Knockdown of TRPV Genes Affects the Locomotion and Feeding Behavior of *Nilaparvata lugens* (Hemiptera: Delphacidae)

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Abstract

The vanilloid-type transient receptor potential (TRPV) channel is reported to be the molecular target of the commercial insecticide pymetrozine, which specifically disrupts the feeding of plant sap-sucking insects. However, the functions of TRPV channels in plant sap-sucking insects have not been fully elucidated. In the present study, RNA interference was used to investigate the effects of the knockdown of TRPV genes (*Nan* and *lav*) on the mortality, locomotion, and feeding behavior of an important plant-feeding insect pest in rice, the brown planthopper, *Nilaparvata lugens*. Injecting dsRNA of *Nan* and *lav* into fourth-instar nymphs significantly knocked down the target genes. The injection of ds*nan* or ds*lav* did not affect any morphological phenotype (including leg extension) of *N. lugens* nymphs and adults. Knockdown of *Nan* or *lav* resulted in significantly decreased climbing activity against top plants but did not influence the leg-gripping strength of adults. Knockdown of *Nan* resulted in a significantly elevated mortality of *N. lugens* in the observation period of 7 d after injection, whereas no significant difference in survival rates 7 d after injection was found between ds*lav*-injected and ds*GFP*-injected insects. Electropenetrographic (EPG) recordings indicated that knockdown of *Nan* and *lav* reduced the ingestion activity in the rice phloem tissues of *N. lugens*. Knockdown of *Nan* and *lav* significantly reduced the amount of honeydew excreted by *N. lugens*. Our findings indicated a relationship between TRPV and *N. lugens* locomotion and feeding behavior, which may help to fully elucidate the functions of TRPV in insects.

Key words: transient receptor potential, *Nilaparvata lugens*, RNAi, feeding behavior, electropenetrography

Recent research discovered a unique, novel insecticide target, the vanilloid-type transient receptor potential (TRPV) channel, which is the binding target of three insecticides, pymetrozine, pyrifluquinazon, and afidopyropen (Nesterov et al. 2015, Kandasamy et al. 2017, Wang et al. 2019). Nesterov et al. (2015) showed that pymetrozine and pyrifluquinazon activate the *Drosophila* TRPV channel complex, encoded by *Nanchung* (*Nan*) and *Inactive* (*lav*) genes, and silence antennal chordotonal stretch receptor organs that are essential for hearing and gravity sensation. It was found that pymetrozine can also activate Nan-*lav* channels of plant sap-sucking insects, *Acyrthosiphon pisum* (Hemiptera: Aphidoidea) and *Nilaparvata lugens* based on an in vitro cell assay (Kandasamy et al. 2017, Wang et al. 2019). These insecticides were thought to selectively block feeding of plant-sap sucking insects, such as aphids, whiteflies, and planthoppers (Harrewijn and Kayser 1997, Fuog et al. 1998, He et al. 2011a, Raj Boina et al. 2011, Kang et al. 2012, Leichter et al. 2013, Maienfisch 2019). However, there are few reports describing the functions of TRPV genes in plant sap-sucking insects. In this study, the effects of knockdown of *N. lugens* TRPV genes on mortality, locomotion, and feeding behavior were investigated.

The brown planthopper, *N. lugens*, is an important rice pest in Asian countries. *N. lugens* causes severe yield reduction of rice and significant economic loss due to its ingestion of rice plant sap and transmission of plant viruses (Sogawa 1982). Chemical control is a common method to manage *N. lugens* populations in China and other Asian countries. Since 2005, pymetrozine has been widely used for *N. lugens* control in China. Recently, it was reported that field populations of *N. lugens* have developed high-level resistance to pymetrozine (Wu et al. 2018). Therefore, there is an urgent need to understand the toxicity mechanism of pymetrozine to *N. lugens* and the resistance mechanism of the insect to pymetrozine.
Materials and Methods

Insect
The tested planthoppers were collected in paddy fields in Huazhong Agricultural University, Wuhan, China, and were reared continuously on rice seedlings of the Taichung Native 1 (TN1) variety in the laboratory at 28 ± 0.5°C, 70% ± 10% humidity, and a photoperiod of 14:10 (L: D) h.

RNA Extraction and cDNA Synthesis
Total RNA was isolated from the heads of *N. lugens* adults using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. RNA purity was checked using a NanoPhotometer spectrophotometer (IMPLEN, Westlake Village, CA). First-strand cDNA was synthesized from 2-μg total RNA using Transcript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, China) according to the manufacturer’s instructions.

RNA Interference
The cDNA fragments of *N. lugens* Nan (KX249697), *N. lugens* Iav (KX249698), and the green fluorescent protein (GFP) gene were amplified by polymerase chain reaction (PCR) using primers containing the T7 RNApolymerase promoter (Supp Table 1 [online only]). The products were gel purified and used as templates to synthesize dsRNA, using the MEGAscript T7 High Yield Transcription kit (Thermo Fisher Scientific, Waltham, MA). The resulting dsRNAs were dissolved in ultrapure water, and the quality and concentration were determined by agarose gel electrophoresis and a Nanodrop 2000 spectrophotometer. DsRNA injection was performed on fourth-instar nymphs of *N. lugens*. Specifically, 150 ng of dsRNA (5 μg/μl) of Nan, Iav, or GFP was injected into the junction of the prothorax and mesothorax of each fourth-instar nymph following the protocol described by Liu et al. (2015). Surviving numbers and morphology of tested insects on TN1 rice seedlings were recorded at 24-h intervals for 7 d. The treated nymphs were first placed in Petri dishes with moist TN1 rice seedlings for 1 d of recovery, and 15 healthy nymphs were subsequently transferred into a plastic cup (3-cm bottom diameter × 25-cm length) with 10 TN1 rice seedlings. Each treatment was repeated three times. Petri dishes, plastic cups, and feeding chambers with treated nymphs were placed at 26 ± 1°C, 70% ± 10% RH, and a photoperiod 14:10 (L: D) h.

Analysis of RNAi Efficiency
The interference efficiency in the entire body was checked using quantitative real-time PCR (qRT-PCR): five insects that were selected randomly at the seventh day after injection of dsRNA for subsequent RNA extraction and cDNA synthesis. Three biological replicates were conducted in each treatment. The qRT-PCR assay was performed on an ABI Prism 7300 (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq (Takara Biotecno Corporation Co. Ltd, Dalian, China). Nlactin1 (GenBank EU179846.1) was used as an internal standard to normalize cDNA concentrations. The primers for qRT-PCR are presented in Supp Table 1 (online only). Quantitative reactions were performed on three technical replicates. The expression level of the target gene was normalized using the 2^(-ΔΔCt) method (Livak and Schmittgen 2001).

Morphology Observation and Behavioral Assays
The morphological phenotypes of *N. lugens* nymphs and adults after the dsRNA injection were examined. The images of 30 insects 7 d after dsRNA injection were recorded and the angle of hindleg femur-tibia joint was measured by Photoshop CS 8.0.1 version (Adobe Systems, Inc, San Jose, CA) based on each image.

Climbing assays were conducted by the modified method according to the method reported by Nesterov et al. (2015). Ten treated adults (6–8 d after dsRNA injection) were released close to the plants. When 10 adults had climbed on the plants, the vial was slowly inverted. Insects that dropped from the plants were counted immediately. Two biological replicates and each biological replicate consisted of three technique replicates were conducted in each treatment. Gripping scores were calculated as the percentages of the insects gripping onto the plants.

Feeding Behavior and Honeydew Excretion
To measure the effect of knockdown of Nan and Iav on feeding of *N. lugens*, DC electropenetrography (EPG) was used to monitor feeding behavior of individual dsRNA-treated female adult on a susceptible TN1 rice plant. EPG was recorded in a Faraday cage using a Giga-4 DC EPG system with a 107 Ω input resistance and an input bias current of <1 pA (Wageningen Agricultural University, Wageningen, the Netherlands). One TN1 rice plant in the tillering stage was placed in a glass tube, with the whole root being dipped in water, within which a plant electrode of a copper wire (2-mm diameter × 10-cm length) was inserted. One *N. lugens* female, which had emerged after 2–4 d (6–8 d after dsRNA injection) was connected to an insect electrode via the thoracic notum using a gold wire (Ø 18 μm, Wageningen Agricultural University) and a silver conductive glue (Wageningen Agricultural University) and was then carefully placed onto the plant stem. All EPG tests were conducted at 26 ± 1°C and 70 ± 10% RH under continuous light conditions for 6 h. Eighteen replicates were recorded for each treatment and used for final data analysis.

The EPG signals were analyzed using PROBE 3.0 software (Wageningen Agricultural University). The EPG waveform from recordings on plants were classified into seven types according to the categories: np for nonpenetration, Nc (N1 + N2 + N3) for pathway phase (including penetration initiation (N1), styte movement and salivation (N2), and extracellular activity near the phloem region [N3, also known as the X wave]), N4 (N4a + N4b) for stylets in the phloem tissue (including an intracellular activity [N4a], and sustained phloem sap ingestion [N4b]) in the phloem tissue termed phloem ingestion phase, and N5 for stylets in the xylem tissue termed putative xylem ingestion phase (Seo et al. 2009, He et al. 2011a). EPG waveform variables (including nonsequential and sequential variables) were analyzed using the same method as reported by He et al. (2011a). Two nonsequential response variables were calculated similarly to those in Backus et al. (2007). The number of waveform events per insect (NWEI) was the number of times that a given waveform appeared during the recording time per individual. The waveform duration per insect (WDI) was the sum of the durations of all
Knockdown of Nan and Iav Genes Affects the Climbing Behavior of N. lugens

The injection of dsNan and dsIav did not influence any morphological phenotype of N. lugens nymphs or adults (Supp Fig. 1 [online only]). No significant difference in the angle of hindleg femur-tibia joint was found between dsGFP-injected group and dsNan- or dsIav-injected group ($F_{1,18} = 1.81, P = 0.166$; Supp Fig. 1 [online only]).

Climbing assay demonstrated that knockdown of Nan or Iav resulted in significantly decreased climbing activity from the tube bottom up rice plants that were inverted on the tube top ($P < 0.05$; Fig. 3A and B). The climbing score was no more than 30% during 9 h in the dsNan- or dsIav-injected group, whereas higher than 70% after 8h in the dsGFP-injected group. However, knockdown of Nan or Iav did not influence the leg-gripping strength of adults (Fig. 3C). Almost 100% of adults from dsGFP-, dsNan-, or dsIav-injected group can grip the plants and not drop down, after the tube was gently inverted ($Q^2_{2,17} = 0.88, P = 0.6432$; Fig. 3C).

Knockdown of Nan and Iav Genes Affects the Feeding Ingestion and Honeydew Excretion of N. lugens

After suppressing the expression level of Nan and Iav, the activities in the phloem ingestion phase of N. lugens female adults were significantly inhibited when fed TN1 rice plants (Fig. 4; Tables 1 and 2). All dsGFP-injected individuals reached the phloem tissues (N4-a and Na-b), whereas approximately 30% of dsNan- or dsIav-injected adults did not (Fig. 4). The dsNan- or dsIav-injected individuals that reached the phloem still spent significantly less time in phloem phase than did the dsGFP-injected insects (Tables 1 and 2). During the 6-h recording period, the mean waveform duration per insect (WDI) for dsNan-injected individuals was significantly lower than (only 23.6% of) that in the dsGFP-injected group ($F_{1,30} = 2.04, P = 0.001$; Table 1). The dsNan-injected individuals also performed significantly fewer events (NWEI) in phloem tissues than did the dsGFP-injected insects during the 6-h recording period ($F_{1,30} = 0.81, P = 0.023$; Table 1). In contrast, the dsNan-injected individuals performed more activities in xylem tissues: all dsNan-injected individuals reached the xylem tissues (Fig. 4), performed more events in xylem issues ($F_{1,31} = 4.56, P = 0.008$; Table 1), and spent significantly more time ingesting xylem sap than did the dsGFP-injected insects ($F_{1,31} = 11.2, P = 0.004$; Table 1). Moreover, WDI values for the nonpenetration phase and pathway waveforms were significantly increased after dsNan injection ($P < 0.05$; Table 1).

In the dsIav-injected group, WDI for the phloem phase was significantly less, only 63.7% of that in dsGFP-injected insects ($F_{1,30} = 4.25, P = 0.048$; Table 2). NWEI for xylem phase of dsIav-injected individuals was significantly fewer than those of dsGFP-injected individuals ($F_{1,30} = 11.3, P = 0.002$; Table 2). Compared with the dsGFP-injected insects, the dsIav-injected individuals showed a higher NWEI value for xylem phase and a higher WDI value for pathway phase ($P < 0.05$; Table 2).

In N. lugens, the amount of food intake is directly proportional to the amount of honeydew excretion. Knockdown of Nan and Iav significantly reduced the amount of honeydew excreted by female adults of N. lugens after feeding on a TN1 plant for 24 h (34.0 and 24.5% of that in the dsGFP-injected group, respectively, $P < 0.05$; Fig. 5).

**Results**

High Mortality After Suppressing Nan Rather Than Iav

The expression of Nan and Iav genes was effectively knocked down using RNAi technology. The results showed that after dsRNA injection of Nan into fourth-instar nymphs, the transcript level of Nan 7 d after injection was reduced by 75.8% ± 11.6% ($F_{1,5} = 28.34, P = 0.006$; Fig. 1) compared with the control in which dsGFP was injected. The injection of Iav dsRNAs into fourth-instar nymphs resulted in an 46.1 ± 9.8% decrease in transcript levels ($F_{1,3} = 14.78, P = 0.018$; Fig. 1) compared with the dsGFP-injected group.

Survival curves demonstrated that knockdown of Nan resulted in significantly decreased survival of N. lugens ($\chi^2 = 15.93, P < 0.0001$; Fig. 2A), while suppressing the expression level of Iav did not cause significantly different survival of N. lugens ($\chi^2 = 2.09, P = 0.15$; Fig. 2B). After 5 d of injection of dsNan into fourth-instar nymphs, only 26.67 ± 6.67% of insects survived, which is significantly lower than the survival rate of 73.33 ± 3.85% observed in the dsGFP-injected group ($F_{1,3} = 36.75, P = 0.0037$; Fig. 2A).

Statistical Analysis

Real-time qPCR results were analyzed by analysis of variance followed by a multiple comparison of means (LSMEANS with Tukey–Kramer multiple comparison tests) using Proc GLM procedures of SAS software (version 8.01, SAS Institute Inc.). Insect survival curves were made with the Kaplan–Meier method and comparatively analyzed with a log-rank (Mantel–Cox) test using GraphPad Prism software (version 7.0; GraphPad Inc.). The data of climbing assay and gripping assay were analyzed by Mann–Whitney U-test. The femur-tibia angle data, honeydew data, and EPG data between different treatments were compared by SAS Proc GLM procedures. The significance level was set to $P < 0.05$.

**Fig. 1.** Mean transcript levels in whole bodies of Nilaparvata lugens at 7 d after injection with dsRNA of Nan, Iav or GFP. *indicates a significant difference ($P < 0.05$) between dsGFP and dsNan or dsIav treatments. Data were expressed as the mean ± SEM.
Fig. 2. Survival curves of *N. lugens* 1–7 d after injection with dsRNA of *Nan*, *lav* or *GFP*, when fed on rice (A and B). (A) A log-rank (Mantel-Cox) test using GraphPad Prism software showed a significant difference (*) in survival rate between dsGFP and dsNan treatments (*P* < 0.05). (B) A log-rank (Mantel-Cox) test showed no significant difference (ns) in survival rate between dsGFP and dsIav treatments (*P* > 0.05).

Fig. 3. Climbing assay (A and B) and gripping assay (C) for *N. lugens* adults after being injected with dsRNA of *Nan*, *lav* or *GFP*. * indicates a significant difference (*P* < 0.05) between dsGFP and dsNan or dsIav treatments. ’ns’ indicates no significant difference (*P* > 0.05) between dsGFP and dsNan or dsIav treatments. Data were expressed as the mean ± SEM.

Fig. 4. Proportion of adults that reached each EPG waveform during feeding on a rice plant after RNAi. N4 (N4-a + N4-b) for stylets in the phloem tissue (including an intracellular activity (N4-a) and sustained phloem sap ingestion (N4-b) in the phloem tissue) termed phloem ingestion phase; N5 for stylets in the xylem tissue. The number in the parentheses upon each column means the number of the insects reached N4, N4-a, N4-b, or N5 waveform/all the recording replicates.
Data were expressed as the mean ± SEM.

indicates a significant difference (P < 0.05) between dsGFP and dsNan treatments within the same variable. Data were presented as mean ± SEM (n).

*Indicates number of waveform events per insect.

Table 1. Comparison of 6-h EPG response variables of *Nilaparvata lugens* feeding rice plants after injected with dsGFP and dsNan

| Variables                                | dsGFP                  | dsNan                  | F (df), P   |
|------------------------------------------|------------------------|------------------------|-------------|
| 1. Mean time to first probe (min) per insect | 5.5 ± 2.1 (18)         | 5.8 ± 1.8 (18)         | 0.05 (1,35), 0.915 |
| 2. Mean duration of first probe (min) per insect | 48.5 ± 26.8 (18)       | 28.7 ± 9.2 (18)        | 3.44 (1,35), 0.489 |
| 3. Mean number of probes per insect      | 6.9 ± 1.1 (18)         | 8.8 ± 0.8 (18)         | 3.89 (1,35), 0.183 |
| 4. WDI for np (min)                      | 26.9 ± 5.5 (18)        | 76.5 ± 18.2 (18)*      | 9.63 (1,35), 0.013 |
| 5. WDI for pathway (min)                 | 75.3 ± 13.0 (18)       | 114.5 ± 10.5           | 0.87 (1,35), 0.024 |
| 6. NWEI for phloem phase                 | 6.2 ± 0.8 (18)         | 3.4 ± 0.9 (12)*        | 0.81 (1,29), 0.023 |
| 7. WDI for phloem phase (min)            | 209.1 ± 23.7 (18)      | 49.4 ± 20.0 (12)*      | 2.04 (1,29), <0.001 |
| 8. NWEI for xylem phase                  | 2.2 ± 0.4 (16)         | 5.2 ± 1.0 (18)*        | 4.56 (1,33), 0.008 |
| 9. WDI for xylem phase (min)             | 48.4 ± 11.2 (16)       | 119.1 ± 20.2           | 11.2 (1,33), 0.004 |

*Indicates a significant difference (P < 0.05) between dsGFP and dsNan treatments within the same variable. Data were presented as mean ± SEM (n).

*WDI means waveform duration per insect.

*NWEI means number of waveform events per insect.

Table 2. Comparison of 6-h EPG response variables of *Nilaparvata lugens* feeding rice plants after injected with dsGFP and dsIav

| Variables                                | dsGFP                  | dsIav                  | F (df), P   |
|------------------------------------------|------------------------|------------------------|-------------|
| 1. Mean time to first probe (min) per insect | 5.6 ± 2 (18)           | 5.3 ± 1.1 (18)         | 0.02 (1,35), 0.900 |
| 2. Mean duration of first probe (min) per insect | 48.7 ± 26.1 (18)       | 34.3 ± 19.1 (18)       | 0.19 (1,35), 0.667 |
| 3. Mean number of probes per insect      | 7.7 ± 1.5 (18)         | 13.3 ± 2.5 (18)        | 3.57 (1,35), 0.068 |
| 4. WDI for np (min)                      | 29.4 ± 6.8 (18)        | 40.8 ± 8.25 (18)       | 1.09 (1,35), 0.303 |
| 5. WDI for pathway (min)                 | 76.8 ± 12.9 (18)       | 156.7 ± 20.0 (18)*     | 10.6 (1,35), 0.003 |
| 6. NWEI for phloem phase                 | 6.5 ± 0.7 (18)         | 3.2 ± 0.6 (13)*        | 11.3 (1,30), 0.002 |
| 7. WDI for phloem phase (min)            | 209.2 ± 23 (18)        | 133.2 ± 27.4 (13)      | 4.25 (1,30), 0.048 |
| 8. NWEI for xylem phase                  | 2.5 ± 0.4 (17)         | 4.8 ± 0.6 (16)*        | 9.42 (1,32), 0.004 |
| 9. WDI for xylem phase (min)             | 49.8 ± 9.3 (17)        | 69.7 ± 17.8 (16)       | 0.89 (1,32), 0.353 |

*Indicates a significant difference (P < 0.05) between dsGFP and dsIav treatments within the same variable. Data were presented as mean ± SEM (n).

*WDI means waveform duration per insect.

*NWEI means number of waveform events per insect.

**Fig. 5.** Mean amount of honeydew per day excreted by a *N. lugens* female adult after being injected with dsRNA of Nan, Iav, or GFP. *Indicates a significant difference (P < 0.05) between dsGFP and dsNan or dsIav treatments. Data were expressed as the mean ± SEM.

**Discussion**

Pymetrozine, an insect TRPV agonist, can effectively control plant sucking insect pests. Pymetrozine-treated aphids immediately stopped feeding and eventually died from starvation (Harrewijn and Kayser 1997). Pymetrozine was slower-acting against *N. lugens* nymphs (He et al. 2011a) and was less toxic to *N. lugens* adults, but strongly suppressed the offspring numbers (Tsujimoto et al. 2015). Pymetrozine and another insect TRPV agonist, afidopyropen, can directly bind to insect Nan protein, rather than Iav protein (Kandasamy et al. 2017). In the present study, 73.3% of the dsNan-injected individuals of *N. lugens* died at 5 d after dsRNA injection into fourth-instar nymphs. Current knowledge indicates that the Nan subunit could be a better binding target for discovery of novel insecticides for the control of plant sap-sucking insect pests.

Our previous EPG studies demonstrated that pymetrozine disturbed the feeding behavior of rice brown planthoppers and green rice leafhoppers, primarily through inhibition of phloem sap ingestion (He et al. 2011a,b). In this study, similar effects of knockdown of TRPV genes on phloem ingestion of *N. lugens* were observed. Our results suggested that there could be a relationship between TRPV and phloem feeding behavior in *N. lugens*. Additionally, knockdown of TRPV genes also affected the climbing activities of *N. lugens* against top plants. Therefore, it remains unknown whether there is direct or indirect action of TRPV on feeding of *N. lugens*. A direct effect acts on the physiological systems regulating feeding progress, while an indirect effect acts on other systems (such as locomotion behavior) causing the feeding inhibition. There are few reports indicating a direct function of TRPV on insect feeding. In *Drosophila*, Nan is expressed (independently of Iav) in labellar neurons as a mechanosensor for food hardness detection (Jeong et al. 2016). *Drosophila* flies prefer softer food at the expense of sweetness; the Nan mutant flies show reduced preference for softer food (Jeong et al. 2016). Notably, Iav mutant flies did not show
a significant defect in their preference for the softer food (Jeong et al. 2016). It cannot be ruled out that TRPV directly functions on feeding behavior in plant sap-sucking insects. Similarly, it cannot be ruled out that pymetrozine may act on insect feeding systems by targeting TRPV or some additional molecular targets.

While Nan and lav are co-expressed specifically in chordotonal stretch receptor neurons as subunits of a heteromeric TRPV channel, the individual distribution of insect TRPV is broader (Salgado 2017). For instance, lav without Nan in Drosophila motor neurons regulates synaptic development and synaptic transmission (Wong et al. 2014). Nan without lav is required in Drosophila to avoid dry air (Liu et al. 2007). In N. lugens, Nan and lav were detected with different expression patterns in different developmental stages: lav was expressed highly in the early nymphal stages, whereas Nan was not (Mao et al. 2018, Wang et al. 2019). In the present study, knockdown of Nan and lav resulted in same phenotypes in morphology and locomotion but in different phenotypes in survival and feeding behaviors: knockdown of Nan caused a high mortality of N. lugens whereas lav did not; the inhibition rate of the phloem phase duration in dsNan-injected individuals was much higher than in dslav-injected ones. These results might indicate that Nan and lav in N. lugens could play both dependent and independent roles under some conditions.

Our findings and current knowledge expand our interest in exploring the functions and applications of insect TRPV. It appears that the Nan protein could be more focused than the lav protein for discovery of novel active compounds. However, the issue of coexpression or independent distribution of Nan and lav in insects seems considerably complex. Whether the relationship between TRPV and N. lugens feeding is direct or indirect warrants further research. Phloem ingestion behavior of plant sap-sucking insects can be affected by multiple factors and regulated via different signaling pathways. The potential mechanisms of inhibition of phloem ingestion after knockdown of N. lugens TRPV could be: 1) a link to the chemosensory signaling system in antennae or stylets before probing or during ingestion; 2) the neuromuscular system in mouthparts that controls the movement of plant fluid and saliva; or 3) some additional signaling pathways (such as serotonin signaling pathways) related to insect feeding behavior. Future research to determine such a mechanisms is warranted.

Supplementary Data
Supplementary data are available at Journal of Integrated Pest Management online.

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