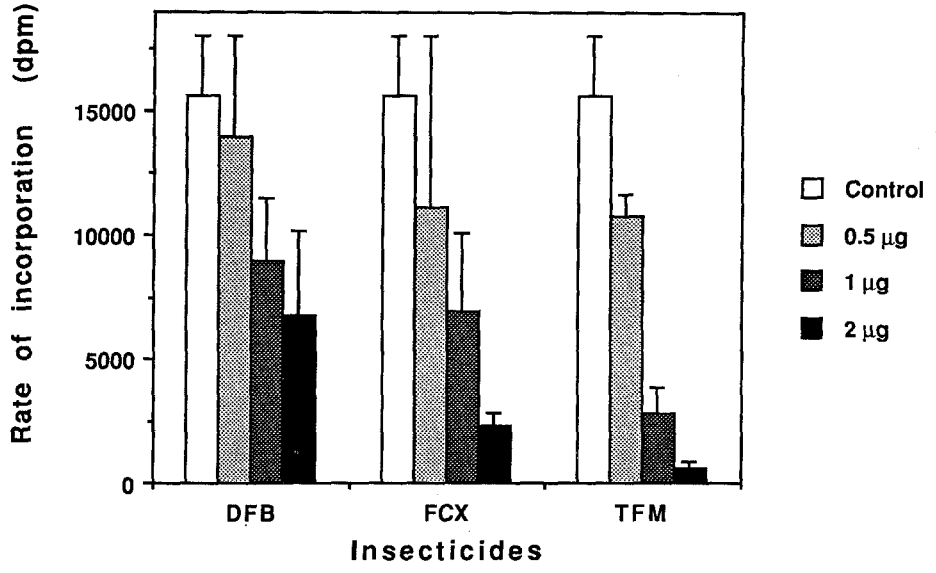

Figure 2. The in vivo incorporation of N-(1-^{14}C)acetylglucosmine (dpm/2h/explant) into the sternal abdominal cuticle of 1-day-old *T. molitor* pupae. Treatment was by injection into newly ecdysed pupae with solvent (control), flucycloxuron, triflumuron and diflubenzuron ($m \pm s$, $n = 3-6$).

cuticle in particular, at days 1 and 5 coinciding with pupal post-ecdysial cuticle and adult pre-ecdysial cuticle, respectively (fig.1). After FCX treatment there were still two important periods of incorporation but there was a significant ($p < 0.05$) reduction in their values compared to controls. The inhibition of NAG incorporation by FCX was significant and varied according to age and dose. The effect of FCX seemed more marked on the adult pre-ecdysial cuticle than on the pupal post-ecdysial cuticle. The potencies of two other BPU compounds have been examined by measuring the incorporation of NAG into chitin of 1-day-old pupae treated in the same way. The results of NAG incorporation, presented in figure 2, suggest that TFM was more effective than FCX and DFB.

Effect on tritiated leucine incorporation into proteins. The *in vivo* incorporation of tritiated leucine into proteins of the sternal abdominal cuticle was determined in control and treated pupae. 1-day old pupae after FCX-treatment showed no significant ($p < 0.05$) difference between control and FCX treatment (fig. 3).

Discussion and conclusion

FCX, a member of the BPU group, has been found to be effective on several crop pests[10]. Our results indicate

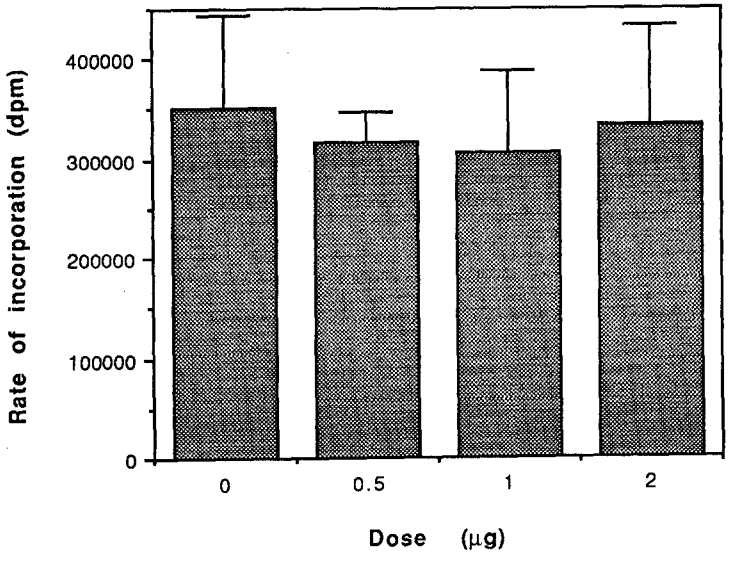

Figure 3. The in vivo incorporation of tritiated leucine (dpm/2h/explant) into proteins of the sternal abdomonal cuticle of 1-day-old *T. molitor* pupae after treatment at pupal ecdysis with solvent (control) and flucycloxuron ($m \pm s$, $n = 3-4$)

that FCX is toxic when injected into newly ecdysed pupae of *T. molitor*. The effects observed in FCX-treated pupae are similar to those commonly induced by other BPUs[1-3]. DFB, the most extensively investigated BPU derivative, prevents the incorporation of radiolabelled precursors into chitin in vivo[1-3] and in vitro[16]. This compound was found to interfere with chitin biosynthesis, resulting in death at adult emergence by failure in ecdysis. FCX treatment results in a reduction in the thickness of secreted cuticle, and inhibits the incorporation of radiolabelled NAG into chitin in *T. molitor*. Thus, as reported in *Spodoptera littoralis*[11], FCX acts by preventing chitin biosynthesis, confirming the primary mode of action of the BPU insecticides. The effect of FCX on protein synthesis has not been tested in insects. As indicated by the incorporation of tritiated leucine, FCX treatment had no effect on protein synthesis in the sternal cuticle of *T. molitor* pupae, which is in accordance with previous results using other BPUs[17-19]. Lastly, comparing the potencies of two other compounds, DFB and TFM, DFB is less potent an inhibitor of chitin synthesis than either FCX or TFM.

1 Cohen, E., Ann. Rev. Ent. *322* (1987) 71.
2 Ishaaya, 1., in: Pesiticides and Alternatives, p. 365. Ed. J. E. Casida. Elsevier, Amsterdam 1990.
3 Wright, J. E., and Retnakaran, A., Chitin and benzoylphenyl ureas. Dr. W. Junk Publishers, Dordrecht 1987.
4 Soltani, N., Delbecque, J. P., and Delachambre, J., Pestic. Sci. *19* (1983) 615.
5 Soltani, N., Besson, M. T., and Delachambre, J., Pestic. Biochem. Physiol. *21* (1984) 256.
6 Soltani, N., Delbecque, J.P., Delachambre, J., and Mauchamp, B., Intern. J. Invert. Reprod. Develop. *7* (1984) 323.
7 Soltani, N., Quennedey, A., Delbecque, J. P., and Delachambre, J., Archs. Insect Biochem. Physiol. *5* (1987) 201.
8 Soltani, N., Delachambre, J., and Delbecque J. P., Gen. comp. Endocr. *76* (1989) 350.
9 Soltani, N., Annals Soc. Entomol. Fr. (N.S.) 26 (1990) 575.
10 Scheltes, P., Hoffman, T. W., and Grosscurt, A.C., Proceedings of the Brighton Crop Protection Conference, Pests and Diseases (1988) 559.
11 Grosscurt, A. C., Haar, M. T., Longsma, B., and Stocker, A., Pestic. Sci. *22* (1988) 51.
12 Abbott, W. B., J. econ. Ent. *18* (1925) 265.
13 Finney, D. J., Probit analysis. Cambridge University Press, London 1971.
14 Martoja, R., and Martoja, M., Initiation aux techniques de l'histologie animale. Ed. Masson, Paris 1967.
15 Sridevi, R., Bajai, P. and Dutta-Gupta, A., Invert. Reprod. Develop. *14* (1988) 177.
16 Nakagawa, Y., Matsutani, M., Kurihara, N., Nishimura, N., and Fujita, T., Pestic. Biochem. Physiol. *42* (1992) 242.
17 Hunter, E., and Vincent, J. F. V., Experientia *30* (1974) 1432.
18 Ker, R. F., J. Insect Physiol. *23* (1977) 39.
19 Saxena, S., and Kumar, V., Indian J. exp. Biol. *19* (1981) 669.