Research Article

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Electrical penetration graph evidence that pymetrozine toxicity to the rice brown planthopper is by inhibition of phloem feeding

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Abstract

BACKGROUND: Pymetrozine is a valuable novel insecticide for control of sucking insects, including the brown planthopper *Nilaparvata lugens* (Stål), one of the most serious pests on rice. This study was conducted to elucidate the action mechanisms of pymetrozine on the feeding behaviour of the planthopper.

RESULTS: The activity test showed that pymetrozine primarily functioned as an antifeedant that caused starvation and death in *N. lugens*, rather than having neurotoxicity. Pymetrozine-treated insects died at a significantly slower speed than insects treated with starvation. Electrical penetration graph (EPG) data indicated that pymetrozine significantly increased the duration of non-probing periods and had a strong inhibition to phloem ingestion. The inhibition was strongly dose dependent, resulting in a complete suppression of the activity in the phloem region when the pymetrozine concentration was increased to 400 mg L^{-1}. Starvation caused by inhibition of phloem ingestion might be a major toxicity mechanism of pymetrozine. EPG data also showed that pymetrozine had no significant effect on stylet movement and duration of xylem sap ingestion.

CONCLUSION: The study revealed that pymetrozine disturbed the feeding behaviour of *N. lugens* mainly by increasing the non-probe period and inhibiting phloem ingestion. The inhibition resulted in a slow death similar to starvation.

Keywords: pymetrozine; *Nilaparvata lugens*; electrical penetration graph; feeding behaviour; phloem; xylem

1 INTRODUCTION

Pymetrozine is a selective insecticide with the basic structure of a pyridine azomethine. It is an effective chemical against plant-sucking insects, such as aphids, whiteflies, leafhoppers and planthoppers.[1] Pymetrozine exhibits a novel mode of action against sucking insects[2] and can be used in integrated pest management programmes.[3] In aphids, pymetrozine treatment results in death after a few days, without showing the classical symptoms of neuronal intoxication. Observation of feeding behaviour in aphids demonstrated that topical application with pymetrozine resulted in immediate feeding inhibition.[2] That study indicated that, when either topically or systemically applied, pymetrozine could quickly inhibit aphid feeding by preventing the insects from inserting their stylets into the plant vascular system.[2] Kaufmann *et al*.[4] suggested that pymetrozine acted via a novel mechanism that might be linked to the signalling pathway of serotonin. Ausborn *et al*.[5] showed that pymetrozine selectively affected chordotonal mechanoreceptors in *Locusta migratoria* (L.) and was probably responsible for the conspicuous lifting and extension of walking legs in the locust. In spite of the wide use of pymetrozine, however, the exact mechanism by which pymetrozine affects feeding behaviour in sucking insects is still unknown.

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is an economically important pest of rice in rice-growing countries and regions in Asia. The damage and economic loss caused by the insect result from direct feeding on rice plant sap and/or transmission of ragged stunt virus and grassy stunt virus.[6] Chemical control is still a major method for suppressing *N. lugens* populations in China. Because *N. lugens* has developed significant resistance to imidacloprid and other conventional insecticides since 2005 in China and other Asian countries,[7,8] it is urgent to screen available alternative insecticides for resistance management. Currently, rice growers in China are starting to apply pymetrozine in rice fields for control of *N. lugens*. Efficacy tests

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in the laboratory and field showed that pymetrozine has a high efficacy and long residual activity against *N. lugens*, but it is a slow-acting insecticide.[9] However, the detailed mode of action of pymetrozine in *N. lugens* has not been thoroughly studied yet.

Electrical penetration graphs (EPGs) have been used as an effective tool to study the feeding behaviours of sucking insects. McLean and Kinsey[10] first developed an alternating current (AC)-based EPG technique to monitor the stylet penetration behaviour of an aphid. A direct current (DC)-based EPG technique was later developed by Tjallingii,[11] which provided more detailed waveforms than the AC-EPG. Several studies have been conducted to evaluate the plant penetration behaviour of *N. lugens* using DC-EPG.[12–16] These studies were useful for explaining the relationship between the stylet penetration behaviour and corresponding EPG waveforms. An especially useful classification was developed by Seo *et al.*[17] that characterised the EPG signals of *N. lugens* on the basis of observations of the actual position of the stylet tips of *N. lugens* within the rice tissue through microsectioning after a laser stylectomy had been conducted during the DC-EPG recording. Currently, the EPG is used to study the effect of pymetrozine on the feeding behaviour of aphids.[2,4]

The major objective of this study was to explore the effect of pymetrozine on the feeding behaviour of *N. lugens*. Results might be helpful in the interpretation of the toxicity mechanism of pymetrozine to *N. lugens*, and may provide valuable information for field application of pymetrozine.

2 MATERIALS AND METHODS

2.1 Insects

Colonies of *N. lugens* used in the study were originally collected in 1995 from a rice field near Hangzhou, Zhejiang Province, China, and were reared continuously on rice seedlings of the TN1 variety in the laboratory without exposure to insecticides. The planthoppers were maintained at 26 ± 1 °C and a 16 : 8 h light : dark photoperiod.

2.2 Pesticides

Pymetrozine 250 g kg^{-1} WP (Jiangsu Anpon Electrochemical Co., Ltd) was diluted with distilled water to a series of concentrations, 0, 1.56, 6.25, 25, 100 and 400 mg AI L^{-1}.

2.3 Toxicity of pymetrozine to *Nilaparvata lugens*

The rice seedling dipping method[18] was adopted for testing the activity of pymetrozine against *N. lugens*. Ten TN1 rice seedlings were planted in a soil pot (7 cm diameter × 6 cm height) (Fig. 1). Each pot was a replicate, and three replicates were used per concentration. The tests used 5–6 concentrations of pymetrozine and a water-only control. The rice seedlings (ca 10 cm high, second leaf stage) were dipped into appropriate insecticide test solutions for 30 s and then air dried for approximately 1 h. The soil surface in each pot was covered with a layer of dry cotton for convenience in checking the mortality of hoppers. A plastic tube (6 cm diameter × 30 cm height) (Fig. 1) made from PVC transparent film was used to surround the rice seedlings, with the bottom inserted into soil and the upper end covered with cheesecloth for air circulation. Ten third-instar nymphs were transferred to the rice seedlings inside each tube. The insects in the starvation treatment were provided with water (water-saturated cotton) only in the tubes (no rice seedling). The treatments and test insects were held at 26 ± 1 °C and a 16 : 8 h light : dark photoperiod. Mortality in the treatments was determined daily. Those insects that fell on their back and were unable to recover normal posture were counted as dead.

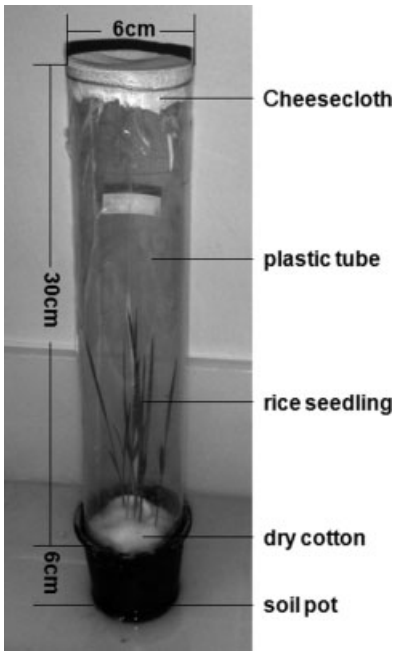

Figure 1. Soil pot with rice seedlings for testing the activity of pymetrozine against *Nilaparvata lugens*.

2.4 Electronic monitoring of the feeding behaviour of *Nilaparvata lugens*

The EPG of *N. lugens* on rice was recorded within a Faraday cage using a Giga-4 DC EPG amplifier with a 10^9 Ω input resistance and an input bias current of less than 1 pA (Wageningen Agricultural University, Wageningen, The Netherlands). The experimental treatments included five concentrations of pymetrozine (1.5625, 6.25, 25, 100 and 400 mg L^{-1}) and a water-only control. Rice seedlings were treated by dipping in different concentrations of pymetrozine, as in the toxicity test. One day after treatment, long-winged females were allowed to settle on the plants for EPG recording. Prior to the experiments, long-winged females were provided water only (water-saturated cotton) for 2 h. One end of a gold wire (20 µm diameter × 10 cm length) was attached to the dorsal thorax of the insect with water-soluble silver conductive glue. The other end of the wire was connected to the amplifier through the EPG probe. A copper wire (2 mm diameter × 10 cm length) was inserted into the soil to serve as the plant electrode. The female attached to the gold wire was then carefully placed onto the stem of the rice plant. The gain of the amplifier was set at 50×, and the plant voltage was adjusted to obtain an output voltage of between −5 and +5 V. EPG recording data were saved in a personal computer. The EPG signals were analysed using the PROBE 3.0 software (Wageningen Agricultural University, Wageningen, The Netherlands). A different, freshly treated plant (with pymetrozine or control) and a different insect were used for each replication. A total of 10–15 recordings were collected for each concentration of pymetrozine treatment and were used for final data analysis. All EPG tests were conducted at 26 ± 1 °C and 70 ± 5% RH under continuous light conditions. To determine suitable EPG recording duration, a preliminary EPG test was conducted by testing planthoppers feeding on untreated plants for 4 and 10 h.

The EPG waveforms from *N. lugens* recorded in the tests were characterised using the correlations found in Seo *et al.*[17] who classified the EPG signals of *N. lugens* into seven distinctive waveforms. The EPG waveforms for the stylet penetration

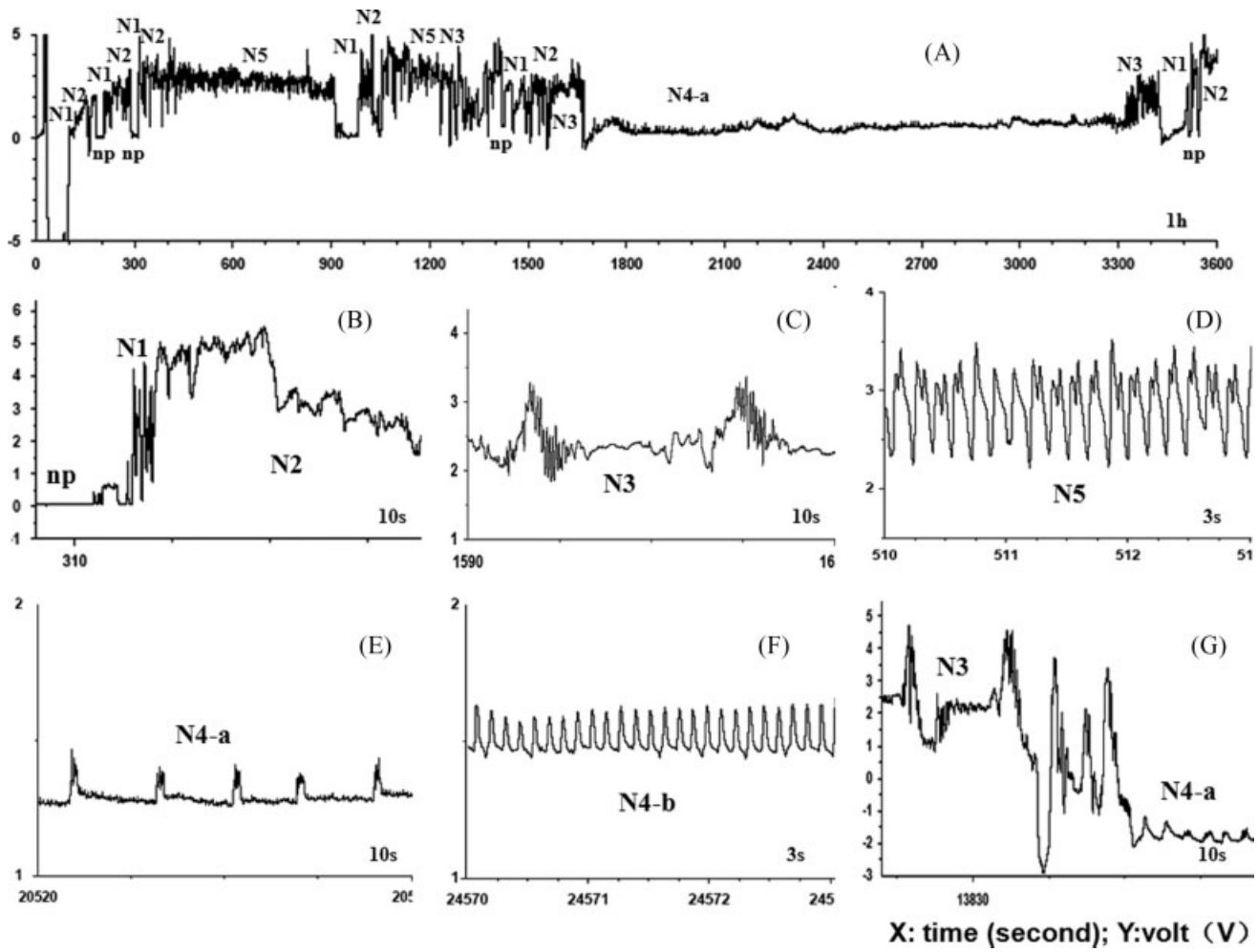

Figure 2. Typical EPG waveforms identified from *Nilaparvata lugens*, showing the general picture during 1 h of recording (A), the characteristic waveform in detail (B to F) and the transients from N3 to N4-a (G). N1, penetration initiation; N2, stylet movement; N3, extracellular activity near the phloem region; N4-a, intracellular activity in the phloem region; N4-b, phloem sap ingestion; N5, activity in the xylem region.

behaviours of *N. lugens* were assigned to the following groups: np, non-penetration; N1, penetration initiation; N2, salivation and stylet movement; N3, extracellular activity near the phloem region; N4-a, intracellular activity in the phloem region; N4-b, phloem sap ingestion; N5, activity in the xylem region (Fig. 2).[17] In addition, an N4 waveform (including N4-a and N4-b waveforms) was used as the activity in the phloem region, and an Nc waveform was used as a pathway period in this study for the combined activities of N2 and N3 waveforms.

EPG waveforms were analysed using a method similar to that of Sarria *et al*.[19] Four parameters of non-sequential variables of each EPG waveform were analysed (see Figs 5–7 and 10), including total duration, average duration, number of occurrences and proportion of each waveform phase in whole recording time. The total duration of each EPG waveform represents the sum of durations of all occurrences of the waveform within the observation time. The mean duration of each EPG waveform was the average duration of each occurrence of a waveform within the observation time. The number of occurrences of each EPG waveform represented the number of times that a waveform occurred during the observation time. The percentage of each waveform phase in all recording time was counted by dividing the total duration of each waveform by the whole recording time. Additional parameters of EPG waveforms (see Fig. 9) were analysed. These sequential variables included the transition period from the start of the experiment to the first probe (N1), the interval between the first and second N1 probing, the number of probes before the first N4, the duration of the first N4, the interval from the first probing to the first N4-a and the interval from the first probing to the first N4-b. Moreover, the percentages of insects reaching the phloem and xylem phases after being treated with water (CK) and different concentrations of pymetrozine were also calculated (see Fig. 8).

2.5 Statistical analysis

EPG data were statistically analysed using SAS.[20] The least significant difference (LSD) procedure was conducted to examine differences in the EPG variables between control (CK) and pymetrozine treatments at different concentrations. Relationships between pymetrozine concentrations (log transformation) and EPG variables were examined using regression analysis. Statistical significance was set at $P < 0.05$. PoloPlus software was used for probit analysis of dose–mortality response data and calculations of LC_{50} values.[21] Graphs were drawn using SigmaPlot.[22]

Table 1. Toxicity of pymetrozine to *Nilaparvata lugens* after different time periods

Time after Treatment (h)	Slope (± SE)	LC_{50} (95% CL) (mg L^{-1})	n^a	χ^2 (df)[b]
24	–	>400	131	–
48	0.96 (±0.25)	461.99 (204.78–3291.94)	131	0.018 (2)
72	1.16 (±0.25)	244.40 (134.11–702.37)	131	0.86 (2)
120	1.49 (±0.27)	116.59 (74.52–204.31)	131	1.47 (2)
168	1.82 (±0.37)	63.94 (42.99–96.71)	131	3.56 (2)
240	1.59 (±0.49)	70.62 (47.60–107.20)	131	7.92 (2)*

[a] Number of insects used.
[b] The χ^2 value followed by an asterisk (*) indicates a poor fit of the data to the probit model ($P < 0.05$).

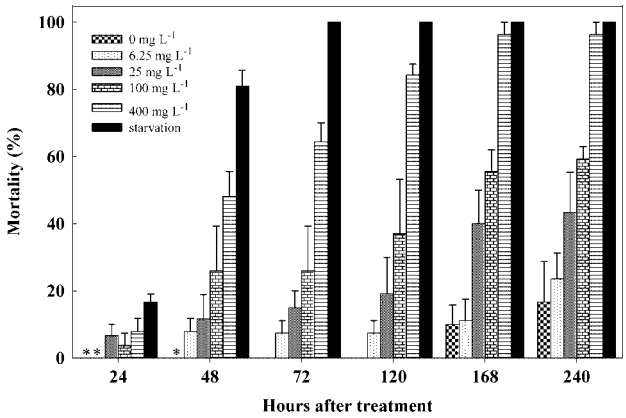

Figure 3. Mortality of third-instar nymphs of *Nilaparvata lugens* treated with pymetrozine or starvation. * indicates 0%.

3 RESULTS

3.1 Activity of pymetrozine WP against *Nilaparvata lugens*

By using the rice seedling dipping method, the toxicity of 250 g kg^{-1} pymetrozine WP to third-instar nymphs of *N. lugens* was compared with control (CK: 0 mg L^{-1} pymetrozine) and starvation treatments (water only, no rice seedling feeding) (Fig. 3 and Table 1). After treatment for 24 h, the mortality of the third-instar nymphs in each pymetrozine treatment at different concentrations was less than 10%, while the mortalities in the CK and the starvation treatment were 0 and 16.67% respectively. After 48 h, the mortality in each pymetrozine treatment was less than 50%, while the mortalities in the CK and the starvation treatment were 0 and 80.95% (Fig. 3). The LC_{50} values of pymetrozine to *N. lugens* at 24 h and 48 h were greater than 400 mg L^{-1}. At 72 h after treatment, the mortality in the CK remained 0%, and the mortality in the starvation treatment reached 100%. The 72 h mortality in the highest concentration (400 mg L^{-1}) of pymetrozine treatment was only 64.0%, and the LC_{50} value of pymetrozine was 244.40 mg L^{-1}. After 120 h, the mortality in CK was still 0%. The LC_{50} value (116.59 mg L^{-1}) of pymetrozine was approximately half that at 72 h. At 168 h after treatment, the mortality of CK slightly increased to 11.54%. The LC_{50} value was 63.94 mg L^{-1}, which was 3.8-fold less than that at 72 h. At 240 h after treatment, the mortality in the CK was less than 20%, while the mortality in the 400 mg L^{-1} pymetrozine treatments reached 96%. The 240 h LC_{50} value was 70.62 mg L^{-1}, but the toxicity data were not in good fit to the probit model ($\chi^2 = 7.92$, df = 2). The bioassay data showed that pymetrozine was slow acting against *N. lugens* and was significantly slower than the starvation in causing mortality.

3.2 EPG and feeding behaviour in pymetrozine-treated *Nilaparvata lugens*

Based on multiple observations of the probing and feeding waveforms of *N. lugens* on untreated rice seedlings for 4 or 10 h, it was confirmed that all of the valid recordings (14/14, 100%) had at least one sustained phloem ingestion waveform (N4 >10 min) during the 4 h recording period. When the recording period was extended to 10 h, the total durations of the np, N3 and N4 waveforms increased significantly, while the durations of the N1, N2 and N5 waveforms in the 4 h period were not significantly different from their durations in the 10 h period (Fig. 4). Thus, the 4 h EPG recording period was long enough to observe and quantify the distinctive waveforms (np, N1, N2, N3, N4 and N5) of *N. lugens* with different treatments.

If the planthopper was motionless during the np waveform period, the voltage level remained at nearly zero to form a flat line. If the insect walked on the rice seedling, an irregular fluctuating waveform was produced. During the 4 h recording period, the total and average durations of the np waveform of the CK were 37.1 ± 6.8 min and 3.8 ± 1.2 min respectively, much shorter than those on treated plants dipped with pymetrozine ($P < 0.05$) (Figs 5A and 6A). The numbers of np occurrences were not significantly different between the control and each pymetrozine treatment ($P = 0.28$) (Fig. 7A). Regression analysis revealed a significant linear relationship between total or average duration of the np waveform and pymetrozine concentrations ($P < 0.01$, $R^2 = 0.84$ or 0.82). The durations of np waveforms increased as pymetrozine concentrations increased (Figs 5A and 6A).

The N1 waveform, defined as penetration initiation activity, indicated that the insect had begun to pierce the plant tissue with its stylets. The durations of the N1 waveform were always very short, with a mean value of no more than 0.1 min. Results indicated that the insects on pymetrozine-treated plants and the insects in the CK showed similar activities for the numbers of N1 occurrences ($P = 0.20$) (Fig. 7B).

During the N2 period, it is presumed that the insect extends its mouthparts to the vascular bundle for feeding, which includes penetration of plant cells and salivating. The N3 waveform occurred as the tips of the stylets reached a location near the extracellular region of the phloem, and ended as the stylets moved towards the phloem. In this study, the Nc waveform was used as the pathway period to represent combined N2 and N3 waveforms. Results showed that significant differences existed in the number of N3 occurrences between CK and some pymetrozine treatments at 6.25, 25 and 400 mg L^{-1} ($P = 0.0001$) (Fig. 7C). However, there was no significant difference in the total duration of the Nc waveform among all of the treatments ($P = 0.13$) (Fig. 5B), and a good fit to either linear or exponential model could not be established (linear: $P = 0.13$, $R^2 = 0.48$; exponential decay: $P = 0.13$, $R^2 = 0.48$).

During the 4 h recording period, not all insects showed an N4-a waveform (intracellular activity in the phloem region) or N4-b waveform (phloem sap ingestion). The percentage of tested insects that reached the N4-a period appeared to be negatively related to the concentration of pymetrozine (Fig. 8), although this relationship did not significantly fit the linear or exponential decay models (linear: $P = 0.13$, $R^2 = 0.59$; exponential decay: $P = 0.14$, $R^2 = 0.57$). A similar decreasing tendency was also

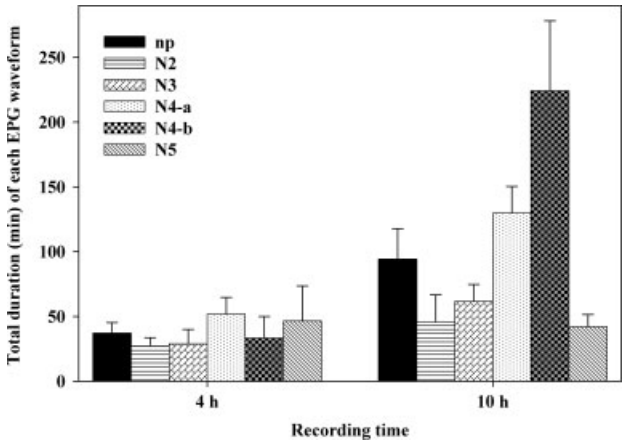

Figure 4. The total duration (min) of each EPG waveform of *Nilaparvata lugens* on untreated rice seedlings during a 4 or 10 h recording period.

observed in the percentage of tested insects that reached the N4-b period (linear: $P = 0.09$, $R^2 = 0.83$; exponential decay: $P = 0.11$, $R^2 = 0.79$) (Fig. 8). Compared with the control, analyses of the total and average durations showed a significant reduction in the N4-a waveform in all pymetrozine treatments at different concentrations ($P < 0.0001$; $P = 0.0055$) (Figs 5D and 6B). In addition, the numbers of the N4-a waveform in all five pymetrozine treatments (from high to low concentration) were 0.0 ± 0.0, 1.0 ± 0.5, 1.6 ± 0.9, 0.9 ± 0.4 and 2.6 ± 1.4 respectively (Fig. 7D), which were significantly lower ($P < 0.0001$) than that of the control (7.6 ± 1.8). Similarly, significant differences were detected in the total duration ($P < 0.0001$) (Fig. 5E), the average duration ($P < 0.001$) (Fig. 6C) and the numbers of N4-b occurrences ($P < 0.0005$) (Fig. 7E) between CK and the pymetrozine treatments at different concentrations. When the pymetrozine concentration was increased to 100 and 400 mg L^{-1}, no N4-b waveform was detected in any treated planthoppers. Regression analyses showed significantly negative relationships between log dose of pymetrozine and the EPG parameters, including the total durations (Figs 5D and 5E), the average durations (Figs 6B and 6C), the maximum durations (Figs 6D and 6E), the number of occurrences (Figs 7D and 7E) of the N4-a and N4-b periods and the total duration of the phloem phase N4 ($P = 0.0001$, $R^2 = 0.996$) (Fig. 5F).

When the planthopper starts xylem ingestion activity, the EPG exhibits the typical N5 waveform (Fig. 2D). Results showed that all tested insects reached the xylem region (Fig. 8), except for two planthoppers that failed to show N5 after being treated with pymetrozine at 400 mg L^{-1}, the highest concentration tested in this study. During the 4 h recording period, the total duration of the N5 waveform was not significantly different between the CK and pymetrozine treatments ($P = 0.96$) (Fig. 5C). Although significant differences existed in the average durations ($P = 0.0056$) (Fig. 6F) and the numbers of occurrence of N5 ($P = 0.0028$) (Fig. 7F) between CK and some pymetrozine treatments at 6.25, 25 and 400 mg L^{-1}, the dose response did not reach a good fit to either the linear ($P = 0.28$ and 0.11, $R^2 = 0.28$ and 0.51) or the exponential model ($P = 0.19$ and 0.13, $R^2 = 0.38$ and 0.48).

In addition, six sequential variables were analysed and compared with the control (Figs 9A–F). Results showed no significant difference ($P > 0.05$) among the treatments for the transition time from the starting point to the first N1 (Fig. 9A) and from N1 to the first N4-a (Fig. 9E) or N4-b (Fig. 9F) and the number of probes before the occurrence of the first N4-a (Fig. 9D). Similarly, no significant linear or exponential regression relationship was detected between log dose of pymetrozine and the interval from the first to the second N1 probe (Fig. 9B), although this variable was much higher than that of CK ($P = 0.04$) (Fig. 9B) when the insects were treated with 400 mg L^{-1} of pymetrozine. Analysis of the first N4 period revealed a significant exponential correlation (Fig. 9C) between log dose and the first N4 period ($P = 0.019$, $R^2 = 0.88$). The planthoppers treated with higher concentrations of pymetrozine might take longer to reach the phloem region (from N1 to N4-a) (Fig. 9E), but the differences among the treatments were not significant ($F = 0.62$, $P = 0.65$). Pymetrozine at two high concentrations (100 and 400 mg L^{-1}) could totally suppress N4-b activity (Fig. 9F). Compared with the CK, the other three low concentrations of pymetrozine had no significant effect on the transition interval from N1 to N4-b ($F = 0.85$, $P = 0.49$).

Results (Fig. 10) showed that, as the pymetrozine concentrations increased, the percentage of the np periods in all the recording time (4 h) showed a significant increase. The results also showed that pymetrozine had a significantly negative effect on N4 periods, while the percentage of the Nc and N5 periods were not significantly influenced by different concentrations of pymetrozine (Fig. 10).

4 DISCUSSION

Pymetrozine kills sap-sucking insects by a novel mode of action through interference with their feeding behaviour.[2] In this study, feeding data (Figs 5 to 10) obtained from EPG analysis indicated that the feeding behaviour of *N. lugens* on pymetrozine-treated rice plants was significantly inhibited. One such influence was the significant increase in np period. Detection of linearly dose-dependent correlation in this study suggested that planthoppers on pymetrozine-treated plants, especially with high chemical concentrations, spent more time resting or were not as active in feeding as those on plants treated with water only. Because the numbers of np occurrence, the waiting time for the first np and the interval between the first and second probes were not dose dependent or significantly altered by pymetrozine, further analysis was made of the N4 duration, the time related to phloem ingestion, and a significant decrease in N4-a and N4-b activities in pymetrozine treatment was found. The negative effect on N4 activities was exponentially dose dependent. Further, a significant drop in the number of test insects that were able to reach the phloem phase as the pymetrozine dose increased was detected, and hence a significant reduction in N4-a and N4-b occurrences and in the duration of phloem activities within the 4 h test period.

Sap-sucking insects are classified as phloem, xylem or mesophyll cell feeders.[23] Planthoppers are phloem sap feeders.[23] As with the phloem-feeding insects, ingestion of xylem sap has been observed occasionally,[24,25] and in aphids the xylem sap ingestion substantially increased after a period of starvation.[26] In contrast to phloem ingestion, xylem ingestion does not seem to be essential for nutrition, but it might be necessary for compensating for water stress. Therefore, xylem sap ingestion should be considered as 'drinking' rather than feeding.[27] In this study, the characterisation of the N5 waveform, representing the activity in the xylem region, based on the definition of Seo *et al.*,[17] agreed with other reports[13–16] that clearly defined this waveform type as the xylem ingestion waveform. Therefore, the present EPG data could well explain the bioassay data which showed that the pymetrozine-treated planthoppers survived longer than starved planthoppers.

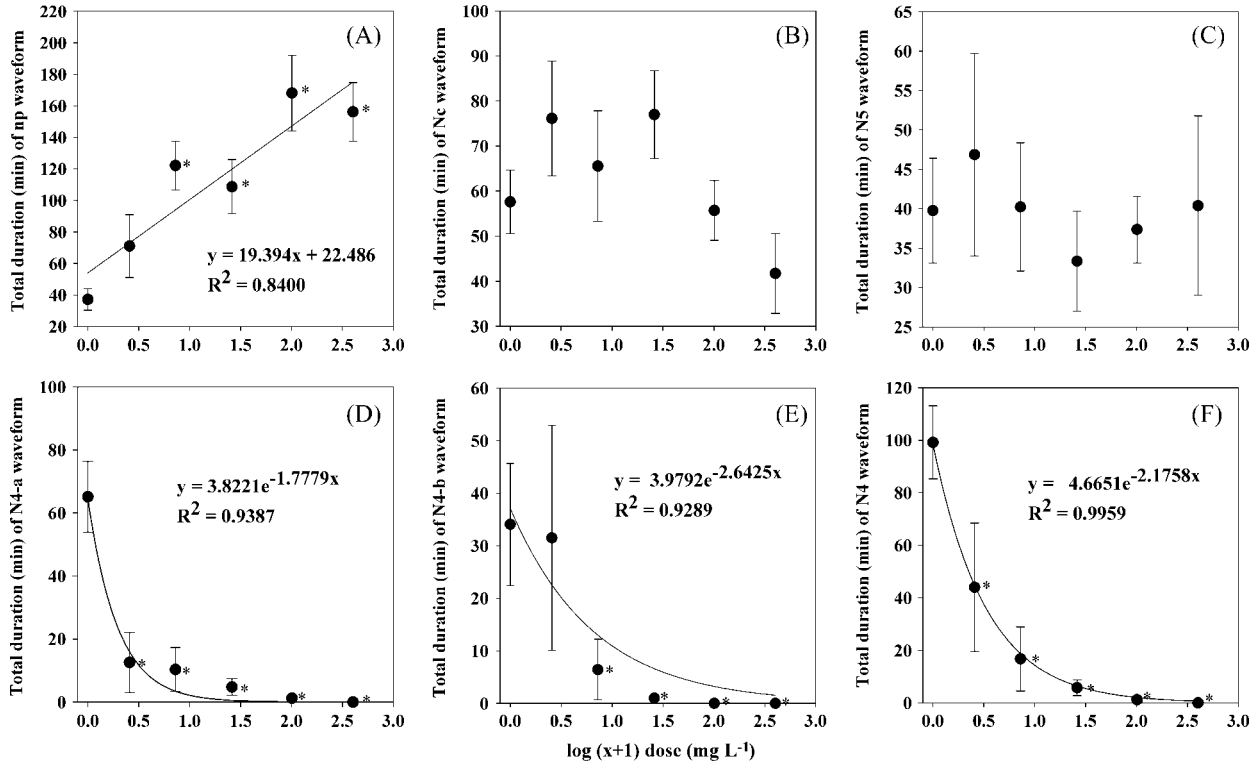

Figure 5. Relationship between pymetrozine concentrations (log) and the total duration of each EPG waveform, including the total duration of the np waveform (A), pathway waveform Nc (B), N5 waveform (C), N4-a waveform (D), N4-b waveform (E) and N4 waveform (F). Each data point is presented as the mean ± SE. An asterisk (*) by a data point denotes a significant difference between the CK and this value ($P < 0.05$).

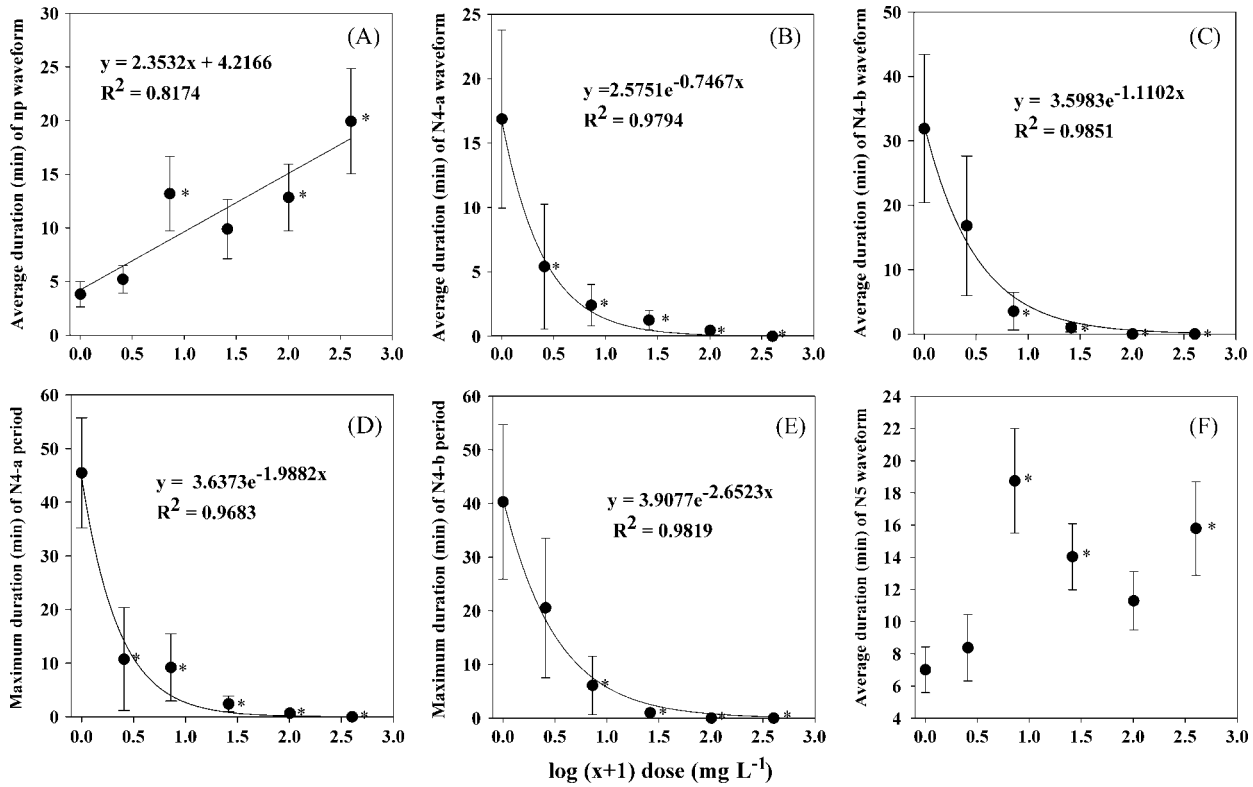

Figure 6. Relationship between pymetrozine concentrations (log) and the average or maximum duration of EPG waveforms, including the average duration of the np waveform (A), the average duration of N4-a (B), the average duration of N4-b (C), the maximum duration of N4-a (D), the maximum duration of N4-b (E) and the average duration of N5 (F). Each data point is presented as the mean ± SE. An asterisk (*) by a data point denotes a significant difference between the CK and this value ($P < 0.05$).

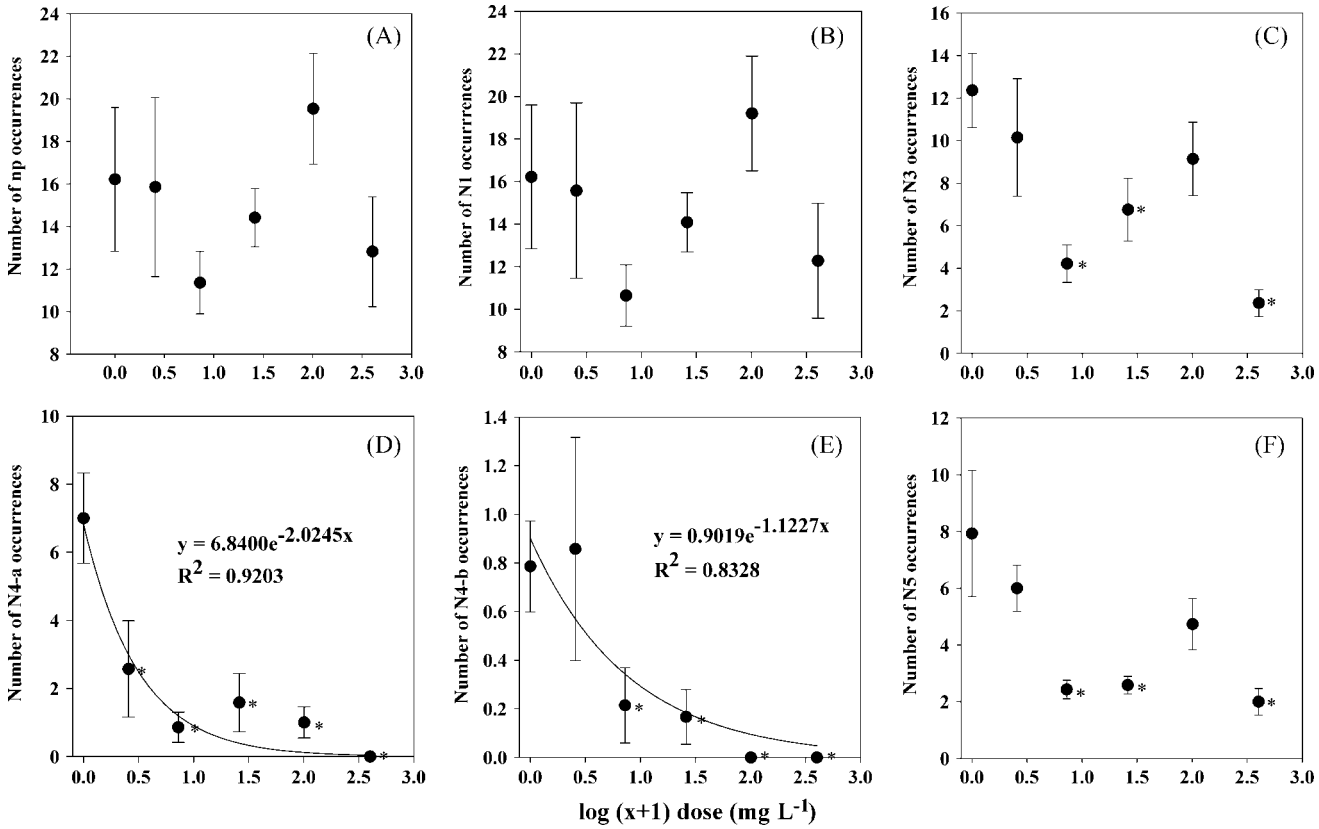

Figure 7. Relationship between pymetrozine concentrations (log) and the number of occurrences of each EPG waveform, including the number of np waveforms (A), N1 waveforms (B), N3 waveforms (C), N4-a waveforms (D), N4-b waveforms (E) and N5 waveforms (F). Error bars indicate ± SE. An asterisk (*) by a data point denotes a significant difference between the CK and this value ($P < 0.05$).

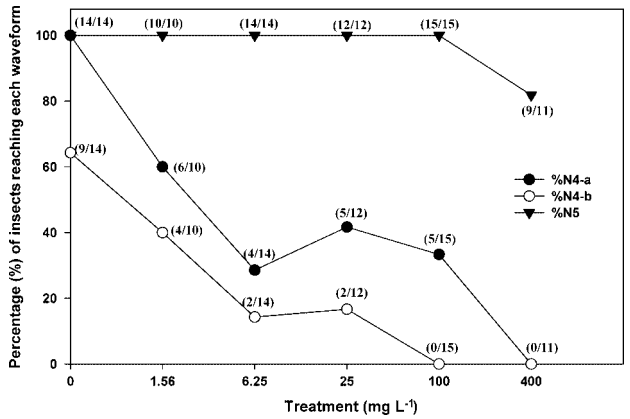

Figure 8. The proportions of insects reaching the phloem and xylem phases after being treated with water (CK) and different concentrations of pymetrozine. The numbers in parentheses by each data point denote the number of insects reaching N4-a, N4-b or N5 waveforms/the number of total valid replicates.

Although phloem feeding was significantly reduced, pymetrozine-treated *N. lugens* maintained comparable durations of the N5 waveform, suggesting that 'drinking' of xylem sap remained at a level similar to that observed in the control. Thus, the pymetrozine-treated planthoppers, even without phloem sap feeding, were still able to rely on xylem sap ingestion to maintain water balance. Limited nutrients in the xylem sap could keep the insect alive for a while and delay death. This phenomenon could explain why the death of pymetrozine-treated *N. lugens* was significantly slower than the death of those treated with starvation (Fig. 3).

The present findings on the effect of pymetrozine on the feeding behaviour of *N. lugens* could help in further exploration of the target of the action of pymetrozine against plant-sucking insects. Based on the observations, it was speculated that stylet penetration of *N. lugens* was not suppressed on a pymetrozine-treated rice seedling. If the N5 waveform is presumed to be the ingestion waveform of xylem sap, the function of forcing xylem sap through the insect's food canal was normal when feeding on pymetrozine-treated plants, indicating that the food canal was not blocked to any degree. Pymetrozine-treated plants might be distasteful to the planthopper. Further studies are needed to examine any influence on the plant by pymetrozine. Several studies have suggested that pymetrozine is a neuroactive insecticide, but its site of action in the nervous system is unknown.[2,4,5] Harrewijn and Kayser[2] concluded that pymetrozine does not have a general toxic effect on aphids but selectively interferes with the nervous regulation of feeding behaviour. Kaufmann *et al*.[4] revealed that the mechanism by which pymetrozine affected the central and/or peripheral nervous systems might be linked to the signalling pathway of serotonin. The 5-HT, like pymetrozine, inhibited stylet penetration and strongly enhanced the action of pymetrozine in *Myzus persicae* Sulzer.[4] In contrast to sucking insects, *Locusta migratoria* responded to pymetrozine with unique symptoms. In addition to feeding cessation, the insect also showed lifting and stretching of its hindlegs.[5] These studies indicated that the novel mechanism of pymetrozine was related to the neuromuscular system.

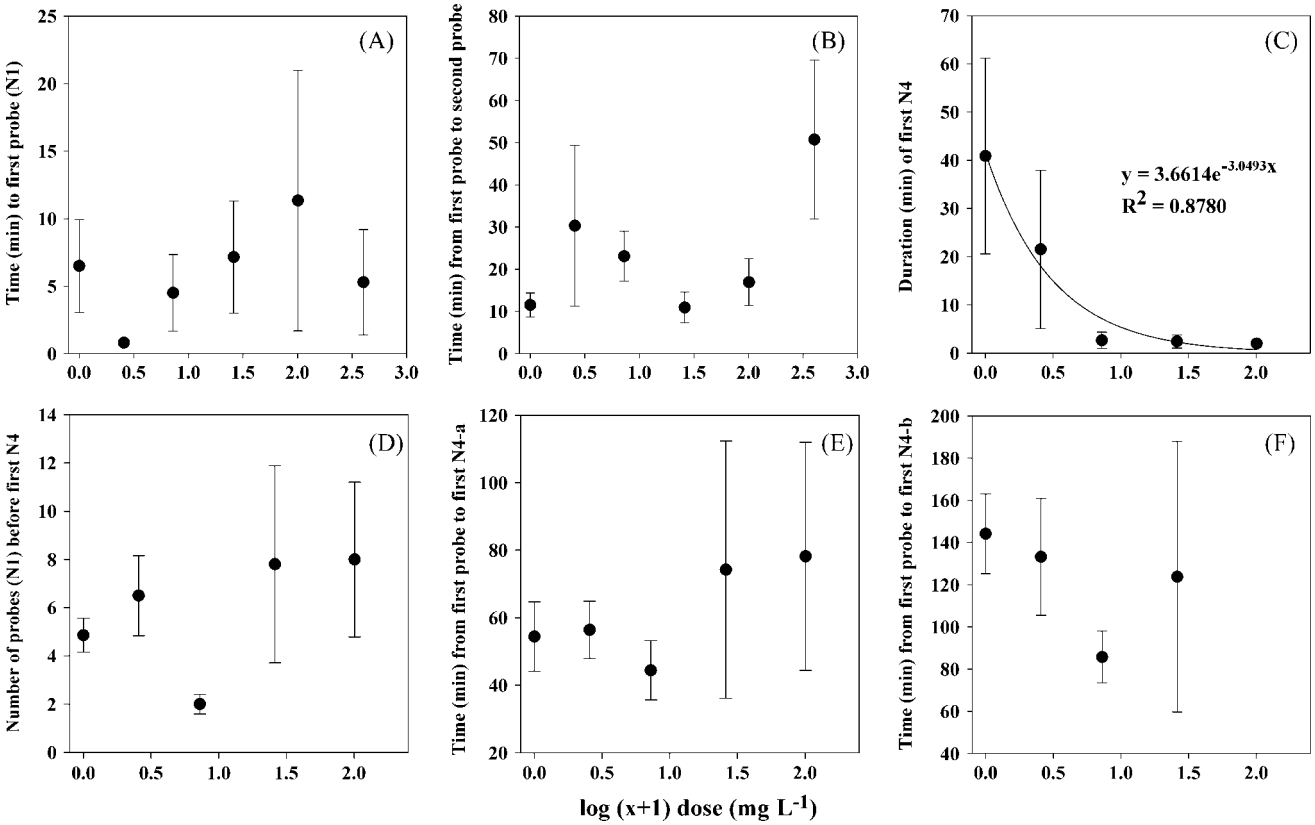

Figure 9. Relationship between pymetrozine concentrations (log) and sequential variables of each EPG waveform, including the time from the start of the experiment to the first probe (A), the interval from the first probe to the second probe (B), the duration of the first N4 (C), the number of probes before the first N4 (D), the time from the first probe to the first N4-a (E), and the time from the first probe to the first N4-b (F). Error bars indicate ± SE.

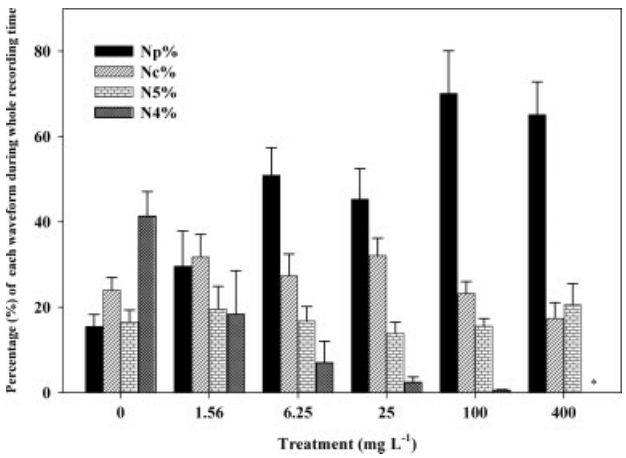

Figure 10. Percentage of each waveform phase in the whole recording time (4 h) after the pymetrozine treatments at different concentrations. An asterisk (*) indicates that 0% of the insects reached the corresponding EPG waveform.

Based on the present observations, it is suggested that the inhibition mechanism of pymetrozine to *N. lugens* might be related to regulation of phloem-feeding behaviour. The information on pymetrozine toxicity from this study may be useful for future studies to determine the concrete mechanism by which pymetrozine acts against sucking insects. The authors intend to design an experiment to examine whether pymetrozine directly acts on insect salivary secretion or indirectly acts on insect behaviour through alteration of food tastefulness. In view of the cost factor, a formulation of pymetrozine was used in this study to simulate field application, and consideration of the potential side effects from additives of this formulation might be valid. The authors plan to use technical-grade pymetrozine to eliminate the potential side effects of formulation reagents in future studies in order to gain an accurate understanding of the mode of action of pymetrozine against planthoppers. Besides the rice seedling dipping, other application methods, such as topical application and injection, will be used for further verification and understanding of the biological meaning of some waveforms, such as N4-a and N5 waveform types.

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