Short Communication

Antagonistic Effect of 20-Hydroxyecdysone to an Insect Growth Regulator, Buprofezin, in *Nilaparvata lugens* Stål

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An insect growth regulator, buprofezin (2-*tert*-butylimino-3-isopropyl-5-phenylperhydro-1,3,5-thiadiazin-4-one, Applaud®, Fig. 1) has recently been shown to kill brown rice planthopper (*Nilaparvata lugens* Stål) nymphs by inhibiting chitin biosynthesis from *N*-acetyl-D-glucosamine and the following cuticle deposition.[1,2] No lethal effect of buprofezin has been reported in the adults, but their egg-laying is remarkably reduced by feeding them with buprofezin-treated rice plants.[3] More recently, the prostaglandin biosynthesis in buprofezin-fed *N. lugens* adults has been shown to be strongly inhibited, resulting in the suppression of egg-laying,[4] as well as in *Henosepilachna vigintioctopunctata* Fabricius.[5] Due to a low probability that this compound can exhibit two intrinsic activities, there may be a relationship between the two types of effects of buprofezin on *N. lugens*. The present study was designed to clarify this hypothesis and finally revealed that 20-hydroxyecdysone could counteract both the nymphicidal and oviposition-inhibitory effects of buprofezin in *N. lugens*. Consequently, buprofezin seems to act on the ecdysone metabolism in *N. lugens* to result in death during molting and the suppression of egg-laying.

N. lugens nymphs of the 5th instar were used within 1 hr after the last nymphal molt. Forty nymphs were fed on rice plants at 25°C under 60～70% relative humidity and 16 hr daily illumination. Buprofezin treatment was performed according to the methods previously described.[1,2] The mortality of the nymphs treated with 0, 5 and 50 ppm of buprofezin was 8～17, 43～47 and 70%, respectively, 6 days after the treatment with 24 hr feeding (Table I). However, 20-hydroxyecdysone (200 ng/insect) applied topically to the nymphs after 2 days

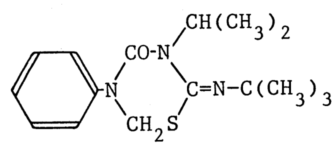

FIG. 1. Structure of Buprofezin (Applaud®).

TABLE I. EFFECT OF BUPROFEZIN AND 20-HYDROXYECDYSONE ON *Nilaparvata lugens* STÅL NYMPHS

Treatment	Mortality (%) (Mean ± S.E.)
Untreated	16.7 ± 6.7
Untreated +20-hydroxyecdysone (after 1 day)[a]	20.0 ± 0.0
Buprofezin 5 ppm[b]	46.7 ± 8.8
Buprofezin 5 ppm +20-hydroxyecdysone (after 1 day)[a]	33.4 ± 3.3
Untreated	13.4 ± 6.7
Untreated +20-hydroxyecdysone (after 2 days)[a]	16.7 ± 6.7
Buprofezin 5 ppm[b]	43.3 ± 12.0
Buprofezin 5 ppm +20-hydroxyecdysone (after 2 days)[a]	20.0 ± 5.8
Untreated	7.5 ± 4.8[††]
Untreated +20-hydroxyecdysone (after 2 days)[a]	22.5 ± 2.5[††]
Buprofezin 50 ppm[b]	70.0 ± 0.0**
Buprofezin 50 ppm +20-hydroxyecdysone (after 2 days)[a]	37.5 ± 2.5*,[††]

[a] 20-Hydroxyecdysone (200 ng/20 nl of methanol/insect) was applied to the nymphs 1 or 2 days after 24 hr feeding with untreated and buprofezin-treated rice plants.

[b] *N. lugens* nymphs were fed for 24 hr with rice plants previously sprayed with 5 or 50 ppm of buprofezin and then subsequently fed with untreated plants.

Statistical evaluation was carried out by using Student's *t* test.

* $p<0.01$, ** $p<0.001$ compared with the untreated group; † $p<0.01$, †† $p<0.001$ compared with the buprofezin-treated group.

(or 1 day but less effective) surprisingly reduced the mortality of the buprofezin-fed nymphs (Table I). Most of the nymphs treated with 5 ppm of buprofezin and half of those treated with 50 ppm of buprofezin were saved by the topically applied 20-hydroxyecdysone (200 ng/insect). This suggests that 20-hydroxyecdysone can be antagonistic to buprofezin-induced mortality in *N. lugens* nymphs. As shown in our previous papers,[1,2] the nymphicidal (or larvicidal) action of buprofezin has been attributed to the inhibition of chitin biosynthesis and the subsequent cuticle deposition in *N. lugens*, as well as in the less susceptible *H. vigintioctopunctata*, in which 20-hydroxyecdysone was again antagonistic to buprofezin-induced mortality of the larvae. Interestingly, processes such as chitin biosynthesis and cuticle deposition are well proven to be regulated by 20-hydroxyecdysone.[6]

N. lugens adults were used within 24 hr after their emergence. Forty pairs were fed on rice plants previously sprayed with 0 ~ 50 ppm of buprofezin suspension throughout the experiment for 6 days.[4] The buprofezin feeding caused no remarkable change in the mortality of *N. lugens* adults (Table II). However, their egg-laying was markedly reduced by buprofezin (Table II), as was also shown in previous papers.[3,4] When 20-hydroxyecdysone (0.1 ng/insect) was injected into the untreated and the buprofezin-treated *N. lugens* adults on the 5th day of buprofezin feeding, it again cancelled out the effect of buprofezin. As shown in Table II, the number of eggs laid during the following 24 hr was not affected by 20-hydroxyecdysone at all in the untreated adults (25.9 ± 3.6 and 23.4 ± 3.8 eggs/female with and without 20-hydroxyecdysone, respectively), but that of the buprofezin-fed adults was only 5.2 ± 2.0 eggs/female, which was significantly increased by an injection of 20-hydroxyecdysone to result in the normal rate of egg-laying (19.0 ± 3.5 eggs/female). Thus, the ecdysis hormone, 20-hydroxyecdysone, well antagonized buprofezin suppression of the egg-laying of *N. lugens* adults as well as the mortality effect on the nymphs.

TABLE II. EFFECT OF BUPROFEZIN AND 20-HYDROXYECDYSONE ON THE OVIPOSITION OF *N. lugens* FEMALE ADULTS

Treatment	Mortality (%)	Number of eggs laid by a female (Mean ± S.E.)
Untreated	20	25.9 ± 3.6††
Untreated + 20-hydroxyecdysone[a]	0	23.4 ± 3.8†
Buprofezin 50 ppm[b]	14	5.2 ± 2.0**
Buprofezin 50 ppm + 20-hydroxyecdysone	0	19.0 ± 3.5†

[a] 20-Hydroxyecdysone (0.1 ng/20 nl of methanol/insect) was injected into the thorax of *N. lugens* female adults on the 5th day of buprofezin treatment.

[b] Newly emerged adults of *N. lugens* were fed with rice plants previously sprayed with 50 ppm of buprofezin throughout the experiment.

Statistical evaluation was carried out by using Student's *t* test.

** $p<0.001$ compared with the untreated group; † $p<0.01$, †† $p<0.001$ compared with the buprofezin 50 ppm group.

The amount of 20-hydroxyecdysone in various insects has been determined to indicate that its distribution in adult insects is limited to their ovaries and mature (or laid) eggs.[7] The site of 20-hydroxyecdysone synthesis is likely to be the ovaries, since an ovariectomy abolishes the peaks of 20-hydroxyecdysone in *Bombyx mori* and *Aedes aegypti* adults.[8,9] In *Locusta migratoria* and *Diploptera punctata* adults, its titers have recently been observed only toward the end of egg development in the ovary just before oviposition.[10,11] However, the role of 20-hydroxyecdysone in adult insects is not yet known. The present results may indicate that 20-hydroxyecdysone plays a role in ovulation or stimulates the egg-laying of *N. lugens* (Table II), as has been reported for prostaglandin E_2.[4,5]

As already mentioned, buprofezin exhibited two types of effects on *N. lugens* and other insects.[1~5,12] Both these effects of buprofezin were well cancelled by 20-hydroxyecdysone, where the applied 20-hydroxyecdysone seemed to act as its titer in *N. lugens*. If so, the primary action of buprofezin is considered to relate to the disturbance of ecdysone metabolism in *N. lugen*. The inhibition of chitin and prostaglandin biosyntheses by buprofezin should be studied by means of its antagonism to 20-hydroxyecdysone. Some benzoylphenyl urea insecticides, which are categorized as chitin biosynthesis inhibitors,[13)] have also suppressed the egg-laying of insects.[14,15)] Their effects on insects[13~17)] are worth investigating in relation to anatgonism to 20-hydroxyecdysone.

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