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Studies on the Mode of Action of Buprofezin II. Effects on Reproduction of the Brown Planthopper, *Nilaparvata lugens* STÅL (Homoptera: Delphacidae)

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The effects of buprofezin on the life span and reproduction of *N. lugens* were examined by allowing adults of different ages to feed on rice plants treated with the chemical. Irrespective of adult age at the time of inoculation on the treated plants, the number of nymphs of the next generation was decreased to 0–6% of the control by 250 ppm buprofezin and to 0–2% by 1,000 ppm. The treatment to adults within 24 hr of emergence considerably inhibited oviposition without interrupting ovarian development and shortened the life span of female adults. Although the treatment to older adults did not affect oviposition, it inhibited the embryo development of deposited eggs, which reached the eye pigmentation stage but failed to hatch.

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* STÅL is one of the important insect pests of rice plants in Southeast and East Asia because the pest not only causes direct damage by sucking the sap but also transmits several viral diseases (DYCK and THOMA, 1979; BRADER, 1982). As the insect's resistance to organophosphorous and carbamate insecticides has developed making control more difficult (NAGATA et al., 1979; LIN et al., 1979; KILIN et al., 1981; NAGATA, 1982; HOSODA, 1983), much effort has been devoted to the development of new insecticides.

Buprofezin ("Applaud®"; 2-*tert*-butylimino-3-isopropyl-5-phenyl 3, 4, 5, 6-tetrahydro-2H-1, 3, 5-thiadiazine-4-one) is a new insecticide which has shown a high level of activity in field trials in the control of *N. lugens* in Japan and Malaysia in spite of its weak killing activity on adults (KANNO et al., 1981; KAJIHARA et al., 1982). As a possible application of buprofezin to control *N. lugens*, efficient nymphcidal activity has been found even at a concentration of less than 1 ppm (ASAI et al., 1983).

Besides being nymphcidal, buprofezin is believed to have an effect on adults, because the number of progeny was drastically reduced when adults were inoculated on rice plants sprayed with the insecticide (KANNO et al., 1981). It also showed a weak ovicidal activity (ASAI et al., 1983). In this paper, to better understand the mechanisms

involved in the progeny reduction by buprofezin treatment, we examined the material's effect on reproduction of *N. lugens* adults of different ages.

MATERIALS AND METHODS

Insect. The brown planthopper, *Nilaparvata lugens* was reared on rice seedlings (Kinmaze cultivar) in an air-conditioned cabinet (25±1°C; 60–70% R. H.; 16–8 hr light-dark) in the Biological Research Center of Nihon Nohyaku Co., Ltd. The insects were resistant to several insecticides such as diazinon, fenitrothion, BPMC and MTMC (Uchida and Fukada, 1983). Adults at different ages were used for experiments.

Chemical. Buprofezin was synthesized and formulated at the Chemical Research Center of our company, and was prepared as a 25% wettable powder.

Effects on adult life span and progeny number. Rice plants of 6–7 leaf stage grown in a 15 cm diameter pot were sprayed with aqueous suspensions (150 m*l*) of buprofezin of 250 and 1,000 ppm. Three pots, each with five plants, were used for each test plot, and each pot was surrounded by a transparent plastic cylinder (55 cm in height × 16 cm in diameter). Ten pairs of *N. lugens* adults were inoculated on rice plants immediately after the surface had dried. The pots were then placed in a greenhouse and the numbers of surviving adults and hatched nymphs were counted at various intervals up to the 35th and 50th day after inoculation. The hatched nymphs were removed from the pot after counting.

Effect on oviposition. Twenty-seven days after the inoculation of adults to rice plants sprayed with an aqueous suspension of buprofezin, the plants were cut. At this time all females had died, and the number of eggs laid was counted. The developmental stages of the eggs were preliminarily checked under a binocular microscope.

Effect on embryogenesis. Five pairs of 120–144 hr old adults of *N. lugens* were fed for two days on rice plants treated with buprofezin at 1,000 ppm. Then the adults were transferred to other rice plants treated with buprofezin at the same concentration and removed from the pot after one day, during which time eggs were laid. The plants were cut 5, 7, 9, 11 and 13 days after the transfer, eggs laid were dissected under a binocular microscope and occurrences of eye pigmentation in them were examined.

Effect on ovarian development. The ovaries of 24-hr old female adults inoculated on rice plants sprayed as described above were dissected in a saline solution 1, 4 and 7 days after inoculation, and the length of the ovariole was measured under a binocular microscope.

RESULTS

Effect on life span

Table 1 shows the life span of female and male adults of *N. lugens* which were inoculated at different ages on rice plants treated with buprofezin. Mean life span of untreated female adults ranged from 19 to 25 days. When female adults within 48 hr of emergence were inoculated on the treated plants their life span was reduced to less than three-fourths those in untreated control, but no shortening of life span was observed when female adults older than 72-hr were treated. The life span of male adults was little affected by buprofezin.

Table 1. Life span of adult *N. lugens* inoculated on rice plants treated with buprofezin

Adult age[a] (hr)	Buprofezin conc. (ppm)	Life span (day)[b]	
		Male	Female
0– 24	1,000	11.9±4.7	12.0±4.0
	250	12.8±5.6	11.8±4.5
	0	14.9±4.9	20.3±7.3
24– 48	1,000	13.7±4.0	15.6±6.8
	250	11.9±4.1	14.1±4.9
	0	12.8±5.4	20.8±6.6
72– 96	1,000	13.3±4.6	20.3±7.7
	250	12.3±3.8	21.5±7.7
	0	11.9±3.4	19.1±4.7
120–144	1,000	12.5±3.8	21.3±6.2
	250	12.7±4.1	21.7±6.9
	0	13.7±4.3	19.1±7.5
168–192	1,000	14.4±3.7	22.8±6.7
	250	16.1±3.8	23.0±8.0
	0	15.6±4.1	25.4±8.9

[a] Age of adult at time of initial inoculation on buprofezin-treated rice plants.
[b] Mean value±standard error.

Table 2. Number of nymphs produced by adult *N. lugens* which had been treated with buprofezin

Adult age[a] (hr)	Buprofezin conc. (ppm)	Number of nymphs from[b] one pair of adults
0– 24	1,000	0 (0)
	250	0 (0)
	0	245.0 (100)
24– 48	1,000	0 (0)
	250	1.4 (0.6)
	0	231.0 (100)
72– 96	1,000	0.8 (0.4)
	250	8.8 (4.6)
	0	193.0 (100)
120–144	1,000	2.7 (1.6)
	250	10.1 (5.9)
	0	171.5 (100)
168–196	1,000	2.0 (1.1)
	250	9.6 (4.9)
	0	188.9 (100)

[a] Age at time of initial inoculation on buprofezin-treated rice plants.
[b] Values in parentheses are percentages of untreated controls.

Effect on progeny number

Table 2 shows the number of nymphs produced by one female during a period of 50 days when *N. lugens* adults were inoculated on treated rice plants. Notwithstanding

Table 3. Effects of buprofezin on oviposition and embryo development of *N. lugens*

Adult age[a] (hr)	Buprofezin conc. (ppm)	No. of eggs laid by one female	Percentage[b]		
			Egg b.e.[c]	Egg a.e.[d]	Hatched eggs
0– 24	1,000	0.2	0	100	0
	250	0.4	0	100	0
	0	171.6	21.4	61.2	17.4
24– 48	1,000	90.4	23.8	76.2	0
	250	121.3	12.5	86.7	0.8
	0	103.8	2.4	10.4	87.2
72– 96	1,000	197.1	44.4	55.3	0.3
	250	175.0	38.7	60.3	1.0
	0	243.5	13.8	10.6	75.6
120–144	1,000	242.0	50.8	47.5	1.7
	250	194.3	16.6	79.1	4.3
	0	201.4	6.0	28.4	65.6
168–192	1,000	204.9	70.6	28.6	0.8
	250	202.7	53.2	44.4	1.4
	0	283.0	42.0	30.3	27.7

[a] Age of adults at time of initial inoculation on buprofezin-treated rice plants.
[d] Rice plants were cut 27 days after inoculation when all females had died, and unhatched and hatched eggs were counted; their proportions are shown as %.
[c] Unhatched eggs before eye pigmentation stage.
[b] Unhatched eggs after eye pigmentation stage.

the different ages of adults at treatment, the number of hatched nymphs was decreased to 0–6% of the control by 250 ppm buprofezin and to 0–2% by 1,000 ppm. The younger adults seemed more susceptible to buprofezin treatment.

Effect on oviposition and embryogenesis

Table 3 shows the total number of eggs laid by one pair of adults during 27 days of inoculation on the buprofezin-treated plants. When adults within 24 hr of emergence were inoculated on plants treated with 250 and 1,000 ppm buprofezin, the number of eggs greatly decreased to 0.2 and 0.4% of untreated control, respectively. Treatments of older adults did not cause such a reduction. Preliminary observation showed that a considerable proportion of the eggs had developed beyond the eye pigmentation stage even with treatment of 1,000 ppm of buprofezin (Table 3). The retardation of egg development by buprofezin was more clearly elucidated in the experiment shown in Fig. 1. The majority of eggs laid by the adults inoculated on rice plants treated with 1,000 ppm of buprofezin after they were 120–144 hr old developed beyond the eye pigmentation stage but hatchability was very low (Fig. 1).

Effect on ovarian development

Table 4 shows the changes in ovariole length (mm) when 24-hr old female adults were exposed to the plants treated with buprofezin at 250 and 1,000 ppm for 1, 4 and 7 days. The lengths of the ovariole for the three exposure periods were almost the same as those in untreated control.

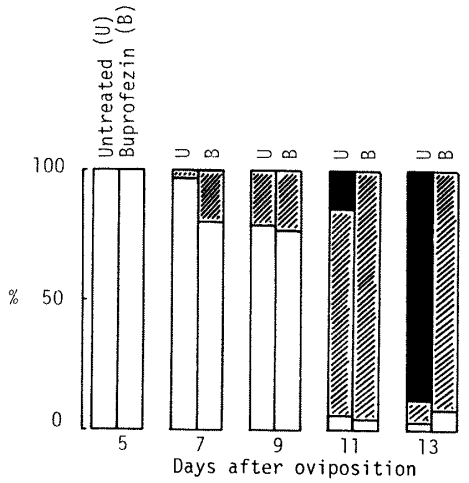

Fig. 1. Comparison of embryogenesis of eggs laid by adult *N. lugens* inoculated on rice plants treated with 1,000 ppm of buprofezin when they were 120–144 hr old, and that of untreated control.

: eggs before eye pigmentation stage.
: eggs after eye pigmentation stage.
: hatched eggs.

Table 4. Effect of buprofezin on ovariole length of *N. lugens*

Buprofezin conc.[a] (ppm)	Ovariole length (mm)[b]		
	Days after inoculation		
	1	4	7
1,000	1.338±0.111	2.313±0.160	2.738±0.325
250	1.583±0.382	1.975±0.126	2.238±0.125
0	1.263±0.025	2.088±0.085	2.388±0.085

[a] 24-hr-old adults were exposed to rice plants treated with buprofezin.
[b] Mean value±standard error.

DISCUSSION

The effect of buprofezin was examined on the life span of *N. lugens* adults of different ages which fed on rice plants treated with the chemical. The life span of inoculated adults older than 48 hr after emergence was not affected (Table 1), as shown also by KANNO (1981). But when younger adults were treated, the life span of females, but not of males was shortened. Thus, buprofezin seems to affect the sexual maturity of young females.

The number of second generation progenies from adults treated with buprofezin was greatly reduced, irrespective of adult age at the time of inoculation, but the cause of reduction differed with adult age. Treatment of 24-hr-old adults (Table 3) caused inhibition of oviposition. Treatment to older adults did not block oviposition, but reduced the hatchability of deposited eggs (Tables 2, 3 and Fig. 1). The reduction of

progeny was also documented in the action of precocene to milkweed bug (Pratt et al., 1980) and that of dimilin to young female adults of yellow-spotted langicorn beetle (Iba, 1981). These compounds suppress maturity by blocking the necessary endocrine secretion or inhibiting chorion formation of the ovary. Buprofezin did not affect the ovarian development even of young adults of *N. lugens* (Table 4), indicating that the mode of action differs from these other compounds. As *N. lugens* can oviposit non-fertilized eggs without copulation (Sugimoto and Yamazaki, 1980), the inhibition of oviposition by buprofezin cannot be due to inhibition of copulation. Buprofezin may act to affect the formation of ovipositing organs or to confuse the ovipositing mechanism.

The proportion of reduction in hatching from eggs laid by adults treated with buprofezin (Tables 2 and 3) was greater than that of eggs treated directly with this chemical (Asai et al., 1983). This may imply that buprofezin mainly affects egg development through the adult body. Embryo development in the eggs laid by buprofezin-treated adults was slightly affected up to the eye pigmentation stage, but the hatchability was greatly reduced (Fig. 1 and Table 2). This suggests that a certain disorder may occur in the embryo development during the period from the eye pigmentation stage to hatching, including cuticle formation just before hatching. Similar actions have been reported for dimilin on stable fly, milkweed bug and housefly (Wright and Spates, 1976; Chang, 1979; Chang and Borkovec, 1980; Redferm et al., 1980).

Buprofezin is similar to dimilin in its action, which interferes with nymphal development (Asai et al., 1983). Dimilin is believed to disturb endocuticle deposition and thereby to inhibit normal cuticle formation (Post and Vincent, 1973; Hajjar and Casida, 1978). Although at present there is no evidence of whether buprofezin acts in a same way as dimilin, many similarities were found in the two compounds, both of them showing weak direct-killing action on adults and eggs (Asai et al., 1983). Effects of buprofezin on cuticle formation will be studied using histological and biochemical methods.

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