Short Communication

Inhibition of Cuticle Deposition and Chitin Biosynthesis by a New Insect Growth Regulator, Buprofezin, in *Nilaparvata lugens* Stål

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Buprofezin (Applaud®, 2-*tert*-butylimino-3-isopropyl-5-phenyl-3,4,5,6-tetrahydro-2*H*-1,3,5-thiadiazin-4-one, Fig. 1) is a new insect growth regulator that effectively controls important insect pests, including the brown rice planthopper, *Nilaparvata lugens* Stål, and the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood.[1~4] *N. lugens* nymphs treated with buprofezin show abnormalities in their ability to shed the exuviae and finally die at the time of molting.[3] Dying nymphs appear to be blackened and dried. Similar poisoning symptoms have been observed on *Pieris brassicae* treated with diflubenzuron or Du 19111.[5,6] Since these compounds have been shown to interfere with the chitin synthesis and the resulting deposition of integumentary cuticle of insects,[6,7] the effect of buprofezin on chitin biosynthesis can be studied by using target insects. In the current paper, the inhibition by buprofezin of cuticle deposition and chitin synthesis in *N. lugens* nymphs is discussed.

N. lugens nymphs were reared on rice plants at 25°C under a daily 16 hr illumination. Female nymphs at the 5th instar were used within 1 hr after the last molt. They were treated with 50 ppm of buprofezin according to the method reported previously.[3] The cuticle deposition in the nymphs was determined by microscopic observation of their stained sections, which were obtained by fixation in Bouin's solution, dehydration with ethanol series, sectioning in parafin block and subsequent staining with Mallory's triple. Under the same conditions, the mortality of the nymphs was also determined. All the untreated nymphs emerged to female adults between 84 and 96 hr after the last nymphal molt (0% mortality). However, the nymphs treated with 50 ppm of buprofezin began to die after 84 hr and none of them emerged (100% mortality).

No histological difference was observed between the untreated and buprofezin-treated nymphs until 72 hr (Fig. 2), when the new cuticle had not then formed well. In the untreated nymphs, the new cuticle was deposited and well stained in red with the Orange G component of Mallory's triple after 84 hr, but not in the treated nymphs (Fig. 2). Even at that time, no abnormality was seen in the appearance and behavior of the treated nymphs. The failure of new cuticle deposition occurred just before the death caused by buprofezin.

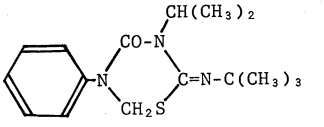

FIG. 1. Structure of Buprofezin.

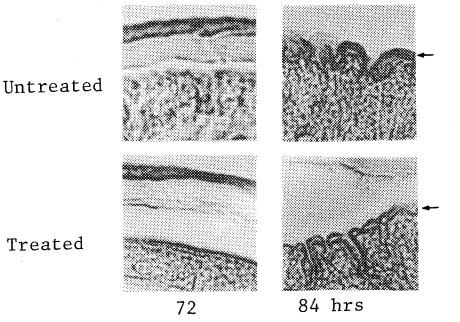

FIG. 2. Micrographs of the Integuments Obtained from Female *Nilaparvata lugens* Nymphs Untreated or Treated with 50 ppm of Buprofezin.

Time and arrows show hours after the last molt and the new cuticle layer, respectively.

TABLE I. INCORPORATION OF RADIOACTIVITY OF [^{14}C(U)]GLUCOSE IN TISSUE FRACTIONS OF UNTREATED AND BUPROFEZIN-TREATED *Nilaparvata lugens* NYMPHS

Fractions	Radioactivity (% of total ^{14}C incorporated)[a]	
	Untreated nymph	Buprofezin-treated nymph[b] (% of the control)
Total	100	100
Haemolymph plus acid soluble	29.8	31.9 (107)
Lipids (95% EtOH extract)	39.5	38.3 (97)
Nucleic acids	4.0	3.3 (83)
NaOH hydrolysate and washings	22.8	24.5 (107)
Chitin	3.8	2.0 (53)

[a] Mean of duplicated experiments. The estimated error is less than 10%.
[b] 10 ppm of buprofezin.

Since chitin is one of the major components of insect cuticle, the chitin biosynthesis in the buprofezin-treated nymphs was also studied by using a radiolabelled precursor. Both buprofezin and the precursor were sucked by the nymphs through Parafilm.® After 2 days, the radioactivity in the nymph bodies was analyzed according to the method of Post and Vincent.[8] The biosynthesis of [^{14}C]chitin from [^{14}C(U)]glucose was inhibited by 47% in the nymphs treated with 10 ppm of buprofezin, more than 70% of which eventually failed to emerge (Table I). The radioactivity in other fractions was less affected by buprofezin.

From these results, buprofezin seems to regulate insect growth by inhibiting the integumentary cuticle deposition or chitin biosynthesis. Other compounds such as polyoxin D and diflubenzuron have also been reported to inhibit both the deposition of insect cuticle and chitin biosynthesis.[9] Structurally unrelated buprofezin has been developed in Japan as an insecticide with a unique spectrum and is useful for controlling important insect pests such as *N. lugens*.

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