Acute Exposure to Imidacloprid Impairs Walking Behavior of *Eocanthecona furcellata* (Hemiptera: Pentatomidae)[1]

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Abstract Imidacloprid, one of the neonicotinoid pesticides, is widely used in various types of farmlands globally, causing negative effects on beneficial insects. The stink bug *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) is an effective generalist predator that provides natural control of Lepidoptera, Coleoptera, and other insect pests. Walking behavior is crucial for these predators; however, the effects of imidacloprid on their walking, and thus searching, behavior remain unknown. We measured walking behavior by using a locomotion compensator to assess the effects of imidacloprid. Acute exposure to imidacloprid significantly reduced the mean walking speed of nymphs (30.6–51.9% depending on instar), adult females (52.0%), and adult males (40.5%). Walking time also was reduced by exposure to imidacloprid in nymphs (27.1–40.5%), adult females (48.2%), and adult males (36.0%), whereas walking distance was reduced in nymphs (8.4–19.8%), adult females (25.2%), and adult males (15.0%). These results reveal that imidacloprid impairs the walking behavior of stink bugs, emphasizing the need to consider the negative effects of neonicotinoid pesticides on the pest control efficacy of natural enemy insects.

Key Words imidacloprid, walking behavior, *Eocanthecona furcellata*

Neonicotinoid pesticides were introduced into the market in the 1990s and rapidly became the fastest growing and most widely used class of pesticides globally (Jeschke et al. 2011; Simon-Delso et al. 2015). They can easily enter the soil and be absorbed by plants, and they are extensively used as seed dressings or foliar sprays in agriculture (Blacquière et al. 2012; Bonmatin et al. 2015; Godfray et al. 2015; Mitchell et al. 2017; Robin and Andreas 2003). Neonicotinoids disrupt the neural activity of insects by binding to nicotinic acetylcholine receptors (Elbert et al. 2008; Matsuda et al. 2001). Furthermore, due to their water solubility and persistence in the environment, neonicotinoids are ingested by herbivorous insects, subsequently exposing predatory insects (Blacquière et al. 2012). Imidacloprid, one of the most common neonicotinoid pesticides, has been restricted for outdoor use in the European Union,

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as have thiamethoxam and clothianidin (Environmental Protection Agency 2015); however, it remains widely used in agricultural production worldwide. Although imidacloprid effectively controls pests, it also adversely affects beneficial insects. Previous studies have shown that imidacloprid can affect many aspects of insect behavior, especially in honey bees and bumblebees, which are important pollinators (Desneux et al. 2007; Kenna et al. 2019; Mengoni Goñalons and Farina 2018; Mustard et al. 2020; Smith et al. 2020; Stanley et al. 2015).

The stink bug *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) is regarded as an important generalist predator of pest insects and is widely distributed in China (Yunnan, Sichuan, Hainan, Fujian, Guangdong, Guangxi), India, Sri Lanka, Myanmar, Malaysia, the Philippines, Indonesia, Japan, and Korea (He et al. 2013; Keerthi et al. 2020; Rider and Zheng 2002; Roca-Cusachs et al. 2020; Shylesha and Sravika 2018). It preys on various soft-bodied arthropods (Gupta et al. 2014), particularly lepidopteran, coleopteran, and heteropteran pests (Keerthi et al. 2020; Lenin and Rajan 2016; Pan et al. 2020; Vanitha et al. 2018; Yu et al. 2021). The stink bug can feed on more than 40 types of pests as both nymphs and adults and can be readily reared in laboratories or natural enemy insect rearing facilities, making it a valuable natural enemy insect with great potential for use in integrated pest management (Guo et al. 2021; Gupta et al. 2014; Pan et al. 2022; Zhang et al. 2023).

Locomotion is a fundamental behavior regulated by the central nervous system, motor neurons, and muscles in animals. Various motor behaviors such as walking, flying, swimming, and crawling, characterized predominantly by rhythmic movement, are critically important for survival and reproduction (De Jongh 2021; Mantziaris et al. 2020; Orlovsky et al. 1999). Insects move to find food, mating partners, and oviposition sites and avoid predators. Walking activity, which consumes less energy than flying, is the primary form of short-distance movement in insects (Shen et al. 2020). Generally, the wingless nymphs and winged adults of *E. furcellata* travel by walking to find food, mates, and oviposition sites on the same or adjacent plants. Therefore, walking behavior is crucial to the survival and reproduction of these stink bugs. However, the effects of widely used imidacloprid on the walking behavior of stink bugs are still unknown. Herein, we examined how acute exposure to imidacloprid might impair the walking behavior of stink bugs by using a locomotion compensator.

Materials and Methods

Insects. *Eocanthecona furcellata* adults were originally provided by GreenLeaf Biological Control Company (Yuxi, Yunnan Province) and were reared in nylon cages (45 cm × 45 cm × 45 cm) under laboratory conditions at 25 ± 1°C, 50–70% relative humidity, and a photoperiod of 14:10 (L:D) h. The eggs of the stink bug were collected into a plastic box containing a moist sponge; all nymphs were reared in cages after hatching. The nymphs of *Tenebrio molitor* L. were provided for stink bug development. Adults 2–7 d after emergence and nymphs were used in the experiments.

Chemicals. Imidacloprid was purchased from Sigma-Aldrich (St. Louis, MO) and diluted with double-distilled water. Field surveys showed that imidacloprid at a dose of 0.04375% (two bags of 70% imidacloprid dissolved in ~16 kg of water) is commonly used to spray in tobacco, cotton, and rice paddy systems. Therefore, 1/500th of the field-realistic dose of imidacloprid (0.875 ppm) was used in the behavioral experiments.

Behavioral bioassay. The walking behavior of the stink bug was tested with a TrackSphere LC-300 locomotion compensator (Syntech, Hilversum, The Netherlands), as described by Piesik et al. (2009). This device is composed of a white sphere (300 mm in diameter) coupled to two servomotors driven by a controller (TrackSphere TRC-01 controller, Syntech). A video camera mounted directly above the sphere monitored the movements of the insect and was connected to the controller that drives the servomotors to compensate for the movements of the insect, so that the image of the insect remained in the center of the visual field. The stink bug's track was recorded by the tracking software TrackSphere^{TM} v 3.1 (Syntech) at a rate of 10 Hz.

Stink bugs were submerged in double-distilled water or imidacloprid for 10 s before being removed and used in behavioral tests after 30 min. Fifteen bugs were used in each treatment group. The mean walking speed, walking time, and walking distance were recorded by the tracking software.

Statistical analysis. All data are presented as means ± SEM, with a threshold level of statistical significance set at $P < 0.05$. An independent sample *t* test was used to analyze the differences in mean walking speed, walking time, and walking distance between control (CTL) and imidacloprid (IMI) groups.

Results

Walking speed. Compared with CTL groups, the mean walking speeds of the IMI groups (centimeters per second, IMI versus CTL) were significantly reduced for first–fifth instar nymphs and adults (first instar: 0.381 ± 0.024 versus 1.191 ± 0.048, $P < 0.001$; second instar: 0.492 ± 0.035 versus 1.012 ± 0.053, $P < 0.001$; third instar: 0.560 ± 0.037 versus 1.832 ± 0.107, $P < 0.001$; fourth instar: 1.093 ± 0.067 versus 2.107 ± 0.061, $P < 0.001$; fifth instar: 1.233 ± 0.103 versus 2.609 ± 0.140, $P < 0.001$; adult female: 2.040 ± 0.099 versus 3.920 ± 0.071, $P < 0.001$; and adult male: 1.493 ± 0.085 versus 3.685 ± 0.103, $P < 0.001$) (Fig. 1).

Walking time. Likewise, compared with CTL groups, the walking times of the IMI groups (seconds, IMI versus CTL) were significantly decreased for first–fifth instar nymphs and adults (first instar: 317.9 ± 22.4 versus 1,174.1 ± 92.0, $P < 0.001$; second instar: 599.3 ± 44.4 versus 1,643.3 ± 72.0, $P < 0.001$; third instar: 709.9 ± 40.5 versus 2,282.5 ± 155.0, $P < 0.001$; fourth instar: 671.3 ± 42.0 versus 1,860.1 ± 71.7, $P < 0.001$; fifth instar: 906.7 ± 79.5 versus 2,240.1 ± 73.8, $P < 0.001$; adult female: 806 ± 69.5 versus 1,673.6 ± 43.1, $P < 0.001$; and adult male: 659.4 ± 63.6 versus 1,833.2 ± 33.5, $P < 0.001$) (Fig. 2).

Walking distance. Compared with CTL groups, the walking distances of the IMI groups (IMI vs. CTL, centimeters) were significantly diminished for the first–fifth instar nymphs and adults (first instar: 11,824.0 ± 987.1 versus 141,346.1 ± 12,698.5, $P < 0.001$; second instar: 28,939.2 ± 2,583.5 versus 168,899.6 ± 15,601.7, $P < 0.001$; third instar: 38,989.2 ± 2,737.4 versus 432,168.7 ± 49,602.4, $P < 0.001$; fourth instar: 73,449.0 ± 6,431.2 versus 390,784.7 ± 17,479.7, $P < 0.001$; fifth instar: 116,342.2 ± 16,840.8 versus 585,366.2 ± 37,418.1, $P < 0.001$; adult female: 165,481.3 ± 16,648.6 versus 657,636.1 ± 23,693.1, $P < 0.001$; and adult male: 101,315.7 ± 11,277.2 versus 676,847.9 ± 25,114.4, $P < 0.001$) (Fig. 3).

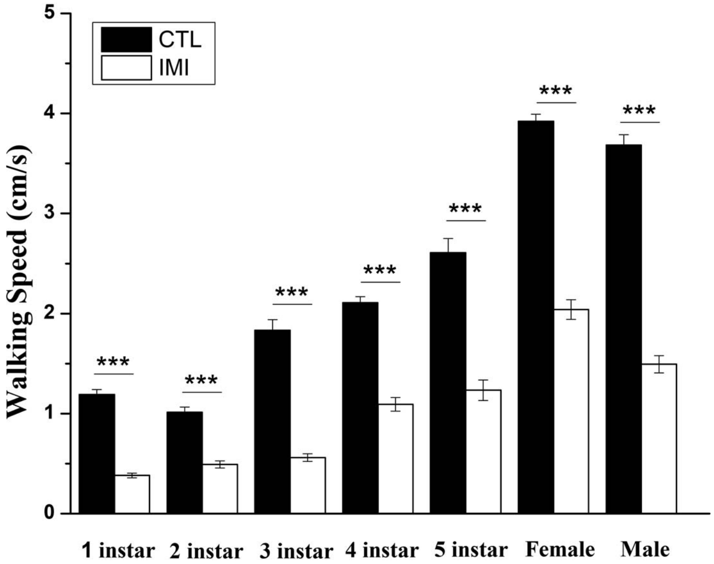

Fig. 1. Reduced walking speed of stink bug.

Discussion

Our study is the first to test how a commonly used neonicotinoid insecticide directly affects the walking behaviors of natural enemy insects. The major findings of our study demonstrate that acute exposure to imidacloprid significantly affected mean walking speed, walking time, and walking distance in both nymphs and

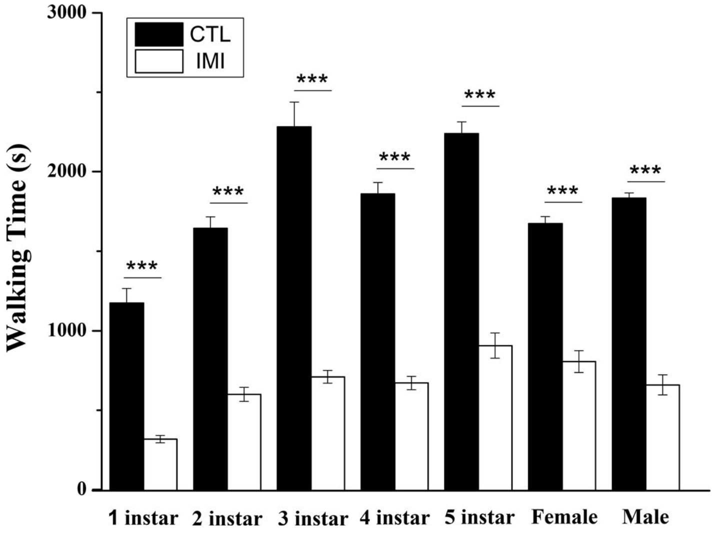

Fig. 2. Decreased walking time of stink bug.

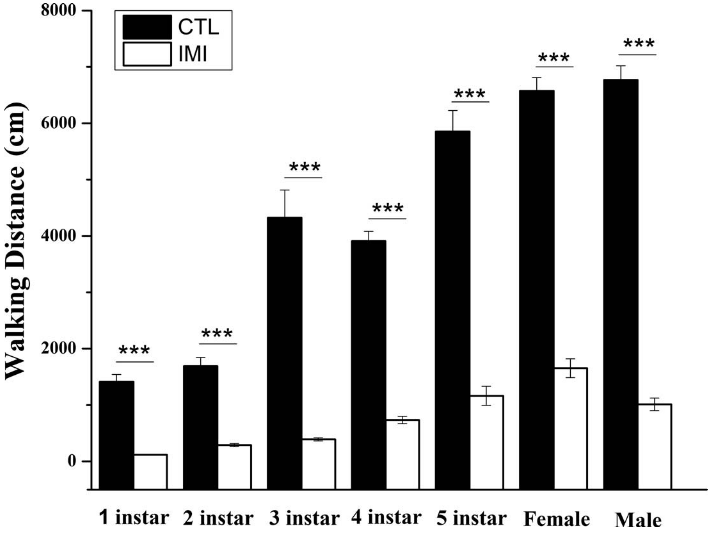

Fig. 3. Decreased walking distance of stink bug.

adults of *E. furcellata* (30.6–52.0% in mean walking speed; 27.1–48.2% in walking time; 8.4–25.2% in walking distance; Figs. 1–3). Together, these results indicate that imidacloprid impairs the walking behavior of *E. furcellata*, suggesting that attention should be paid to the negative effects of imidacloprid when releasing, augmenting, or conserving natural enemy insects in production fields.

It is well known that walking is crucial for insects to move around in search of food, mating partners, oviposition sites, and avoidance of predators. *Eocanthecona furcellata* can encounter nymphs of lepidopteran and coleopteran pests in the same or adjacent crops after being released into fields. Because of the widespread use of imidacloprid in agricultural fields, exposure to this pesticide is unavoidable for the stink bug. Previous studies have shown that imidacloprid impairs social interaction (Desneux et al. 2007), associative learning (Mengoni Goñalons and Farina 2018; Mustard et al. 2020; Smith et al. 2020), flying behavior (Kenna et al. 2019), and effective foraging and pollination services (Stanley et al. 2015) in honey bees and bumblebees. Imidacloprid affects gustatory sensitivity to sucrose and impairs the ability of honey bees to perform the waggle dance (Eiri and Nieh 2012). Imidacloprid reduces the ability of bees to forage and perform homing flights in field situations (Bortolotti et al. 2003; Mommaerts et al. 2010; Schneider et al. 2012). High acute doses (100 and 500 ppb) of imidacloprid affect locomotion in bees (Medrzycki et al. 2003). A recent study showed that imidacloprid at acute doses of 200 and 400 ppb significantly reduces overall daily activity in honey bees (Delkash-Roudsari et al. 2020), and imidacloprid at a dose of 10 ppb reduces flight time (Kenna et al. 2019), showing similar patterns of effects to our findings.

Motor activity is the output of nerve signals and is the result of the combined action of the central nervous system, motor neurons, and muscles (De Jongh 2021; Mantziaris et al. 2020). Acetylcholine is an important excitatory neurotransmitter in the insect nervous system, regulating the input and output of nerve signals (Gauthier

2010; Hu et al. 2010). Previous studies have shown that imidacloprid has a high agonistic affinity for nicotinic acetylcholine receptors, affecting the central nervous system (Elbert et al. 2008; Schmuck et al. 2003). Imidacloprid binds to acetylcholine receptors, inhibiting or blocking nerve signal transmission, which may lead to reduced movement speed, power, and ultimately, shortened movement distance in the stink bugs. In addition, imidacloprid has been shown to induce the down-regulation of genes involved in sugar-metabolizing pathways (Derecka et al. 2013) and could also reduce mitochondrial activity, impair respiratory processes, and cause rapid mitochondrial depolarization (Moffat et al. 2015; Nicodemo et al. 2014), which may contribute to the impaired walking performance in stink bugs. To understand the neural and molecular mechanisms by which imidacloprid impairs walking behavior, further research is needed to determine where and how imidacloprid affects nerves and muscles during signal transmission.

In summary, our results show that acute exposure to imidacloprid impaired the walking behavior of *E. furcellata*, indicating that we should pay attention to the negative effects of neonicotinoid pesticides on the pest control efficacy of natural enemy insects in the future.

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