Effect of Alsystin and Diflubenzuron on the Rice Water Weevil (Coleoptera: Curculionidae)

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ABSTRACT Two benzoylphenyl ureas (BPU), alsystin and diflubenzuron, were each tested at a dosage of 0.28 kg (AI)/ha in the greenhouse and laboratory on wild-caught adults of *Lissorhoptrus oryzophilus* Kuschel to determine the effects of the chemicals on oviposition and immature progeny. Studies showed significant reductions in the number of immatures that developed when either BPU was applied either to rice foliage or to water (containing submerged rice stems with eggs) immediately after oviposition. Applications of either BPU to water at intervals of 5, 9, and 14 days after oviposition showed no significant reductions in numbers of immatures. There were no significant differences in numbers of eggs deposited following contact of adults with treated foliage, regardless of whether immediate or residual effects (up to 6 days) were examined. Either BPU applied to rice foliage to contact adults or to water immediately after oviposition resulted in significantly fewer first instars. First instars treated directly in a suspension of either BPU showed no mortality.

BENZOYLPHENYL UREAS (BPU), a relatively new class of insecticide that appear to inhibit the synthesis of chitin (Mulder and Gijswijt 1973, Post et al. 1974), have been shown to have ovicidal or larvicidal effects. They have been successfully used in experiments to control many economically important insect species such as the ash weevil (*Myllocerus undecimpustulatus maculosus* [Desb.]) (Thangavelu 1982), boll weevil (*Anthonomus grandis grandis* Boheman) (McLaughlin 1978), codling moth (*Cydia pomonella* [L.]) (Elliott and Anderson 1982), gypsy moth (*Lymantria dispar* [L.]) (Granett and Dunbar 1975), soybean looper (*Pseudoplusia includens* [Walker]) (Reed and Bass 1980), and several stored-product pests (McGregor and Kramer 1976).

The rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is a major pest of rice in many of the rice-growing areas of the United States, including California (Lange and Grigarick 1970), and is also a pest of rice in Japan (Koboyashi et al. 1980). The adults feed on rice leaves; females either crawl down or swim to the leaf sheath below the water line, where oviposition takes place. Most eggs are inserted into the leaf-sheath tissue above the crown, although eggs are occasionally found in the roots. After eclosion, first instars mine the leaf sheath for approximately 1 day, emerge, and settle through the water to the soil surface (Bowling 1972). Larvae then enter the soil and feed on and in the roots until pupation occurs (Grigarick and Beards 1965). Larval feeding damage to the roots is of major economic importance since rice yields can be significantly reduced.

BPU's appear to be potentially useful for control of rice water weevil larvae because of their highly selective activity on insects, low toxicity to vertebrates including man and certain nontarget organisms (MacGregor et al. 1979), rapid dissipation with low residual levels in the aquatic environment (Booth and Ferrell 1976, Ivie et al. 1980), and their relative ease of handling and application.

We conducted our study to analyze the susceptibility of different life stages of the rice water weevil to alsystin and diflubenzuron. Four experiments were conducted during the late spring and summer of 1982 and 1983.

Materials and Methods

Experiments 1 and 2. Greenhouse tests were conducted in 1982 (Experiment 1) and 1983 (Experiment 2). Both BPU's were applied to plant foliage or water in order to provide information on the life stages of the rice water weevil affected. The treatment regimes were: application to the rice foliage (Treatments 1–4, Experiment 1, and Treatments 1–3, Experiment 2); application to water immediately after oviposition (Treatments 5–8, Experiment 1, and Treatments 4–6, Experiment 2); application to water 5 days after oviposition (Treatments 7–9, Experiment 2), 9 days after oviposition (Treatments 9–12, Experiment 1), and 14 days after oviposition (Treatments 10–12, Experiment 2); and direct application to first instars (Treatments 13–15, Experiment 2). Evaluation of the affected stage was determined by the number of larvae surviving on rice roots.

Possible effects on plant growth by either BPU or from weevil larvae or both were analyzed by measuring the plant wet weight, height, root length, number of tillers, dry root weight, and root volume.

Rice variety 'M-9' was grown in 950-ml plastic pots. Sandy loam soil was sifted through a 3.2-mm mesh screen and steam sterilized. Each pot was filled with 710 ml of this soil, and $(NH_4)SO_4$ was added (561 kg/ha) based on the soil surface area of the pot. The prepared pots were arranged in a galvanized metal tank in five randomized complete blocks. The tank was flooded with tap water to a level about 5 cm above the top of each pot. Eight rice seeds (presoaked for 24 h) were sown in each pot. Thirteen days after seeding, plants in each pot were thinned to two rice plants. Copper sulfate was applied in doses of 8.4 kg/ha to the tank water for algae control.

Foliage treatments were made before introduction of adult weevils. Each pot was covered with a plastic petri dish containing a 2.5-cm hole in the center, through which the two rice plants protruded. Cotton was inserted into the hole around the plants to ensure that no spray reached the water below the cover. Five pots of plants, each representing a replication for each treatment, were evenly arranged on a flat surface. The BPU suspension, either alsystin (25% WP, Mobay, Kansas City, Mo.) or diflubenzuron (25% WP, Thompson-Hayward, Kansas City, Kans.) was applied to potted plants in a dose of 0.28 kg (AI)/ha and 112 liters of H_2O/ha. Plyac® (Fisher, Fair Lawn, N.J.) was added to the spray suspension in a dose of 0.33 ml/liter of spray, and was also sprayed on the accompanying controls. The treatments were applied with a hand-held atomizer using pressurized CO_2. The plants were dried for 2 h following application and the petri dish covers and cotton were removed. Each plant was staked to prevent the foliage from contacting the water.

Following foliage treatment, the metal tank was drained so that the water level was 1.3 cm below the top of each pot. Each pot was individually flooded and maintained this way throughout the remainder of the experiments. This handling prevented any movement of chemical from one container to the next.

Polyacetate cylinders 45.7 cm high with a diameter slightly smaller than that of the pot were used to confine the adult weevils. The cylinders were pushed into the soil of each pot and four (Experiment 1) or eight (Experiment 2) female weevils were placed inside each cylinder. All weevils were hand-collected from a rice field near Biggs, Calif. Perforated paper cups were placed over the tops of the cylinders to prevent escape of the weevils. Weevils were allowed to feed on and oviposit in the rice foliage for 48 h (Experiment 1) or 24 h (Experiment 2) and then were removed along with the cylinders. This confinement procedure was used for all treatment regimes except Treatments 13 to 15 of Experiment 2.

Following removal of the adult weevils, spray suspensions were formulated for the water treatments at the same dose of AI as the foliar treatment; however, the volume was changed to 225 liters/ha. The suspension was applied on the appropriate day with a glass pipette.

Treatments 13 to 15 of Experiment 2 were applied to first instars after they emerged from the plant. Untreated plants were prepared in paper cups as will be described for Experiments 3 and 4. Following emergence, larvae were placed in a spray suspension (formulated exactly as those used for container water application) for 2 h. Untreated rice plants were prepared in the greenhouse as described and incorporated in a randomized complete block design interspersed with other treatments of Experiment 2. The "treated" first instars were then transferred to these plants with a pipette and placed under water but on top of the pot soil. Approximately 30 larvae were added to each pot in this manner. Three weeks following oviposition, the roots of the plants and soil from all treatments and controls were washed into a 0.84-mm mesh screen and immature weevils were counted. These plants were measured and weighed.

Experiments 3 and 4. In Experiment 3, the effect of both BPU's on oviposition of the weevil after initial exposure to treated rice foliage was determined in two types of trials. Evaluation was based on the number of eggs found in the submerged plant tissue. In Experiment 4 the effects of both BPU's on the weevil were compared when adults contacted treated foliage and eggs were exposed in early or late periods of development while in stem tissue. Evaluations were made by counting first instars that emerged from the rice plant.

Preliminary preparations for Experiments 3 and 4 were identical. Rice plants were grown in the greenhouse in 1983 in plastic trays ca. 35 cm by 28 cm in size. Soil was prepared as before and each tray was filled with 10 cm of soil. The trays were flooded to a depth of 5 cm and seeded with 50 (rice variety 'M-9') seeds. Thirteen days after seeding, plants in each tray were thinned to 15 plants that were randomly distributed within the tray. Two of these trays were evenly arranged within a 2.32-m^2 (1.52 by 1.52 m) area. Treatments were applied by a CO_2-pressurized hand-held atomizer to the plants in the trays in a dose of 0.28 kg (AI)/ha and 112 liters H_2O/ha. Plyac was added to the spray suspension at 0.33 ml/liter of spray and was included in the control. The entire 2.32-m^2 area, including plants, was sprayed, ensuring even coverage. Plants were dried for 2 h following treatment.

Two plants were removed from the tray and the roots were washed to ensure that the treated area of the plant was not washed accidentally. Paper cups (280-ml capacity) were filled with water 2 cm from the top. Roots were trimmed on both plants up to the seed and then placed in the water and secured so that at least 5 cm of foliage above the seed was under water. Small wooden sticks pushed through the cups 1.5 cm from the top were used as supports for the plants, which were tied to the sticks. Eight wild-caught adult weevils were

added to each cup. Another paper cup was inverted over the plants to confine the weevils. The cups were then transferred to the galvanized tank and arranged in a randomized complete block design with five replications per treatment.

After a 24-h oviposition period on treated plants in Trial 1 of Experiment 3, the weevils were killed. The plants were immediately frozen and later thawed for egg counts. Each plant was immersed in boiling water for 5 min and then placed in 95% EtOH for 24 h. After 24 h, the alcohol was removed and replaced by fresh 95% EtOH. This procedure (Everett and Trahan 1967) removed all pigmentation from the plant and allowed easy observation of the eggs, which remained a highly visible ivory color against a dull white background (bleached plant). Eggs were counted and recorded.

Plants in Trial 2 were prepared for egg counts in the same manner as in Trial 1. After the weevils were allowed to feed and oviposit on the treated plants for 24 h, each set of eight adult weevils per cup was removed and placed in a 130-ml baby food jar containing fresh untreated rice leaves for the weevils to feed on. The jars were placed in a growth chamber set for 14 h light at 30°C and 10 h dark at 26.7°C for 48 h. After 48 h in the jars, the weevils were placed for 24 h on untreated rice plants that were suspended in a paper cup. After the 24-h period, the weevils were removed and again placed in baby food jars with untreated rice leaves. This second set of rice plants was frozen for later egg counts. After 48 h, the weevils were again placed on untreated rice plants suspended in water-filled paper cups and confined for 24 h. After 24 h, the weevils were killed and the plants frozen for later egg counts.

Three treatments were included in Experiment 4. In the first treatment, the rice foliage was sprayed in plastic trays as described previously. In the second treatment, the BPU suspension was introduced into the water in the paper cup containing an untreated rice plant immediately after oviposition. In the third treatment, untreated rice plants were used but the water in the cup was treated 5 days after oviposition.

Eight weevils were confined to two rice plants suspended in water-filled paper cups as previously described. After 24 h, adults were removed; all the plants (cups) were taken to the laboratory and placed in a growth chamber under conditions as described in Experiment 3. Roots were trimmed daily to prevent first instars from migrating from the leaf sheath to the roots. Cups were periodically checked for emerging larvae, and eclosion began 7 days following oviposition. Larval counts were made daily for the next 5 days. Plants were then frozen for later egg counts.

All data were analyzed by two-way analysis of variance (ANOVA). Duncan's (1951) multiple range test was used to separate treatment means at the $P < 0.01$ level.

Table 1. Effect of BPU's on immature rice water weevils in relation to treatment regimes

Treatment regime	Avg no. immatures per two plants
Rice foliage	
1. Diflubenzuron	0.0a
2. Control	14.8c
3. Alsystin	2.4ab
4. Control	14.2c
Water after oviposition	
5. Diflubenzuron	0.0a
6. Control	17.2c
7. Alsystin	0.8a
8. Control	16.2c
Water 9 days after oviposition	
9. Diflubenzuron	14.6c
10. Control	15.6c
11. Alsystin	7.6bc
12. Control	17.2c

Means followed by the same letter are not significantly different ($P < 0.01$; Duncan's [1951] multiple range test).

Results and Discussion

Experiment 1. The number of immature rice water weevils recovered from foliage treatment and water treatment immediately after oviposition was significantly lower than the respective controls (Table 1). Within each treatment regime, no significant differences ($P < 0.01$; ANOVA) between BPU's were observed. Treatments of the rice foliage (Treatments 1 and 3) showed the effects of the BPU's resulting from adult feeding or by contact with leaf residues. Treatment of the water following oviposition (Treatments 5 and 7) indicated contact action on the eggs or first instars. When the water was treated 9 days after oviposition, most larvae had hatched and settled down to the roots in the soil. Neither BPU (Treatments 9 and 11) showed effects significantly different from the control ($P < 0.01$; ANOVA). No significant differences ($P < 0.01$; ANOVA) were noted in plant growth in this experiment at the time larvae were recovered from the soil.

Experiment 2. Five different treatment regimes were used to attempt to identify which stage of the life cycle and what period within a particular stages was affected by the BPU's (Table 2). Treatment of the rice foliage (Treatments 1 and 2) and water after oviposition (Treatments 4 and 5) showed the same effects as seen in Experiment 1; significantly fewer immatures were recovered at the end of the experiment, and no differences were observed between BPU's within a treatment regime ($P < 0.01$; ANOVA).

Delay of the water treatment to 5 days after oviposition resulted in no significant differences ($P < 0.01$; ANOVA) between treatments and controls. These results demonstrated that the effect on the egg occurred shortly after oviposition and not

Table 2. Effect of BPU's on immature rice water weevils and on rice plants in relation to treatment regimes

Treatment regime	Avg no. immatures per two plants	Plant growth characteristics				
		Avg plant wet wt (g) of two plants	Avg plant ht (cm)	Avg root length (cm)	Avg root vol (ml)	Avg dry root wt (g)
Rice foliage						
1. Diflubenzuron	0.0a	31.2cd	54.7bc	22.5e	16.4de	1.26de
2. Alsystin	0.4a	28.7bcd	54.9bc	20.3cde	14.4bcde	0.09abcde
3. Control	15.4bc	25.4abcd	51.4abc	18.5bcde	10.2abcde	0.81abcde
Water after oviposition						
4. Diflubenzuron	0.2a	33.9d	57.2c	20.1cde	17.0de	1.18cde
5. Alsystin	5.0ab	31.3cd	56.3bc	19.8cde	15.2cde	1.10bcde
6. Control	19.8c	16.6abc	47.5abc	12.2ab	7.4abc	0.57abc
Water 5 days after oviposition						
7. Diflubenzuron	18.8c	10.7a	43.3a	10.7a	3.8a	0.26a
8. Alsystin	15.8bc	21.4abcd	49.2abc	14.2abcd	8.8abcd	0.82abcde
9. Control	19.8c	13.7ab	46.9abc	11.6ab	4.6a	0.60abcd
Water 14 days after oviposition						
10. Diflubenzuron	16.2bc	21.3abcd	47.3abc	16.8abcde	9.2abcd	0.77abcde
11. Alsystin	16.2bc	13.9ab	46.1ab	13.0abc	5.8ab	0.48ab
12. Control	12.0abc	21.6abcd	47.2abc	15.8abcde	10.2abcde	0.81abcde
First instar larvae exposure						
13. Diflubenzuron	18.2c	35.0d	55.3bc	21.1de	17.8de	1.37e
14. Alsystin	15.6bc	37.0d	57.2c	20.8de	19.0e	1.35e
15. Control	21.2c	33.1d	56.5c	19.9cde	16.4de	1.31e

Means within a column followed by the same letter are not significantly different ($P < 0.01$; Duncan's [1951] multiple range test).

during the later periods immediately before eclosion (eggs hatch 6 to 7 days after oviposition). Treatment of the water at 14 days after oviposition (Treatments 10 and 11) was directed against the second to fifth instars. No significant differences ($P < 0.01$; ANOVA) in numbers of larvae were found between treated plants and controls.

After direct immersion applications (Treatments 13 and 14), first instars were returned to the growth system. No significant differences ($P < 0.01$; ANOVA were seen in number of larvae recovered from the immersion applications, the 5 and 14 day water treatments, or the controls. Significant differences ($P < 0.01$; ANOVA) were found between the control and treatments for selected measurements of plant growth for the water treatment immediately after oviposition. A general trend of less growth in the controls associated with the two effective treatment regimes (Treat-

Table 3. Effect of BPU's on number of eggs of the rice water weevil laid at intervals after initial adult exposure to treated foliage

Treatment and h after exposure	Trial 1 Avg no. eggs per weevil	Trial 2 Avg no. eggs per weevil
24 h		
1. Diflubenzuron	9.4	8.4a
2. Alsystin	9.5	9.0a
3. Control	9.4	9.9a
96 h		
4. Diflubenzuron	—	16.1c
5. Alsystin	—	14.2bc
6. Control	—	17.6c
168 h		
7. Diflubenzuron	—	9.7a
8. Alsystin	—	9.6a
9. Control	—	11.4ab

Means within a column followed by the same letter are not significantly different ($P < 0.01$; Duncan's [1951] multiple range test).

Table 4. Rice water weevil first instar survival after treatments with BPU's

Treatment regime	Avg no. first-instar larvae per two plants
Rice foliage	
1. Diflubenzuron	0.4a
2. Alsystin	2.8a
3. Control	37.4b
Water after oviposition	
4. Diflubenzuron	0.0a
5. Alsystin	3.6a
6. Control	51.6b
Water 5 days after oviposition	
7. Diflubenzuron	41.2b
8. Alsystin	48.0b
9. Control	34.2b

Means followed by the same letter are not significantly different ($P < 0.01$; Duncan's [1951] multiple range test).

ments 1–6) was noted; this trend reflected the effect of the increased numbers of larvae feeding on the roots of the control plants.

Experiment 3. The possible effect of either BPU in reducing fecundity was examined at three time periods (24, 96, and 168 h) following initial exposure of the adults to treated rice foliage. No significant changes (Table 3) in the number of eggs could be attributed to the BPU's. A significant ($P < 0.01$; ANOVA) almost 2-fold, increase in number of eggs laid at 96 h occurred after the start of the experiment both for the treatments and control, but this probably reflects a characteristic of oviposition behavior.

Experiment 4. This experiment was designed for direct recovery of first instars after emergence from the rice sheath tissue. In this way, treatment effects on the first instars could be observed within 1 day after the normal period of hatching rather than near the end of larval development (Experiments 1 and 2). Significantly fewer larvae were recovered for both BPU's (Table 4) when rice foliage was treated (Treatments 1 and 2) and when the water was treated immediately after oviposition (Treatments 4 and 5). Neither BPU (Treatments 7 and 8) resulted in significant differences ($P < 0.01$; ANOVA) in larval numbers from the control, when the water was treated 5 days after oviposition.

These four experiments demonstrate that the effect of both BPU's on the rice water weevil occurs during the first half of egg development. No effect was observed on eggs during periods of later development or on any instar. The effect can be transferred to the egg from the mother or achieved by direct contact with treated water.

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