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ORIGINAL ARTICLE

Study of the relationship between the content of the rare earth element Eu in rice plants and in *Nilaparvata lugens* (Hemiptera: Delphacidae)

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Abstract The current study investigated changes in the content of the rare earth element Europium (Eu) in roots, shoots and leaves of rice plants and in *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) using an Eu marker and hydroponic culture. The results showed that *N. lugens* infestation significantly reduced Eu content in roots, shoots and leaves of two rice varieties, Shenyou 1 and Xieyou 963. The Eu content in roots, shoots and leaves of the susceptible variety, Shenyou 1, was significantly higher than that in the resistant variety, Xieyou 963. The Eu content of *N. lugens* fed on Shenyou 1 was significantly higher than of those fed on Xieyou 963. In addition, the Eu level was elevated at a higher density of *N. lugens* infestation. Eu content in the bodies of *N. lugens* was related to their weight and honeydew excretion, with a significant positive correlation. Thus, Eu content in the bodies of *N. lugens* can be considered an index of the amount of phloem sap taken in by *N. lugens* because the amount of honeydew excretion is proportional to the amount of phloem sap consumed. The ratios of Eu content in *N. lugens* to that in roots, shoots and leaves of rice plants were elevated at a higher *N. lugens* infestation density. That ratio was maximal in leaves, was intermediate in shoots and was minimal in roots. There was no significant difference in ratios between the two plant varieties. An Eu marker may be useful in the screening of resistant varieties and in the study of the mechanisms of resistance.

Key words markers, *Nilaparvata lugens*, rare earth element Eu, rice

Introduction

In studies of insect population dynamics, marking techniques may provide valuable information for understanding insect migration and dispersal. The source area of a *Nilaparvata lugens* Stål immigrant population was identified by mark–release–recapture using a dye marker (Cheng *et al*., 2003). Schellhorn *et al*. (2004) evaluated a water-soluble fluorescent dye and a resin-based fluorescent pigment that were sprayed on crops to mark beneficial and pest insects, and they monitored the dispersal of marked insects. The use of a natural enemy refuge in the establishment and movement of the *Bemisia* parasitoids *Eretmocerus eremicus* and *Encarsia* spp. was studied by marking a naturally occurring field population of parasitoids with rubidium chloride (RbCl) (Pickett *et al*., 2004). In addition to dye-marking studies, pollen grains and C_3 and C_4 plants have also been used to track movements of natural enemies and migration of cotton bollworm moths (Xu *et al*., 1999; Prasifka & Heinz, 2004; Silberbauer *et al*., 2004). In recent years, data on the population dynamics of beneficial insects, host switching and breeding systems have been obtained using molecular DNA markers (MacDonald & Loxdale, 2004). Enzyme-linked immunosorbent assay (ELISA) has been used to study the movement and feeding activity of insects (Hagler & Naranjo, 2004; Hagler, 2004).

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Eu is a rare earth element with the characteristics of a strong fluorescence, a long half-life and easy uptake by plants. Therefore, it is used as a marker in the quantification of energy flow among rice plants, pests and natural enemies (Wen *et al*., 2001; Hu *et al*., 2002). However, the transfer of Eu between roots, shoots and leaves of rice plants and the body and honeydew of *N. lugens* has not been investigated.

Nilaparvata lugens is a long-distance migratory insect pest, and its populations in rice-growing districts and northern overwintering areas are established annually by wind-borne immigration from the tropics and subtropics (Cheng *et al*., 1979; Kisimoto, 1987; Rosenberg & Magor, 1987; Pender, 1994; Riley *et al*., 1994). The insect is a monophagous pest that threatens rice (*Oryza sativa* L.) production in many Asian countries. *N. lugens* is a typical vascular feeder, primarily sucking phloem sap (Sogawa, 1980), which reduces the chlorophyll and protein content of leaves and lowers the photosynthetic rate (Watanabe & Kitagawa, 2000). *N. lugens* feeds on rice plants by sucking phloem sap and producing copious amounts of honeydew. Components of the ingested phloem sap that are not absorbed are excreted in the honeydew. Therefore, the amount of honeydew produced can be considered an index of the amount of phloem sap taken in by *N. lugens.* Understanding the feeding dynamics of *N. lugens* and its effect on the roots, stems and leaves of different rice varieties using an Eu marker may be important in determining the resistance of rice to *N. lugens* and the migration route of the insect. Thus, the objective of this study was to examine the distribution of Eu among the roots, shoots and leaves of rice plants and to examine which Eu marker may be useful in the screening of resistant varieties and in the study of the mechanisms of resistance.

Materials and methods

Rice variety, insect and Eu

Two rice varieties were used in the experiments: Xieyou 963 (indica rice), which is resistant to *N. lugens* (Wang *et al*., 1999), and Shenyou 1 (japonica rice), which is susceptible to that insect. Plant seeds were sown in cement tanks (height 60 cm, width 100 cm and length 200 cm) in batches. Four-leaf seedlings were removed from the tanks and rinsed with tap water to remove the soil. Single seedlings of similar size without tillers were transplanted in small hills into porcelain pots (height 13 cm and diameter 13 cm) containing Espino hydroponic solution (Mae & Ohira, 1981) with NH_4NO_3, $NaH_2PO_4 \bullet 2H_2O$, K_2SO_4, $CaCl_2$, $MgSO_4 \bullet 7H_2O$, $MnCl_2 \bullet 4H_2O$, $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$, H_3BO_3, $ZnSO_4 \bullet 5H_2O$, $FeCl_3$, $CuSO_4 \bullet 5H_2O$ and citric acid. Details of the cultivation method were described in Wu *et al*. (2003). All of the rice plants were grown in a greenhouse under natural temperature and photoperiods. Eu_2O_3 (Shanghai ManKun Chemical Reagent Ltd., Co. Shanghai, China) was used as the marker for Eu in the experiments.

Experiment

Fifty milligrams of Eu_2O_3 in 1 000 mL of hydroponic solution was added to experimental pots 7 days before insect release and 30 days after transplanting at the late tillering stage. Eu_2O_3 was not added to control pots. The experiment was arranged in a randomized complete block design, with five replicates for each treatment and control. Rice plants were subjected to two densities of *N. lugens* infestation: 30 or 50 fifth instar nymphs were released onto potted rice plants within a nylon cage (30-mesh) 7 days after the addition of Eu_2O_3. Rice plants without exposure to *N. lugens* were used as controls. Live nymphs on treated and control plants were sampled at 3 and 6 days after release to measure their Eu content. Insect mortality was recorded every 24 h after release and dead nymphs were replaced with live ones of the same age to maintain insect density.

Collection of honeydew excretion

Honeydew collection followed the methods described by Pathak *et al.* (1982). A parafilm package was hung on a rice shoot approximately 10 cm above the water surface. A single 5th instar nymph from marked or unmarked plants was placed into each package with 12 replicates for each treatment. The insects were starved for 2 h prior to release. All nymphs used in this experiment were the same age and were weighed before release into a package. The nymphs were kept in the insect ecology laboratory of Yangzhou University, where they were held at $26 \pm 2^{\circ}C$ in a 16 : 8 h L : D illumination cycle. Twenty-four hours later, the package was opened, and the insect was removed. Honeydew was collected using a medical injector (Honqiao Medical Apparatus and Instruments Ltd. Co., Yangzhou, Jiangsu, China).

Measurement of Eu contents in roots, shoots and leaves of rice plants and N. lugens *bodies*

To quantify the Eu content in the roots, shoots and leaves of rice plants and in *N. lugens* bodies, all plants

and insects in a pot were dried in an electric oven at 80°C for 24 h. A 2-g portion of each dried sample was placed in a muffle furnace (LWL Development Ltd., Co. Hong Kong, China) and reduced to ash in 1 h at 800°C. Five milliliters HCl (1 : 1) and 0.2 mL H_2O_2 were placed in a crucible along with the ashed sample. The crucible was heated for 30 min in an electric furnace to dissolve the ashed substance, and the volume was then standardized to 30 mL with 2% HCl. Ten milliliters of the standardized solution was then absorbed and placed in a separatory funnel.

Extraction and back extraction of Eu

The methods of **e**xtraction and back extraction of Eu in the sample solution was described by Yin *et al*. (2000). For extraction, 0.1 g of L-ascorbic acid, 2 mL of 40% NH_4SCN, 2 mL of 20% $C_7H_6O_6S$ and 0.15 mL of $C_{27}H_{28}Br_2O_5S$ (bromothymol blue) were added to the sample solution. The solution was titrated with NH_3H_2O (1 : 1) to aquamarine and then titrated with HCl (1 : 9) to yellow. Five milliliters of CH_3COOH-CH_3COONH_4 buffer solution (pH 5.8) and 20 mL of 0.5% PMBP-C_6H_6 were added to the sample, which was then shaken. The aqueous phase was removed following 10 min of settlement.

Back extraction of sample

Following removal of the water phase, 10 mL of HCl (pH 2.4) was added to the organic phase, and the solution was then allowed to settle for 5 min after shaking. The water phase was poured into a beaker flask, 10 mL of HCl (pH 2.4) was added to the organic phase and that solution was allowed to settle for 5 min after shaking. The water phase, which contained the Eu^{3+} solution, was then poured into a beaker flask.

Sensitization of sample and addition of ligand

Four milliliters of CH_3COOH-CH_3COONH_4 buffer solution (pH 5.8) and 0.05 mL of Gd_2O_3 (1.723 × 104 mol/L) were added to the Eu^{3+} solution after adjusting its pH to 4.5 with HCl or NH_3H_2O. Then 0.4 mL of dibenzoylmethane (DBM), 0.1 mL of cetyl pyridinium bromide (CPB) and 5 mL of $Na_2B_4O_7{\cdot}10H_2O$-NaOH buffer solution (pH 10.0) were added after adjusting the pH to 10.0 with NH_3H_2O. The Eu^{3+} content of the samples was measured with a HITACHI F-4500 fluorescent spectrophotometer (Hitachi Ltd., Co. Chlyoda-ku, Tokyo, Japan). The content of Eu^{3+} in the samples was calculated according to the standard curve based on Eu_2O_3.

Statistical analysis

Two-way analyses of variance (ANOVA) on rice varieties and *N. lugens* densities were performed to compare Eu content in rice leaves, roots, shoots and in insect bodies and honeydew. Multiple comparisons of means were conducted using Fisher's protected least significant difference (PLSD). All analyses were conducted using the GLM procedure of the SPSS II program (SPSS, 2002). In addition, the correlation between Eu content in *N. lugens* bodies and in honeydew was determined.

Results

Changes in Eu contents of rice roots, shoots and leaves of marked and unmarked rice plants under N. lugens *infestation*

Although the Eu content of leaves was not significantly different between rice varieties (Table 1), there were significant differences in the Eu content of roots and shoots at different *N. lugens* densities. The interaction between insect density and rice variety was also significant. *N. lugens* infestation significantly reduced the Eu content in roots, shoots and leaves (Fig. 1). Multiple comparisons showed that the Eu content of roots, shoots and leaves was significantly reduced with increased densities of *N. lugens*. Furthermore, the Eu contents in roots, shoots and leaves of rice plants infested by 60 nymphs were significantly lower than those infested by 30 nymphs. In addition, the Eu contents in roots, shoots and leaves of Shenyou 1 were significantly higher than those of Xieyou 963.

Changes in the Eu content of the roots, shoots and leaves of unmarked rice plants were associated with *N. lugens* density and rice variety (Table 1, Fig. 1). ANOVA results showed that *N. lugens* infestation significantly influenced the Eu content of roots and shoots, but not of leaves.

Eu content in N. lugens *bodies*

In both varieties of rice, the Eu content in the bodies of *N. lugens* was significantly higher on marked rice than on unmarked rice (Table 2). For Shenyou 1 and Xieyou 963, at infestation densities of 30 and 60 nymphs per hill, the Eu content of *N. lugens* on marked rice increased by 6.6- and 5-fold, respectively, compared to an increase of 8.2- and 5.5-fold on unmarked rice. There were significant

Table 1 Analysis of variance of the Eu content data in Figures 1.

Treatment	Source	*F*-ratio	Root *P*-value	*F*-ratio	Shoot *P*-value	*F*-ratio	Leaf *P*-value
Marked rice	*Nilaparvata lugens* density (NLD)	597.8	0.000 1	816.8	0.000 1	603.5	0.000 1
	Rice variety (RV)	1 983.1	0.000 1	1 906.6	0.000 1	731.6	0.000 1
	NLD × RV	91.2	0.000 1	202.2	0.000 1	155.9	0.000 1
Unmarked rice	NLD	300.4	0.000 1	22.2	0.001 0	81.4	0.000 1
	RV	1 826.0	0.000 1	337.0	0.000 1	4.1	0.064 0
	NLD×RV	35.4	0.000 1	1.4	0.260 0	24.7	0.000 1

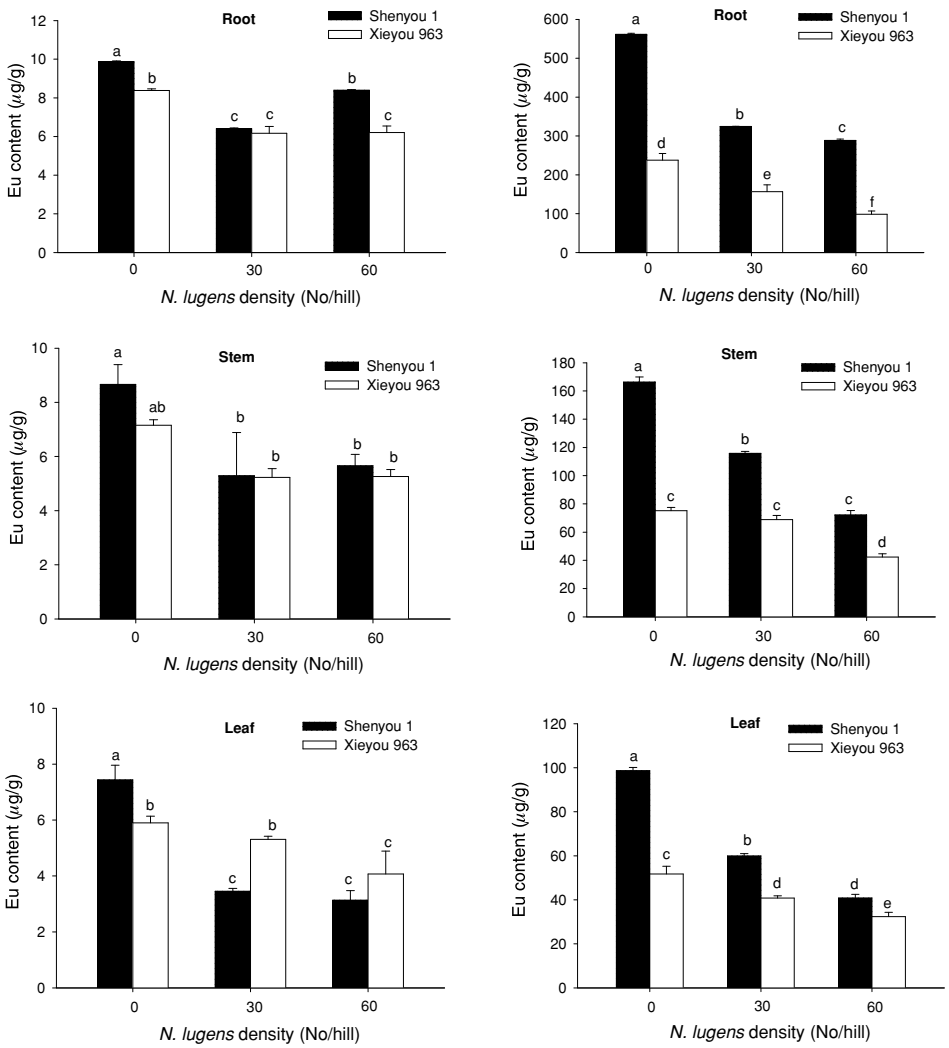

Fig. 1 Eu content of roots, shoots and leaves of unmarked (left) and marked (right) rice plants 3 days after *Nilaparvata lugens* infestation.

Table 2 Eu content in the bodies of *Nilaparvata lugens*.

Rice variety	Eu concentration (mg/L)	*N. lugens* density (no. per hill)	Eu content in the insect body
Shenyou 1	0	30	0.136 ± 0.063^{c}
		60	0.137 ± 0.006^{c}
	50	30	1.033 ± 0.016^{a}
		60	0.807 ± 0.056^{b}
Xieyou 963	0	30	0.102 ± 0.016^{c}
		60	0.123 ± 0.001^{c}
	50	30	0.939 ± 0.025^{a}
		60	0.792 ± 0.081^{b}

The means ± SD followed by different letters in the last column are significantly different at the 5% level.

differences in the Eu content of *N. lugens* between the two treatment densities, with that for the 60-nymph treatment being lower than for the 30-nymph treatment.

The relationship between Eu content, body weight and honeydew excretion in N. lugens

On marked rice of both varieties, there was a significant positive correlation between Eu content in *N. lugens* and body weight (Fig. 2). The Eu content of the insect was also positively correlated with the amount of honeydew produced (Fig. 3). Because the amount of honeydew produced was linearly correlated with body weight (Fig. 4) and because it is also proportional to feeding activity, the

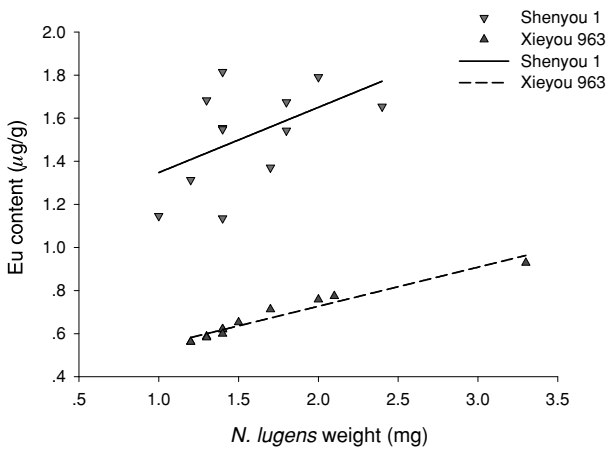

Fig. 2 The relationship between the Eu content in *Nilaparvata lugens* and insect weight (W) on marked rice. $Eu = 0.0015 + 0.0028W$, $r = 0.57$, $P < 0.05$ for Shenyou 1, $Eu = 0.3649 + 0.181W$, $r = 0.98$, $P < 0.01$ for Xieyou 963.

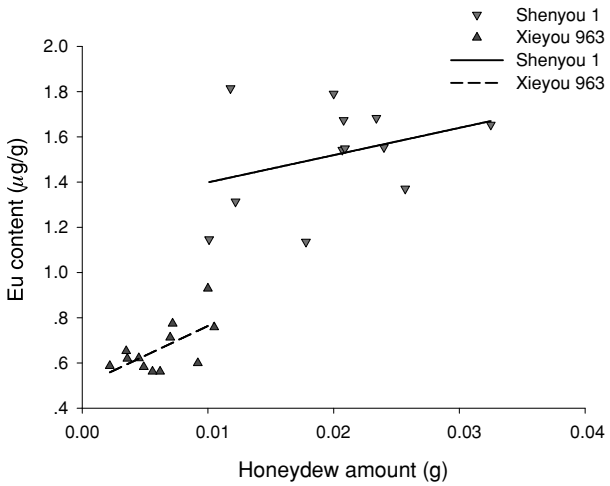

Fig. 3 The relationship between the Eu content in *Nilaparvata lugens* and honeydew amount (H) on marked rice. $Eu = 1.1033 + 46.194H$, $r = 0.59$, $P < 0.05$ for Shenyou 1, $Eu = 0.5401 + 26.302H$, $r = 0.63$, $P < 0.05$ for Xieyou 963.

Eu content in the bodies of *N. lugens* can be considered to be an index of feeding activity of the insect. However, on unmarked rice regressions between *N. lugens* weight and Eu content and between insect weight and honeydew excretion were not significant (Fig. 5, Fig. 7). Only the amount of honeydew excreted was significantly correlated with the Eu content of *N. lugens* for Shenyou 1 (Fig. 6). The lack of significance for these regressions may result from the low level of Eu in unmarked rice; therefore, the Eu content of unmarked plants is of little

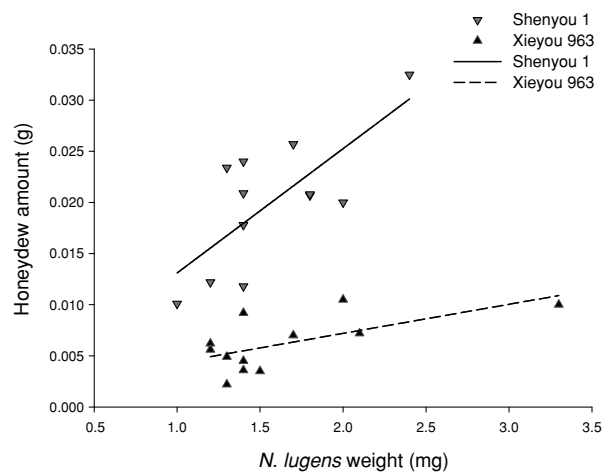

Fig. 4 The relationship between *Nilaparvata lugens* weight (W) and honeydew amount (H) on marked rice. $H = 0.0095 + 0.0121W$, $r = 0.74$, $P < 0.05$ for Shenyou 1, $H = 0.0015 + 0.0028W$, $r = 0.63$, $P < 0.05$ for Xieyou 963.

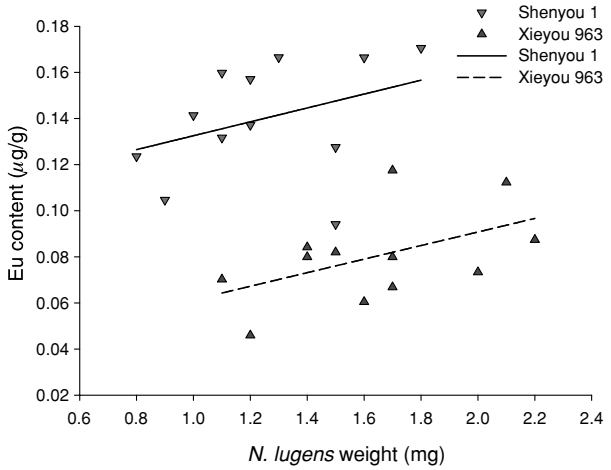

Fig. 5 The relationship between the Eu content in *Nilaparvata lugens* and insect weight (W) on unmarked rice. $Eu = 0.1024 + 0.0301W$, $r = 0.36$, $P > 0.05$ for Shenyou 1, $Eu = 0.0319 + 0.029W$, $r = 0.50$, $P > 0.05$ for Xieyou 963.

use for examining the feeding activity of *N. lugens* or the physiological and biochemical responses of the different rice varieties to *N. lugens* infestation.

The ratios of the Eu content in N. lugens *to that in roots, shoots and leaves of rice plants*

For the two varieties of marked rice, the ratio of the Eu content in *N. lugens* to that in roots, shoots and leaves

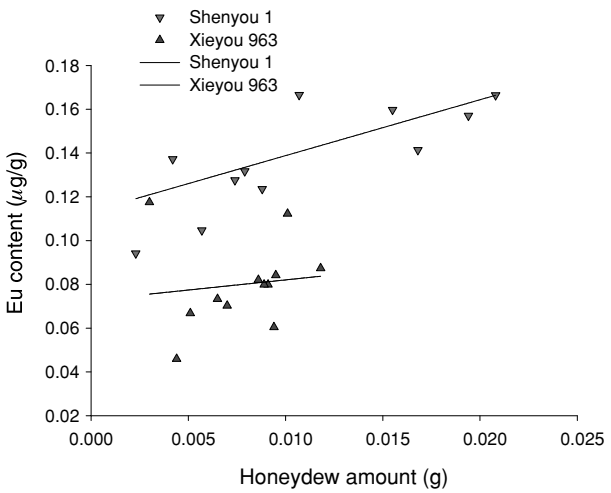

Fig. 6 The relationship between the Eu content in *Nilaparvata lugens* and honeydew amount (g) on unmarked rice. $Eu = 0.1132 + 2.5555H$, $r = 0.63$, $P < 0.05$ for Shenyou 1, $Eu = 0.0728 + 0.9278H$, $r = 0.12$, $P > 0.05$ for Xieyou 963.

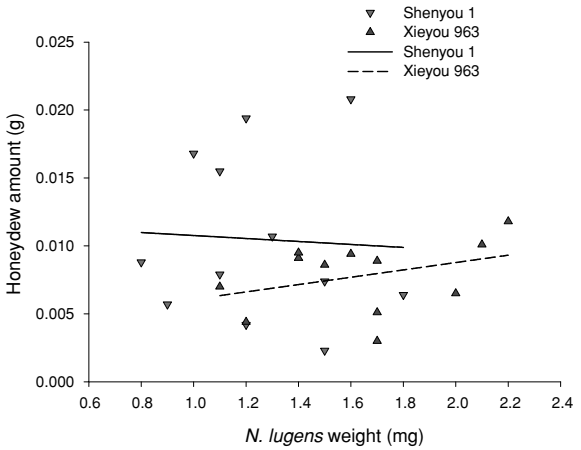

Fig. 7 The relationship between *Nilaparvata lugens* weight (W) and honeydew amount (g) on unmarked rice. $H = 0.0118 - 0.0109W$, $r = 0.053$, $P > 0.05$ for Shenyou 1, $H = 0.0034 + 0.0027W$, $r = 0.35$, $P > 0.05$ for Xieyou 963.

increased with an increase in *N. lugens* density. This ratio was highest for leaves, intermediate for shoots and lowest for roots (Table 3). Values of these ratios were similar for the two varieties of rice. However, for unmarked rice, the ratios showed no similar patterns related to rice variety, plant part or *N. lugens* density.

Discussion

In ecology, trophic relations within food chains are often investigated qualitatively or quantitatively using some

Table 3 Ratios of the Eu content in *Nilaparvata lugens* to the Eu content in roots, shoots and leaves of rice plants.

Treatment	Rice variety	*N. lugens* density	Root	Shoot	Leaf
Marked rice	Shenyou 1	0	0.002 6	0.009 1	0.015
		30	0.004 6	0.013 0	0.025
		60	0.005 2	0.021 0	0.037
	Xieyou 963	0	0.002 7	0.008 6	0.012
		30	0.004 2	0.009 4	0.015
		60	0.006 6	0.015 0	0.020
Unmarked rice	Shenyou 1	0	0.013 0	0.015 0	0.018
		30	0.021 0	0.025 0	0.039
		60	0.016 0	0.023 0	0.043
	Xieyou 963	0	0.008 9	0.010 0	0.012
		30	0.011 0	0.014 0	0.014
		60	0.012 0	0.014 0	0.018

type of marker. However, most markers are radioactive substances, such as uranium, ^{12}C or ^{32}P, which are expensive and environmentally hazardous. The rare earth element Eu has the advantages of high selectivity and recovery rate, ease of use and affordability (Yin *et al*., 2000). Therefore, some investigators have used Eu to study energy flow among rice – *Sogatella furcifera* – *Pirata subpiraticus* and the damage rate of insect pests to rice plants (Wen *et al*., 2001; Hu *et al*., 2002; Lu *et al*., 2002). The present experiment demonstrated that Eu absorbed by roots can be transferred to shoots and leaves and subsequently to insect bodies. Eu uptake was significantly lower in the resistant variety of rice, Xieyou 963, than in the susceptible variety, Shenyou 1. Thus, the Eu marker can be used to study the dynamics of phloem sap uptake by planthoppers and the mechanism of insect pest resistance in rice varieties and has wider applicability to food chain studies.

The amount of honeydew excreted by *N. lugens* is proportional to the amount of phloem sap taken in (Du & Ding, 1991; Huang & Feng, 1993). The present findings demonstrated that the Eu content in the bodies of *N. lugens* was positively correlated with insect weight and the amount of honeydew. Thus, the Eu marker can be used to estimate feeding activity of *N. lugens*.

Our findings also showed that the Eu content in the susceptible variety of rice, Shenyou 1, was significantly higher than in the resistant variety, Xieyou 963. This suggests that physiological and biochemical processes, such as water uptake by roots, assimilation and transport, differ between the resistant and susceptible varieties. Consequently, the Eu content of *N. lugens* fed on the susceptible variety was significantly higher than of those fed on the resistant variety. It has been demonstrated that the amount of phloem sap taken in and the excretion of honeydew by *N. lugens* is significantly higher in insects fed on the susceptible variety than on the resistant variety (Padgham & Woodhead, 1989). The effect of *N. lugens* infestation on the physiology and biochemistry of the resistant variety is different from its effect on the susceptible variety (Wang *et al*., 2006; Liu *et al*., 2008). Therefore, we suggest that an Eu marker may be useful as a screening method for resistant varieties, although more plant varieties must be tested to determine the utility of the method.

This experiment only used two varieties and it is hard to determine if universal differences in Eu contents among rice varieties are related to resistance levels to planthoppers, because the capacity of rice varieties taking Eu might be independent from the level of resistance to planthoppers. In addition, the feeding rate of planthoppers and capacity of rice plants to take in Eu are affected by other environmental factors, such as temperature, crop stage and so on. Therefore, all these factors should be taken into consideration if the result of this experiment is used in practice. Thus, a large sample of varieties and the combined effect of more factors will be further investigated.

Acknowledgments

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