Short Communication

Mode of Action of Buprofezin on the Twenty-eight-spotted Ladybird, *Henosepilachna vigintioctopunctata* Fabricius

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Buprofezin (2-*tert*-butylimino-3-isopropyl-5-phenyl-perhydro-1,3,5-thiadiazin-4-one, Applaud®) is an insect growth regulator effective in controlling *Nilaparvata lugens* Stål and *Trialeurodes vaporariorum* Westwood.[1～3] The compound kills *N. lugens* nymphs at the time of molting by inhibiting the chitin biosynthesis and the subsequent cuticle deposition in integument.[4,5] Although no lethal effect of buprofezin on adult insects has been observed, it strongly suppresses the egg-laying of *N. lugens* female adults.[6] Recently, its similar effect on the twenty-eight-spotted ladybird, *Henosepilachna vigintioctopunctata* Fabricius, was found, and the mode of action of buprofezin against larvae and adults of *H. vigintioctopunctata* will be discussed in this paper.

Fourth instar larvae of *H. vigintioctopunctata* were used 1～2 hr after molting. They were fed on tomato leaves at 25°C under daily 16 hr illumination. Buprofezin (0.15～7.5 μg/insect) in 20 nl of methanol was injected into 20 larvae, and their mortality was determined 7 days after treatment. From the obtained dose-mortality curve, the LD_{50} value was calculated: LD_{50}=2.3 μg/insect (155 μg/g of insect body weight). In order to measure the chitin biosynthesis by *H. vigintioctopunctata* larvae, the precursor, *N*-acetyl-D-[1,6-^{3}H]-glucosamine (33.1 mCi/mmol, New England Nuclear, U.S.A.), of chitin was injected into the larvae (0.1 μCi/20 nl/insect) 2 days after a treatment with 0 or 4 μg/insect of buprofezin. The radioactivity in the [^{3}H]chitin synthesized by the larvae for 24 hr was determined according to the methods reported previously.[5,7] In the larvae treated with buprofezin, the chitin biosynthesis was inhibited by 74.4% (Table I). When tomato leaves immersed in 50 ppm of buprofezin for 30 sec were fed to *H. vigintioctopunctata* larvae, 55% of them died after 3～7 days. On the 2nd day of treatment, the biosynthesis of [^{3}H]chitin from *N*-acetyl-D-[1,6-^{3}H]glucosamine (0.3 μCi/60 nl/insect) was inhibited by 42.1% in the buprofezin-fed larvae (Table I). Thus, the lethal effect of buprofezin on *H. vigintioctopunctata* larvae can be attributed to the inhibition of chitin biosynthesis, as already reported for *N. lugens* nymphs.[4,5] Indeed, the poisoning, symptoms of *H. vigintioctopunctata* larvae intoxicated with buprofezin were quite similar to those reported for other chitin biosynthesis inhibitors.[8,9] The dying larvae appeared to be blackened and finally died before pupation.

Female adults of *H. vigintioctopunctata* usually begin to deposit their eggs 3 days after

TABLE I. LETHAL ACTIVITY OF BUPROFEZIN AND ITS EFFECT ON CHITIN BIOSYNTHESIS FROM *N*-ACETYL-D-GLUCOSAMINE IN *H. vigintioctopunctata* LARVAE

Buprofezin treatment	Mortality (%)	[^{3}H]Chitin biosynthesis from *N*-acetyl-D-[^{3}H]-glucosamine dpm/insect (% of the control)
Injection (μg/insect)		
0	0	5,047±1,364[a] (100)
0.15	0	—[b]
0.5	10	—
1.5	40	—
4	—	1,290±578* (25.6)
7.5	70	—
Feeding (ppm)		
0	0	18,390±4,430 (100)
10	30	—
50	55	10,648±2,148 (57.9)

[a] Mean±S.E.
[b] Not determined.
* $p<0.05$ compared with the untreated control.

TABLE II. EFFECT OF BUPROFEZIN, ASPIRIN AND PROSTAGLANDIN E_2 ON THE OVIPOSITION OF *H. vigintioctopunctata* ADULTS

Treatment	Mortality (%)	Number of laid eggs/female (mean ± S.E.)
Untreated	0	190.8 ± 23.8[a]
Buprofezin 1,000 ppm feeding	0	112.8 ± 13.6*
Aspirin 1,000 ppm feeding	0	124.0 ± 15.7
Untreated	0	86.6 ± 3.8[b]
Untreated + prostaglandin E_2 (1 ng)/μ	0	90.5 ± 10.5
Buprofezin 1,000 ppm feeding	0	63.5 ± 5.7*
Buprofezin 1,000 ppm feeding + prostaglandin E_2 (1 ng)[c]	0	89.5 ± 13.4

* $p < 0.05$ compared with the untreated control.
[a] Total number of eggs laid for 7 days.
[b] Total number of eggs laid during 4 ~ 7th days of buprofezin feeding.
[c] Prostaglandin E_2 (1 ng) was injected into the abdomen of *H. vigintioctopunctata* female adults on the 5th day of buprofezin feeding.

emergence. Therefore, 3-day-old adults were used throughout the experiment. As mentioned above, no lethal effect of buprofezin was observed against the adults, even when 20 pairs of them were reared on tomato leaves immersed in 1,000 ppm of buprofezin suspension for 30 sec. However, their oviposition was suppressed by buprofezin treatment (Table II). The untreated female adults deposited 190.8 ± 23.8 (mean + S.E.) eggs/female for the following 7 days, but those treated with buprofezin laid only 112.8 ± 13.6 eggs/female (59.1% of control, Table II). Surprisingly, an inhibitor of prostaglandin biosynthesis, aspirin, was able to suppress the oviposition of *H. vigintioctopunctata* in a similar manner (Table II). This strongly suggests that the suppression of oviposition in *H. vigintioctopunctata* can be attributed to the inhibition of prostaglandin biosynthesis. Indeed, prostaglandin E_2 has been shown to play a role in stimulating the egg-laying of insects such as *Acheta domesticus* and *Teleogryllus commodus*.[10,11] In order to clarify this, 1 ng of prostaglandin E_2 was injected into the abdomen of *H. vigintioctopunctata* female adults, which had been fed along with the same number of male adults for 5 days on the tomato leaves immersed in 0 or 1,000 ppm of buprofezin for 30 sec. The injection of prostaglandin E_2 did not affect the egg-laying of the untreated female adults but accelerated that of the buprofezin-treated female adults (Table II). Consequently, the suppression of egg-laying by buprofezin was well cancelled out by injecting prostaglandin E_2.

From these results, buprofezin seems to have killed *H. vigintioctopunctata* larvae by inhibiting the chitin biosynthesis from *N*-acetyl-D-glucosamine, and to have suppressed the oviposition of the female adults by affecting the biosynthesis of prostaglandin E_2. No insect growth regulator has been previously known to interfere with prostaglandin biosynthesis in insects.

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