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INSECTICIDAL EFFECTS OF EXTRACTS FROM TWO RICE VARIETIES TO BROWN PLANTHOPPER, *Nilaparvata lugens*

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Abstract—Ether extracts from a resistant rice variety, Jingxian 89, and a susceptible variety, Qidaizhan, were significantly toxic to *Nilaparvata lugens* adult females, causing 98% and 73.5% mortalities, respectively. The area covered honeydew droplets excreted by *N. lugens* adult females while feeding on Qidaizhan was 28.5-fold more than the area of those feeding on the resistant variety, Jingxian 89. The honeydew covered areas were reduced 29.7- and 8.8-fold, respectively, after feeding on plants treated with the ether extracts from both varieties. Among the fractions from each variety, fractions 9 (three sterols) from both varieties and fraction 10 (3-nitraphthalic acid) from Jingxian 89 showed significant repellent effects on the planthoppers. Further bioassays revealed that fraction 9 from extracts of both varieties at 0.02 mg/ml or higher deterred the feeding activity of the planthoppers, whereas the deterrent effects of fraction 10 from the extract of Jingxian 89 were 100- to 500-fold stronger than those of fraction 9. Fraction 10, identified as 3-nitraphthalic acid, unique in Jingxian 89, was extremely toxic to *N. lugens* adult females, and the LC_{50} and LC_{90} were 0.00045 and 0.00525 mg/ml, respectively. After extensive bioassays and analysis, we concluded that 3-nitraphthalic acid plays a key role in the resistance to *N. lugens* adult females.

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Key Words—Rice, feeding deterrent, brown planthopper, *Nilaparvata lugens* resistance variety, plant resistance.

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stal), is one of the most important pests of rice worldwide (Oercke, 1994). Resistance in rice varieties has been increasingly recognized as the most desirable and economically feasible control tactic in the management of the brown planthopper. However, crop resistance can only be fully utilized after we understand its mechanism. Previous studies have shown that the chemical mechanisms involved with resistance are diverse and may affect the planthoppers' feeding behaviors in various ways, such as plant surface exploration, probing, or ingestion (Sogawa, 1977, 1982). For example, oxalic acid, which inhibits ingestion in *N. lugens*, is twice as concentrated in resistant rice varieties as in susceptible varieties (Yoshihara et al., 1979). The apigenin-C glycosides have been reported as probing stimulants (Kim et al., 1985) and sucking deterrents (Grayer et al., 1994). Stevenson et al. (1996) believed that these compounds are responsible for rice resistance to *N. lugens.*

Our objective in this study was to determine the mechanism of resistance of a resistant rice variety, Jingxian 89 to *N. lugens* (Tan et al., 1995) by comparison with a susceptible rice variety, Qidaizhan, using the extracts to bioassay the toxicological, repellent, and deterrent effects on adult females of *N. lugens.*

METHODS AND MATERIALS

Rice Plants and Insects. A resistant rice variety, Jingxian 89, and a susceptible variety Qidaizhan, were grown in paddy fields in Dinghu District, Zhaoqing City, Guangdong. The plants had been transplanted on April 15, 1995. *N. lugens* adults, collected from rice fields in Dinghu District, were reared on rice variety Qidaizhan in screen cages (35 × 35 × 80 cm) in a laboratory at 25 ± 5°C and relative humidity 80 ± 10%.

Extraction of Rice Plants. Rice plants were sampled from the field when the plants were 61 days old at boot stage. Fresh leaves (2 kg) with both young and old leaves from each of the two varieties were cut into pieces (about 3 cm long). The leaves were immersed in 95% ethanol for two days, and filtered using double-layer paper filter. This procedure was repeated twice, and the ethanol extracts were combined (about 5 liters). The extract was evaporated under reduced pressure by using a standard vacuum pump. The enriched extract was washed three times with petroleum ether (30–60°C). The postether extract was partitioned three times with ethyl acetate. Dry ether extract, postether acetidin extract and postacetidin water extract were obtained by evaporating under reduced pressure for 2 hr and stored in the refrigerator at 4°C.

The ether extract of each variety was subjected to preliminary separation by flash column chromatography using 100 5-cm columns packed with 400 g of 60- to 100-μm preparative silica gel (Qindao Marine Chemical Plant, Qindao, China). Two-thousand gram leaf equivalents (GLE) of the extract were loaded onto the column. One hundred fourteen fractions (100 ml each) were collected by sequentially eluting the column with 2500 ml each of petroleum ether and 20, 40, 60, 80, and 100% ethyl acetate in petroleum ether. The compounds in these fractions were compared using a thin-layer chromatography system (TLC) with five 10-cm, 0.25-mm-thick silica gel (same as above) plates, with petroleum ether–ethyl acetate (4 : 1) as the solvent system. Plates were examined under an iodine-containing box. Those fractions with same or similar migratory rates in the TLC tests were combined to form a new fraction. Thus, 13 and 12 new fractions were obtained from the ether extracts of Jingxian 89 and Qidaizhan, respectively. These fractions were bioassayed for bioactivity on *N. lugens* adults.

Extract Analysis. GC-MS analysis of fractions 9 or 10 was performed on a HP5972 MSD instrument (Hewlett Packard Company) with an electron ionization energy of 70 eV and an ion current of 50 μA using a HP-5 capillary column (50 m $\times$ 0.32 mm $\times$ 0.17 m). Helium was used as the carrier at a flow rate of 1 ml/min. Samples (1 μl) was injected into the column (Ganeswara-Rao et al., 1995). The column temperature was held at 80°C for 5 min, then programmed at a 3°C/min increment to 290°C.

Toxicological Effects of Extracts. Three 20-day-old Qidaizhan seedlings (five leaves and approximately 10 cm high) were dipped separately for 3 sec in 1 GLE/ml solutions of extract from each variety (diluted in acetone) and placed in a 2000-ml beaker. Seedlings treated with acetone alone were used as controls. Ten 1-day-old *N. lugens* adult females were placed in each of the treated or control beakers for about 30 min after the acetone had evaporated. The mortality was recorded 24 hr after the introduction of the planthoppers. Each treatment was replicated five times, and all tests were conducted at room temperature (25 $\pm$ 5°C) and a 12L : 12D photoperiod. Percent mortalities relative to controls were computed according to Zhang (1989): $RM\% = [(TM\% - CM\%)/(100 - CM\%)] \times 100$, where RM is the mortality relative to control; TM is the mortality on treated plants; and CM is the mortality on the untreated plants (control). Percent mortalities were arc sine transformed before analysis of variance (ANOVA) (Gomez and Gomez, 1984), although untransformed mean percentages are reported. Mean percent mortalities were separated using the least significant tests (LSD) (SAS Institute, 1996).

Honeydew Excretion. N. lugens excretes large amounts of honeydew when feeding on the rice plants. However, the amounts of honeydew excreted and the area of the honeydew droplets deposited by the planthoppers vary greatly depending on the developmental stage, host plants, and other biotic and abiotic factors. The planthoppers excrete more honeydew, and the honeydew droplets

cover a larger area, when feeding on preferred host plants or susceptible varieties than when feeding on unpreferred host plants and resistance varieties. The amount of honeydew excreted and the area covered by the honeydew droplets have been used as indirect indicators of host plant preference and resistance (Tan et al., 1995).

Area Covered by Honeydew Droplets. The area covered by honeydew droplets from *N. lugens* adult females after feeding on the plant seedlings was determined as follows, using a feeding chamber (Liu and Zhao, 1996). Ether extracts from each of the two varieties were diluted in acetone. The rice stems were treated with 1 GLE/ml of ether extract from each variety. Control plants were either treated with acetone alone or were untreated. Five 1-day-old adult females were allowed to feed on the rice stems for 24 hr, and the area covered by the honeydew droplets was measured. Each treatment was replicated five times. Areas (square millimeters) covered by honeydew droplets were analyzed, differences between treatments were separated by ANOVA, and means were separated using the LSD test at $P = 0.05$ (SAS Institute, 1996).

Quantity of Honeydew Excreted. The total amount of honeydew excreted over a 24-hr period was measured in the feeding chamber (Hu et al., 1994; Powell et al., 1995). Fractions 9 and 10 from Jingxian 89 extracts and fraction 9 from Qidaizhan extract were recrystallized before the experiment. Artificial diets (10% sucrose + 0.5% glutamic acid) incorporated with either fraction 9 or fraction 10 were used in the bioassay. The amounts of fraction 9 from both rice varieties and fraction 10 from Jingxian 89 in the artificial diets ranged from 0.01 to 1.00 mg/ml, and from 0.002 to 0.00005 mg/ml, respectively. When testing, five 1-day-old *N. lugens* adult females were transferred into the feeding chamber and allowed to feed on a fraction-incorporated artificial diet. Planthoppers feeding on the artificial diets alone were used as controls. *N. lugens* adults were allowed to feed on the diets for 24 hr. The honeydew excreted by *N. lugens* while feeding was collected on the bottom of a plastic flask. After the removal of the adults from the chamber, 2 ml of distilled water was added to the flask to dissolve the honeydew. The solution was stirred for a few seconds and then transferred into a small glass tube. We then added 0.1 ml of 97.1% phenol and 2 ml of 100% sulfuric acid to the tube, and the solution quickly became yellowish brown. After cooling and stirring, the absorbance of the solution at 490 nm was measured. Each treatment was replicated four to five times.

The amount of the honeydew (sucrose equivalent) excreted was calculated using a linear equation, $Y = 3.2209X$, where Y is the amount of the honeydew excretion, and X is the absorbance at 490 nm from a standard curve generated using sucrose. A feeding deterrent index (FDI) (Liu and Zhao, 1996) was used in the rearing experiments and was based on the amount of honeydew excreted. The FDI was calculated as: $FDI = [(HC - HT)/HC] \times 100$, where HC is honeydew in the control, and HT is honeydew in the treatment. The amounts of

honeydew excreted and FDIs were analyzed by ANOVA, and the means were separated using the LSD test as $P = 0.05$ (SAS Institute, 1996).

Numbers of dead and living *N. lugens* adult females were determined after feeding on the treated or untreated plants for 24 hr. Lethal concentrations of fraction 10 extracts for *N. lugens* adult females were calculated by using probit or logit analysis or POLO (LeOra Software, 1994).

Repellent Effects. Three rice seedlings were dipped in appropriate fractions (1 GLE/ml) extracted from each of the two rice varieties. After drying, the seedlings were confined in small cages, and 15 adult females (24 hr old) were introduced into each cage. The location of the planthoppers was examined at 2 hr after introduction. An index based on whether the *N. lugens* adult females were present and feeding on the plant seedlings was calculated using the following formula: $RI(\%) = C/T \times 100$, where RI is the repellent index, C is the surviving *N. lugens* adult females feeding on the seedlings, and T is the total number of *N. lugens* adults tested. Thirteen fractions from Jingxian 89 and 12 fractions from Qidaizhan were tested, and each treatment had five replications.

RESULTS

Extraction of Rice Materials and Analysis. Ether, postether acetidin, and postacetidin water extracts from each rice variety, and a total of 13 and 12 fractions were obtained from the ether extracts from Jingxian 89 and Qidaizhan varieties, respectively. Three major peaks were detected in fractions 9 from Jingxian 89 and Qidaizhan with retention times of 59.05 min (I), 59.70 min (II), and 60.01 min (III). Peaks I, II, and III were then identified as ergosterol, stigmasterol, and β-stigmasterol, respectively. The concentrations of each sterol in the 2 varieties were almost equal according to the peak area. A single major peak was detected in fraction 10 of Jingxian 89 with retention time of 42.37 min (Figure 1), and this peak was identified as 3-nitraphthalic acid. Before this peak, one minor peak was detected with the retention time of 19.07 min, but it has not been identified yet, and its biological and chemical significance is currently unknown.

Toxicological Effects of Extracts. The *RM*'s of *N. lugens* adult females after feeding on the treated plants for 24 hr were significantly different among the three extracts from both Jingxian 89 and Qidaizhan seedlings (Table 1). The percent mortalities caused by the extracts from Jinxian 89 were generally greater than those caused by those from Qidaizhan. However, only the percent mortality for the ether extract from Jingxian 89 (98%) was significantly greater than that from Qidaizhan (73.5%). It is clear that the ether extracts from both varieties have significant insecticidal activities.

Fraction 10 of the Jingxian 89 extract was extremely toxic to *N. lugens*

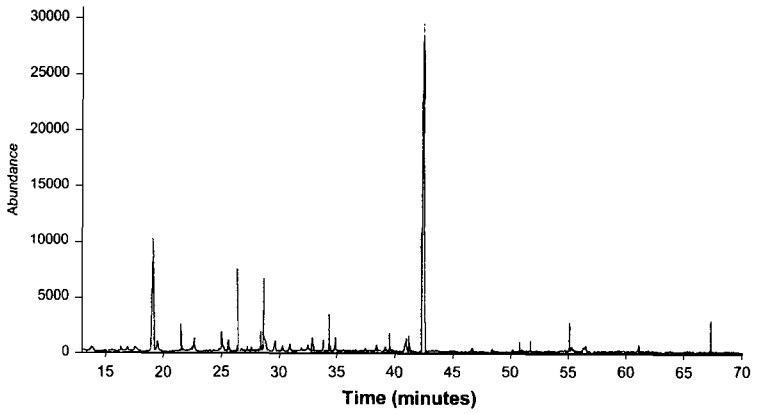

FIG. 1. The chromatogram of fraction 10 from the extract of Jingxian 89. The highest peak was identified as 3-nitraphthalic acid.

adult females (Figure 2). The LC_{50} and LC_{90} were 0.00047 (95% FL: 0.00022–0.00112), and 0.00525 (95% FL: 0.00183–0.11088), respectively.

Area Covered by Honeydew Droplets. The planthoppers fed on the susceptible variety, Qidaizhan, excreted significantly more honeydew than those fed on the resistant variety, Jingxian 89, as indicated by the areas covered by the honeydew droplets after 24 hr of feeding (Table 2). However, the honeydew droplets excreted by *N. lugens* adult females fed on the same susceptible variety (Qidaizhan) seedlings treated with the ether extracts from both varieties decreased dramatically, although the effects of the ether extract from Jingxian 89 were stronger than those of Qidaizhan. The application of acetone alone

TABLE 1. EFFECTS OF THREE TYPES OF EXTRACTS FROM RICE VARIETIES JINGXIAN 89 AND QIDAIZHAN ON *N. lugens* ADULT FEMALES 24 HOURS AFTER TREATMENT[a]

	Mortality (%, mean ± SE)			
Extracts	Jingxian 89	Qidaizhan	*F*	*P*
Ether	97.9 ± 2.0aA	73.5 ± 4.0aB	28.80	<0.001
Post-ether acetidin	18.4 ± 4.5bA	10.2 ± 3.8bA	1.88	0.207
Post-acetidin water	8.0 ± 3.8cA	2.0 ± 2.0cA	0.40	0.545
F	252.93	134.24		
P	<0.001	<0.001		

[a]Means in the same columns followed by the same lowercase letters and in the same row followed by the same uppercase letters are not significantly different ($P = 0.05$, LSD) (SAS Institute, 1996).

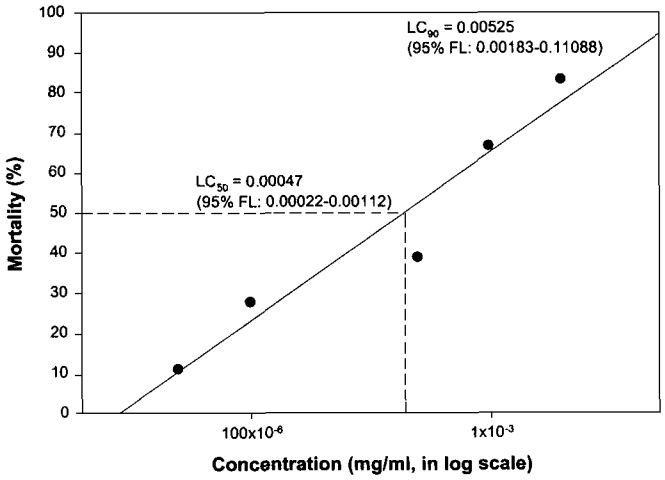

FIG. 2. Toxicity of fraction 10 of extract from Jingxian 89 on *Nilaparvata lugens* adult females after a 24-hr feeding period.

on Qidaizhan did not affect the honeydew excretion or feeding activity of the planthoppers.

Quantity of Honeydew Excreted. The feeding activities of *N. lugens* adult females were significantly affected by feeding on the plant seedlings treated with the fractions (fraction 10 from Jingxian 89 and fraction 9 from both varieties) as indicated by the amount of honeydew (sucrose equivalents) excreted and the FDIs (Table 3 and Figure 3).

TABLE 2. EFFECTS OF ETHER EXTRACTS ON HONEYDEW EXCRETION OF *N. lugens* ADULT FEMALES AFTER FEEDING ON TREATED OR UNTREATED PLANTS FOR 24 HOURS[a]

Treatments	Area covered by honeydew droplets (mm^2/adult, mean ± SE)
Qidaizhan sprayed with ether extract from Jingxian 89	1.86 ± 0.10c
Qidaizhan sprayed with ether extract from Qidaizhan	6.26 ± 0.21b
Qidaizhan sprayed with acetone	57.66 ± 1.85a
Untreated Jingxian 89	1.94 ± 0.21c
Untreated Qidaizhan	55.24 ± 0.78a
F	1030.16
P	<0.001

[a]Means followed by the same letter in the same column are not significantly different ($P = 0.05$, LSD) (SAS Institute 1996).

TABLE 3. DETERRENT EFFECTS OF FRACTION 10 OF JINGXIAN 89 EXTRACT ON *N. lugens* ADULT FEMALES AFTER A 24-HOUR FEEDING PERIOD AS SHOWN BY AMOUNT OF SUCROSE EQUIVALENT[a]

Extract concentration (mg/ml)	Honeydew excreted (μg sucrose/adult, mean ± SE)	FDI
Control	29.12 ± 8.24a	0.00
0.00005	24.86 ± 5.43ab	14.63
0.00010	13.91 ± 2.85bc	52.23
0.00050	3.22 ± 0.76c	88.94
0.00100	3.09 ± 0.92c	89.39
0.00200	2.00 ± 0.75c	93.16
F	7.87	
P	<0.001	

[a]Means followed by the same letter in the same column are not significantly different ($P = 0.001$, LSD) (SAS Institute 1996).

Fraction 10 from Jingxian 89 showed significant feeding deterrent effects on the planthoppers. The amounts of honeydew excreted by the planthoppers after feeding on the seedlings treated with fraction 10 differed significantly among the five concentrations ($F = 7.87$; $df = 5, 24$; $P < 0.001$) (Table 3). The planthoppers excreted significantly less honeydew when feeding on the plants treated with the concentrations of 0.0001 mg/ml or higher. Similarly, the FDIs increased when concentrations of the fraction increased.

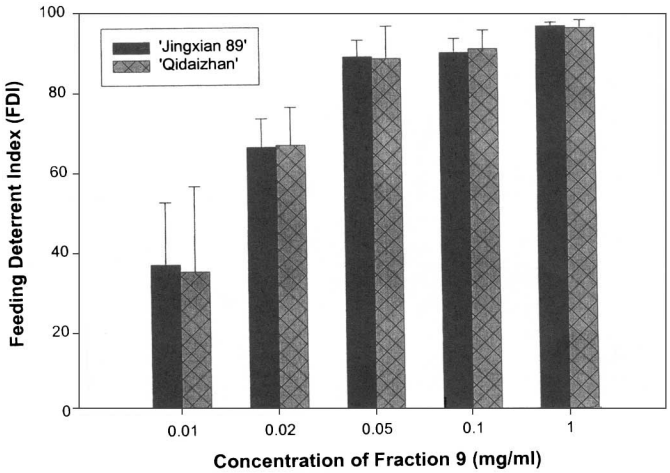

FIG. 3. Feeding deterrent effects of fraction 9 from the extracts of the two rice varieties, Jingxian 89 and Qidaizhan to *Nilaparvata lugens*.

Fraction 9 from both varieties also showed strong feeding deterrent effects on the planthoppers. The FDIs, computed from the amount of honeydew excreted after feeding on the plants, differed significantly between concentrations (F = 9.05; df = 4, 15; $P < 0.001$ for Jingxian 89; F = 3.43; df = 4, 15; P = 0.035 for Qidaizhan) (Figure 3). We consider the extract has feeding deterrent effects if the FDI is 50% or greater. Therefore, fraction 9 caused significant feeding deterrent effects when applied at 0.02 mg/ml or higher. However, the FDIs between the same concentrations of the two fractions from the two rice varieties were not significantly differences ($P > 0.05$).

Repellent Effects. Among the 13 fractions from Jingxian 89, the repellent effects were significantly different (F = 9.48; df = 13, 56; $P < 0.001$), with the strongest effects shown by fractions 9 and 10 (Table 4). The repellent indices among the 12 fractions from Qidaizhan (there is no fraction 10) were also significantly different (F = 5.98; df = 12, 52; $P < 0.001$), with fraction 9 showing a strong repellent effect. All other fractions in both Jingxian 89 and Qidaizhan had no or weak repellent effects on *N. lugens* adult females. These data further support the results from other bioassays, as mentioned above, that the compound, 3-nitraphthalic in fraction 10, which is unique in Jingxian 89, is probably responsible for the resistance of the variety to *N. lugens* adult females.

TABLE 4. REPELLENT EFFECTS OF FRACTIONS FROM JINGXIAN 89 AND QIDAIZHAN ON *N. lugens* ADULT FEMALES AS INDICATED BY REPELLENT INDEX[a]

Fractions	Repellent index (%, mean ± SE)	
	Jingxian 89	Qidaizhan
1	37.3 ± 3.4ab	38.7 ± 3.9ab
2	32.0 ± 2.5abc	34.7 ± 3.9a-d
3	28.0 ± 2.5bc	32.0 ± 5.3a-e
4	25.3 ± 3.9bc	33.3 ± 2.1a-e
5	28.0 ± 3.9bc	29.3 ± 3.4b-e
6	30.7 ± 3.4abc	28.0 ± 2.5cde
7	38.7 ± 4.4a	32.0 ± 2.5a-e
8	28.0 ± 4.4bc	30.7 ± 4.5a-e
9	5.3 ± 2.5d	8.0 ± 1.3f
10	2.7 ± 1.6d	—
11	22.7 ± 1.6c	26.7 ± 4.2de
12	30.7 ± 3.4abc	40.0 ± 3.0a
13	26.7 ± 4.7c	24.0 ± 3.4e
Control	30.7 ± 2.7abc	37.3 ± 1.6ab

[a]Means followed by the same letter in the same column are not significantly different (P = 0.05, LSD) (SAS Institute 1996).

DISCUSSION

Shigematsu et al. (1982) have demonstrated that sterols and asparagine from the rice plants are endogenous factors related to resistance against *N. lugens* by some rice varieties in Japan. As we mentioned above, we found similar compounds (including three sterols) from both rice varieties affecting the feeding behavior of the planthoppers and even causing high mortality. The question is which compound is responsible for the resistance in Jingxian 89. After the extensive bioassays and analyses of the extracts, we now have a better understanding of the resistant variety, Jingxian 89. Firstly, because the ether extracts from both varieties, Jingxian 89 and Qidaizhan, were highly toxic to the planthoppers and strongly deterred the feeding activities of *N. lugens* adult females, as indicated by honeydew excretion, the ether extracts cannot be totally responsible for the resistance in Jingxian 89. Second, three sterols were present in fraction 9 of both varieties with similar concentrations, and both showed significant feeding deterrent and repellent effects on the planthoppers. Therefore, the fraction 9 in Jingxian 89 is not responsible to the resistance to the planthoppers. Finally, 3-nitraphthalic acid is unique in fraction 10 of Jingxian 89, which has great toxicity and feeding deterrent effects against the planthopper. We concluded, therefore, that it is responsible for the resistance to *N. lugens* adult females.

Utilization of resistant rice varieties is one of the major measures in the management of rice pests. Tan et al. (1995) reported that Jingxian 89 is resistant to *N. lugens* but did not reveal its resistance mechanism. The present study quantified the feeding deterrent effects of the extracts and also revealed that 3-nitraphthalic acid may play a key role in the resistance of Jingxian 89 to *N. lugens*.

There are two kinds of resistance, constitutive resistance and induced resistance. Previous studies showed that biological factors (e.g., insects, fungi, bacteria, and viruses) and nonbiological factors (e.g., pesticides) could bring out induced resistance of multiple crops to pests (Lou and Cheng, 1997). If 3-nitraphthalic acid could be introduced into a rice variety or the concentration of the compound could be manipulated and increased, the resistance of the variety to *N. lugens* could be enhanced. At present, we are attempting to obtain purified 3-nitraphthalic acid to verify our results in this study, to conduct further laboratory bioassays and field tests on *N. lugens* and other pests, and to study whether 3-nitraphthalic acid is induced by the feeding activities of *N. lugens*. We also plan to study the function and action mechanism of the compound to *N. lugens* and to determine how the planthoppers adapt to the compound. After finishing these studies, we will be able to better understand the interactions between resistant rice varieties and *N. lugens* and to incorporate the information into programs for breeding resistant rice varieties.

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