

Sublethal Effects of Flonicamid and Thiamethoxam on Green Peach Aphid, *Myzus persicae* and Feeding Behavior Analysis

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The green peach aphid, *Myzus persicae* Sülzer, is an important sap-sucking pest of many plants, including Chinese cabbage. The objective of the present study was to determine the effects of sublethal concentrations of two insecticides (flonicamid and thiamethoxam) and the action mechanisms on the feeding behavior of *M. persicae*. The median lethal concentrations (LC₅₀) of flonicamid and thiamethoxam for adult *M. persicae* were 2.56 and 4.02 mg/L, respectively. The sublethal concentrations of flonicamid were 0.44 mg/L (LC₁₀) and 1.25 mg/L (LC₃₀), and those of thiamethoxam were 1.19 mg/L (LC₁₀) and 2.45 mg/L (LC₃₀). The developmental period of *M. persicae* nymphs was 5.9 days at LC₁₀ and 6.1 days at LC₃₀ for both insecticides compared to 5.7 days for the control. Adult longevities at LC₁₀ and LC₃₀ of flonicamid were 13.2 and 13.7 days, respectively. Adult longevity at LC₁₀ of thiamethoxam was 14.7 days. Control adult longevity was 11.6 days. Total fecundity was higher at LC₁₀ (41.8 offspring/female) and LC₃₀ (43.0 offspring/female) of flonicamid, and at LC₁₀ (42.1 offspring/female) of thiamethoxam than that of the control (29.5 offspring/female). Feeding behavior analysis using an electrical penetration graph showed that sublethal doses of flonicamid and thiamethoxam had significant effects on the duration of phloem ingestion. However, higher doses of flonicamid induced starvation by inhibition of phloem ingestion and higher doses of thiamethoxam induced contact toxicity rather than inhibition of feeding behavior. This study provides the basis for a more efficient use of these pesticides in Korea.

Key words: electrical penetration graph, flonicamid, *Myzus persicae*, sublethal concentration, thiamethoxam

The green peach aphid, *Myzus persicae* (Sülzer), is an economically significant pest in many temperate regions of the world. It causes direct damage to a broad range of arable and horticultural crops and transmits more than 100 plant viruses [Blackman *et al.*, 2000]. Recently, neonicotinoid-class insecticides have been used as one of the main insecticides for the control of green peach aphid [Nauen *et al.*, 1998]. Neonicotinoids mimic the mode of action of nicotine, acting as agonists on nicotinic acetylcholine receptors in postsynaptic nerve membranes [Chao *et al.*, 1997]. The increasing popularity of these compounds reflects their rapid action, high systemicity, long residual activity, high potency, and effectiveness against species resistant to other insecticide classes [Wollweber and Tietjen, 1999; Foster *et al.*, 2003].

Flonicamid (IK1220; *N*-cyanomethyl-4-trifluoromethyl-nicotinamide) is a novel, selective aphicide discovered by Ishihara Sangyo Kaisha, Ltd (Tokyo, Japan), and developed worldwide jointly with FMC. This compound belongs to the pyridinecarboxamide group, a novel class of chemical pesticides for controlling aphids that are resistant to other insecticides. The main insecticidal mechanism of flonicamid is starvation due to the inhibition of stylet penetration into plant tissues [Morita *et al.*, 2007]. Thiamethoxam [(*EZ*)-3-(2-chloro-1,3-thiazol-5-yl-methyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine] is classified according to the pharmacophore as *N*-nitroguanidine neonicotinoid [Elbert *et al.*, 2008]. Thiamethoxam is presently one of the most effective chemicals for the control of sucking pests such as aphids, whiteflies, thrips, some microlepidoptera, and a number of coleopteran species [Sharma and Lal, 2002].

Exposure to sublethal doses of pesticides may result in both behavioral and physiological changes in individuals. The physiological changes caused by exposure to sublethal

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concentrations of insecticides can reduce developmental rate, longevity, fecundity, and fertility [Stark and Banks, 2003]. Previous authors have reported that exposure to or feeding on sublethal concentrations of imidacloprid reduces growth, survival, and reproduction in many hemipteran insect pests [Mowry and Ophus, 2002; Lashkari *et al.*, 2007; Wang *et al.*, 2008]. In contrast, exposure to or feeding on plants treated with imidacloprid increased the fecundity of various insects [James, 1997; James and Price, 2002; Wang *et al.*, 2005]. Behavioral changes caused by exposure to or feeding on sublethal concentrations of an insecticide can also result in reduced feeding or searching behavior [Stark and Banks, 2003]. For example, sublethal concentrations of imidacloprid act as anti-feedants for many hemipteran insects, including aphids, whiteflies, and green leafhoppers [Nauen *et al.*, 1998; Mowry and Ophus, 2002] and reduces acquisition and/or transmission of potato leafroll virus by *M. persicae* [Mowry and Ophus, 2002].

Electrical penetration graphs (EPGs) are an effective tool to study the feeding behaviors of sucking insects such as aphids [Morita *et al.*, 2007; Seo *et al.*, 2007; Yang *et al.*, 2010]. When feeding in phloem, the aphid stylet penetrates between the epidermal and mesophyll cells overlying the vascular tissues of the phloem until a suitable nutritional site is found [Sauge *et al.*, 2002]. Some aphid species initiate feeding by touching their proboscis to the plant surface, salivating, and then sucking back some fluid. This provides an opportunity to sample surface chemicals [Hashiba and Misawa, 1969]. Thus, aphids determine whether a plant is suitable for feeding after several probing attempts [Cui *et al.*, 2010] observed that IPP-10 (a novel neonicotinoid insecticide) had both contact and systemic activity, with sublethal effects resulting in reduction in *Rhopalo siphumpadi* feeding behavior, growth rate, and fecundity. Morita *et al.* [2007] used the EPG technique to investigate the main insecticidal mechanism of flonicamid and found that starvation is due to the inhibition of stylet penetration into plant tissues.

The purpose of this study was to determine the effects of sublethal concentrations of flonicamid and thiamethoxam on developmental period of nymphs, adult longevity, and fecundity of *M. persicae*. In addition, the feeding behavior of *M. persicae* on Chinese cabbage treated with various concentrations of flonicamid and thiamethoxam was also observed.

Materials and Methods

Test insects. A susceptible strain of *M. persicae* was initially obtained from Korea Research Institute of Chemical Technology (Daejeon, Republic of Korea) in

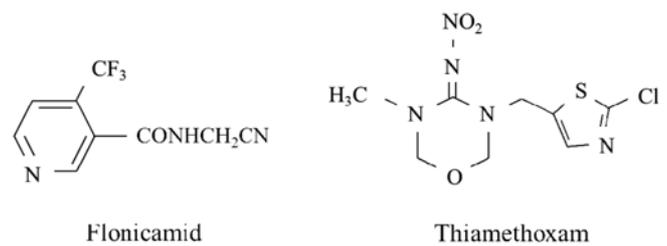


Fig. 1. The chemical structures of flonicamid and thiamethoxam.

1998 and was maintained in the laboratory without exposure to any known insecticides. The strain was reared through several generations in plastic containers (30 cm × 30 cm × 30 cm). They were provided with 3-week-old Chinese cabbage (*Brassica rapa* L. cv. Chunchujeonguk) as food under the following conditions: 21±2°C, 50–60% RH and a 16L: 8D photoperiodic cycle.

Insecticides. Flonicamid (commercial formulation Setis®WG 10%, DONGBU Hannong Chemical Co., Daejeon, Korea) and thiamethoxam (commercial formulation Actara®WG 10%, Syngenta Korea Ltd., Seoul, Korea) were used in all experiments. The chemical structure of the two insecticides is shown in Fig. 1. Serial dilutions were prepared in distilled water. Solutions were used immediately after preparation to minimize any chemical decomposition.

Lethal toxicity bioassays. Five different concentrations each of flonicamid (0.06, 1, 10, 16.75, and 33.5 mg/L) and thiamethoxam (1, 5, 7, 10, and 50 mg/L) were prepared. Both insecticides were diluted in distilled water. Chinese cabbage leaf discs (5.5 cm diameter) were cut out with a sharp metal cylinder. Discs were dipped for one minute in each solution and left to air dry at room temperature. Discs were then transferred into Petri dishes (55×15 mm). Fifteen *M. persicae* adults were transferred to each dish with the aid of a soft brush. The Petri dishes were maintained in a growth chamber at 21±2°C and 50–60% RH. The mortality was evaluated 48 h after exposure to thiamethoxam and 96 h after exposure to flonicamid. Bioassays were repeated three times for each concentration of each insecticide.

Sublethal assays. Using the experimental protocol described above, we exposed female *M. persicae* to sublethal concentrations of flonicamid and thiamethoxam. To ensure low mortality, LC₁₀ (0.44 and 1.19 mg/L) and LC₃₀ (1.25 and 2.45 mg/L) concentrations of flonicamid and thiamethoxam, respectively, were used.

Nymph developmental period, adult longevity, and fecundity. To compare the effect of the sublethal concentrations of two insecticides estimated in above section on *M. persicae* nymph developmental period, 50

first-instar nymphs born within 6 h were selected and placed individually in fifty Petri dishes containing Chinese cabbage leaf discs treated with insecticides. Nymphs were observed throughout their development and total duration until adult emergence and survival were recorded. Nymph mortality was calculated as the difference between the total number of nymphs at the beginning of the experiment and the total number of emerging adults. After the final ecdysis, the aphids were transferred to leaf discs in new dish using a fine paintbrush. The dishes were placed in clear plastic containers lined with moist paper towels and held in a growth chamber. Adult survival and the number of F1 progeny were recorded daily. Aphids were considered dead if they did not move after prodding. The experiment was repeated three times on different dates.

Electrical recording of aphid feeding behavior. The probing and feeding behaviors of *M. persicae* adults feeding on Chinese cabbage treated with flonicamid or thiamethoxam were monitored using an 8-channel Direct Current-Electrical Penetration Graph (DC-EPG) [Tjallingii, 1978; 1988]. In brief, starved aphids were connected individually, via their dorsum, to thin gold wire (2–4 cm length, 20- μ m diameter; Goodfellow, Cambridge, UK) using silver conductive paint (RS Components Ltd., Northants, UK) and connected to the input probe of the EPG. The voltage was supplied to each plant via a copper electrode inserted into the compost. EPG recording was carried out using a Giga-8 EPG amplifier system with 1 G Ω input resistance (EPG Systems, Wageningen, The Netherlands). A plant electrode was inserted into the soil of each potted plant and connected to the plant voltage output of the Giga-8 EPG device. After wiring and attachment to the system, aphids were suspended and starved for 1 h before initiation of monitoring. Recordings were made simultaneously on eight plants, four of each cultivar, placed randomly within a Faraday cage at 21 \pm 2°C under electric florescent lighting. Aphids and plants were used only once and then discarded. Feeding behavior was measured for 7 h. Data acquisition and waveform analyses were performed by PROBE 3.0 software (WF Tjallingii, Wageningen University, Wageningen, The Netherlands). For traces that were fully analyzed, electrical signals and their correlations with aphid behavior were scored based on the categories described by Tjallingii [1990]: non-probing (stylets external to the plant), xylem ingestion (waveform G), and phloem activities (pooling together waveforms E1 and E2 reflecting salivation into sieve elements and phloem ingestion, respectively). When an aphid failed to exhibit a certain waveform within the recording period, the aphid was recorded as having taken the entire recording period

to reach the waveform, as suggested by Prado and Tjallingii [1997].

Three parameters of non-sequential variables of each EPG waveform were analyzed, including total duration, number of occurrences, and proportion of each waveform phase in the 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 number of occurrences of each EPG waveform represented the number of times that a waveform occurred during the observation time. The percentages of insects reaching the phloem and xylem phases after being treated with flonicamid and thiamethoxam were also calculated.

Statistical analysis. Adult *M. persicae* mortality data from three replicate experiments were pooled for each concentration tested and subjected to log-probit regression analysis (PROC PROBIT program) (SAS, ver. 9, SAS Institute, Cary, NC) after correcting for control mortality to calculate the concentration for sublethal effects. For EPG data, the proportions of traces analyzed or rejected for aphids feeding on Chinese cabbage treated with flonicamid or thiamethoxam were subjected to a χ^2 test. To determine the effects of flonicamid or thiamethoxam on aphid probing and feeding behaviors, non-parametric analyses were performed using Duncan's test.

Results

Insecticidal activity and determination of sublethal concentrations. The mortality rates of *M. persicae* adults when treated with different concentrations of flonicamid or thiamethoxam were investigated (Fig. 2). No mortality occurred 24 h after treatment with flonicamid. However, mortality increased with increasing concentration of flonicamid after 48 h. *M. persicae* adults treated with 10 mg/L thiamethoxam had 50% mortality 24 h after treatment. After 48 h, the recommended concentration (50 mg/L) induced more than 90% mortality. Mortality of the control group in all bioassays was below 2%. LC₉₀ values for flonicamid against *M. persicae* were 14.70 mg/L and those for thiamethoxam were 13.58 mg/L. LC₁₀ and LC₃₀ values for flonicamid were 0.44 and 1.25 mg/L, while those of thiamethoxam were 1.19 and 2.45 mg/L, respectively (Table 1). The median lethal concentration (LC₅₀) of flonicamid and thiamethoxam for adult *M. persicae* was 2.56 and 4.02 mg/L, respectively.

Effect of sublethal concentrations on nymph developmental period, adult longevity, and fecundity. Developmental period of nymphs that fed on plants treated with flonicamid or thiamethoxam was delayed compared with those that fed on control plants treated

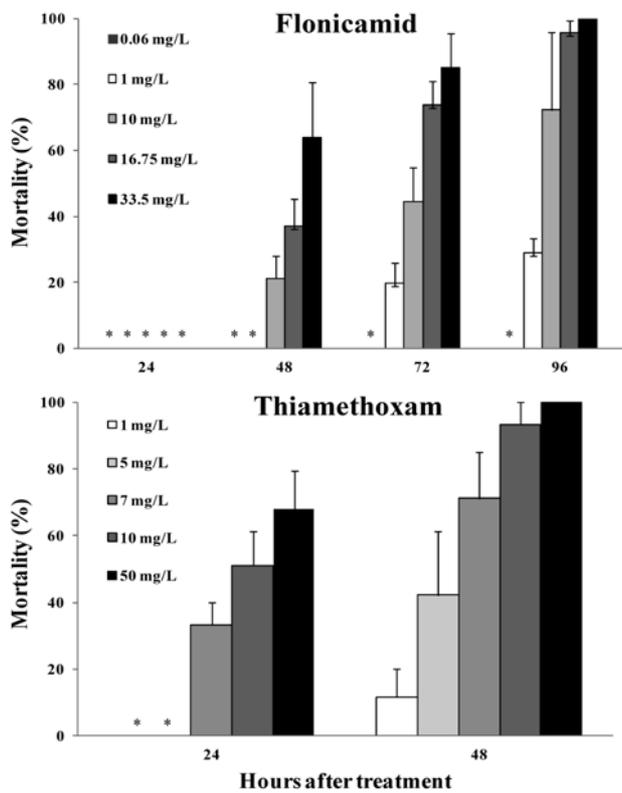


Fig. 2. Percent mortality of adult female *M. persicae* treated with flonicamid and thiamethoxam. An asterisk (*) indicates 0% mortality.

with distilled water (Table 2). However, there was no significance. The developmental period of *M. persicae* nymphs was 5.9 days at LC₁₀ and 6.1 days at LC₃₀ for both insecticides, whereas control nymphs took 5.7 days.

The effects of sublethal concentrations of flonicamid or thiamethoxam on adult longevity and fecundity of *M. persicae* were investigated (Table 3). Exposure to flonicamid (LC₁₀ and LC₃₀) increased adult longevity to 13.2 and 13.7 days, respectively. However, there was no significant difference compared with the control (11.6 days). Exposure to thiamethoxam significantly increased adult longevity at LC₁₀ to 14.7 days, but LC₃₀ was not significantly different from the control. Total fecundity was higher when insects were treated at LC₁₀ (41.8±16.1 offspring/female) and LC₃₀ flonicamid (43.0±13.7) and

Table 2. Effect of sublethal concentrations of flonicamid and thiamethoxam on the developmental period of first instar *M. persicae* nymphs

Insecticide	Conc. (mg/L)	n	Nymphal period (days) (Mean ± SD)
Flonicamid	LC ₁₀ (0.44)	50	5.9±0.57 ^a
	LC ₃₀ (1.25)	50	6.1±0.56 ^a
Thiamethoxam	LC ₁₀ (1.19)	50	5.9±0.73 ^a
	LC ₃₀ (2.45)	50	6.1±0.74 ^a
Control	-	50	5.7±0.67 ^a

^aThe same letter within a column are not significantly different at $p=0.05$ by Duncan's multiple range test (SAS Institute, 2010).

LC₁₀ thiamethoxam (42.1±13.0) than when they were given water (29.5±12.4). Significant effects were not observed for LC₃₀ of thiamethoxam. Mean daily fecundity was higher at LC₁₀ and LC₃₀ flonicamid than the control, whereas sublethal concentrations of thiamethoxam did not affect fecundity. Fig. 3 shows the effect of sublethal concentrations of flonicamid or thiamethoxam on survival rate (panel A) and fecundity (panel B) of *M. persicae* adults.

EPG and feeding behavior of *M. persicae*. To further elucidate the effects of flonicamid and thiamethoxam on *M. persicae*, feeding activities and feeding behavior were studied using the EPG technique. An overview of the EPG probing process of *M. persicae* adults for one hour is shown in Fig. 4A. Different waveforms represented different behaviors. The waveforms and their correlation with aphid behavior are described as follows: NP, non-penetration; G_x xylem phase; E1, salivation, and E2, sap feeding (Fig. 4B–E).

All EPG recordings were done for 7 h. The total durations of the NP waveform of LC₁₀ and LC₃₀ flonicamid were 37.1±33.2 and 32.4±46.7 min, respectively, which were shorter than the control (42.7±32.1 min) (Fig. 5A). However, the difference was not significant. The numbers of NP waveform occurrences produced by aphids feeding on Chinese cabbage treated with flonicamid were lower than those on control leaves (Fig. 6A). The total durations

Table 1. Toxicity of flonicamid and thiamethoxam against adult female *M. persicae* of a susceptible strain

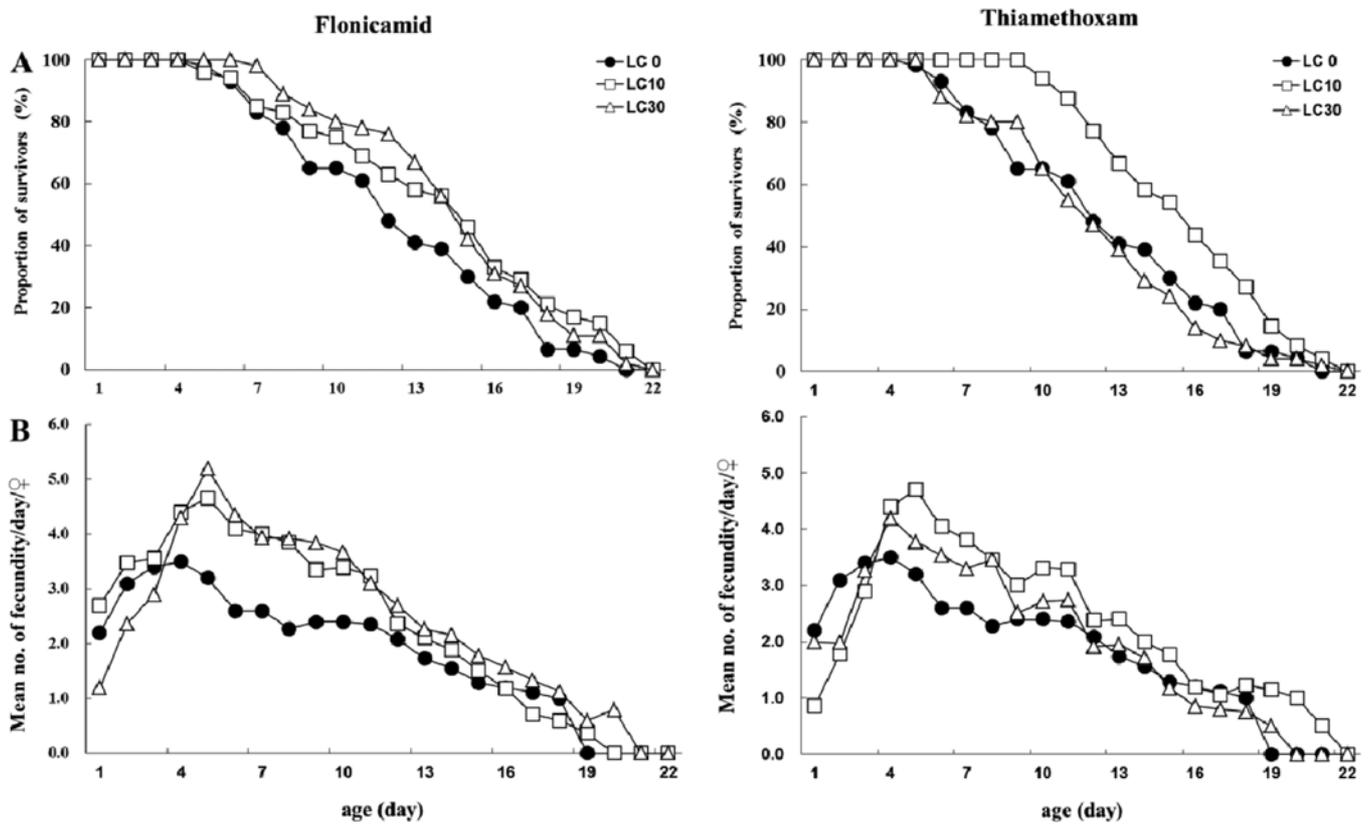
Insecticide	Sublethal conc. (mg/L)		Lethal conc. (mg/L)		Slope (±SE)	χ^2	RC ^a (mg/L)
	LC ₁₀	LC ₃₀	LC ₅₀	LC ₉₀			
Flonicamid	0.44 (0.27–0.65)	1.25 (0.89–1.65)	2.56 (1.97–3.22)	14.70 (11.41–19.93)	1.69±0.13	17.14	33.5
Thiamethoxam	1.19 (0.85–1.53)	2.45 (1.97–2.90)	4.02 (3.45–4.61)	13.58 (11.29–17.28)	2.43±0.21	22.60	50

^aRecommended concentration (Agrochemicals Use Guide Book, Korea Crop Protection Association, 2010).

Table 3. Effect of sublethal concentrations of flonicamid and thiamethoxam on adult longevity and fecundity of adult female *M. persicae*

Insecticide	Conc. (mg/L)	n	♀ longevity (days) (Mean ± SD)	Total fecundity/♀ (Mean ± SD)	Fecundity/day/♀ (Mean ± SD)
Flonicamid	LC ₁₀	48	13.2±4.9 ^{ab}	41.8±16.1 ^b	3.1±0.7 ^b
	LC ₃₀	45	13.7±4.0 ^{ab}	43.0±13.7 ^b	3.1±0.7 ^b
Thiamethoxam	LC ₁₀	48	14.7±3.4 ^b	42.1±13.0 ^b	2.8±0.7 ^{ab}
	LC ₃₀	49	11.3±4.1 ^a	32.0±13.7 ^a	2.8±0.7 ^{ab}
Control	-	46	11.6±4.4 ^a	29.5±12.4 ^a	2.5±0.6 ^a

^aThe same letter within a column are not significantly different at $p=0.05$ by Duncan's multiple range test (SAS Institute, 2010).

**Fig. 3. Effect of sublethal concentrations of flonicamid and thiamethoxam on survival rate (A) and daily fecundity (B) of adult female *M. persicae*.**

of the NP waveform of LC₁₀ and LC₃₀ thiamethoxam were 42.4±36.1 and 56.5±43.7 min, respectively. LC₅₀ value of both insecticides was highly increased the total duration of the NP waveforms than LC₃₀ and LC₁₀ values. Interestingly, LC₅₀ of thiamethoxam increased total duration of the NP waveform about two-fold (98.4±106.5 min), whereas LC₅₀ of flonicamid increased it about four-fold (176.9±127.7 min). The numbers of NP occurrence were not significantly different between the control and each thiamethoxam treatment. These results suggest that flonicamid but not thiamethoxam inhibits stylet penetration of plant tissues.

The total duration of the G waveform was not significantly different between the control and sublethal concentrations to half of the recommended concentration of flonicamid (Fig. 5B). However, the recommended concentration (33.5 mg/L, 0.0±0.0 min) and double recommended concentration (67 mg/L, 0.0±0.0 min) of flonicamid completely inhibited the xylem ingestion. In contrast, all concentrations of thiamethoxam showed the xylem feeding behavior and the total duration of the G waveform was similar to the control except for 10 mg/L. However, number of G occurrences were not significantly different between the control and both insecticide treatments.

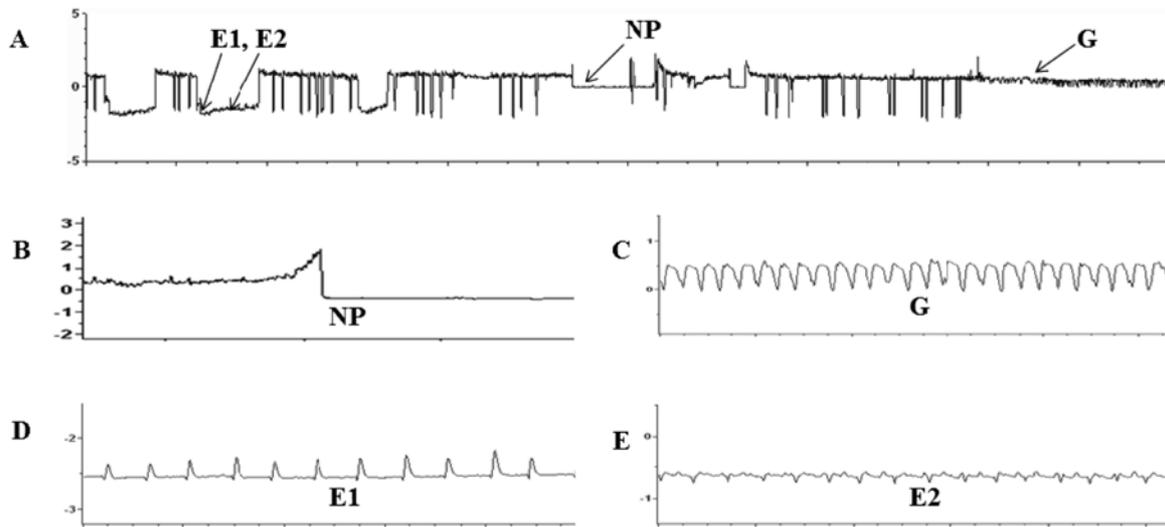


Fig. 4. Typical EPG waveforms of *M. persicae* during 1 h of recording (A) and the characteristic waveform in detail (B to E). NP, non-penetration; G, xylem phase; E1, salivation, and E2, sap feeding region.

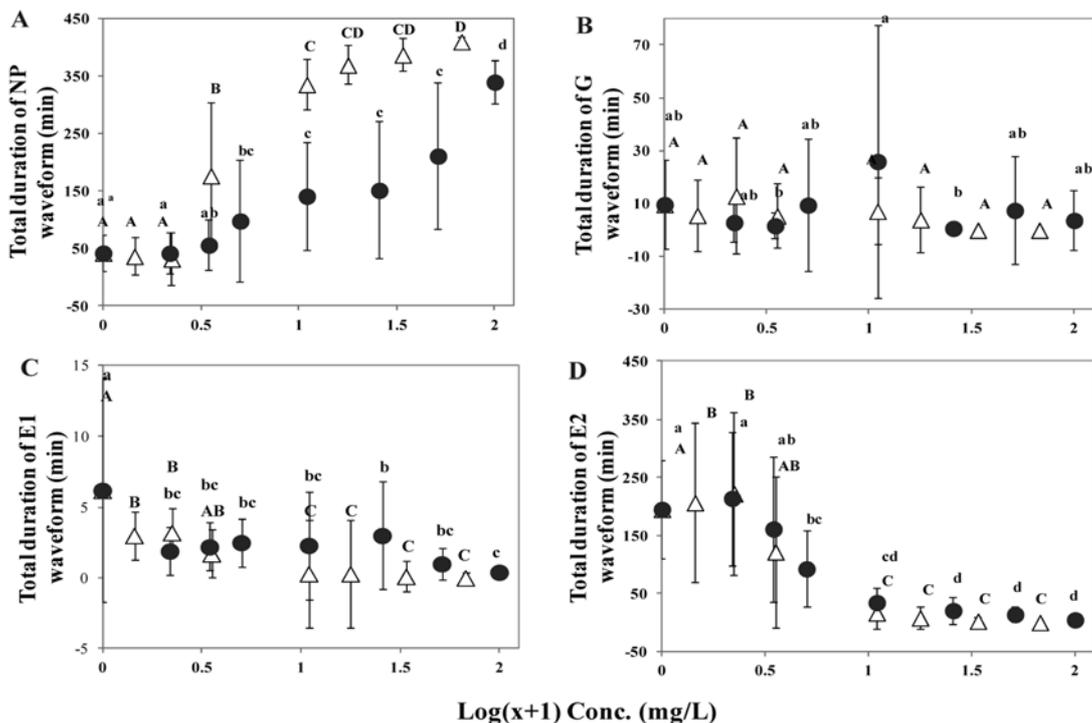


Fig. 5. Relationship between each insecticide concentration (log) and the total duration of each EPG waveform, including the total durations of NP waveform (A), G waveform (B), E1 waveform (C), and E2 waveform (D). Open triangles are flonicamid-treated groups (significance: capital letter) and closed circles are thiamethoxam-treated group (significance: small letter). Error bars indicate \pm SE.

In the phloem phase, E1 and E2 salivations were recognized by dissecting the EPG waveform patterns (Fig. 4D and E). During the E1 period, it is assumed that the insect extends its mouthparts to the vascular bundle for feeding and includes penetration of plant cells and salivating. The total durations of the E1 waveform of sublethal concentrations of flonicamid were shorter than those of the control group and rapidly decrease at more

than 10 mg/L (Fig. 5C). In addition, the numbers of occurrences of the E1 waveform produced by aphids feeding on Chinese cabbage treated with sublethal concentrations of flonicamid (LC_{10} , 4.1 ± 1.9 and LC_{30} , 5.2 ± 2.5) were lower than those of the control group (7.9 ± 5.3) (Fig. 6C). The total durations of the E1 waveform produced by aphids feeding on sublethal concentrations of thiamethoxam (LC_{10} , 1.9 ± 1.5 min and

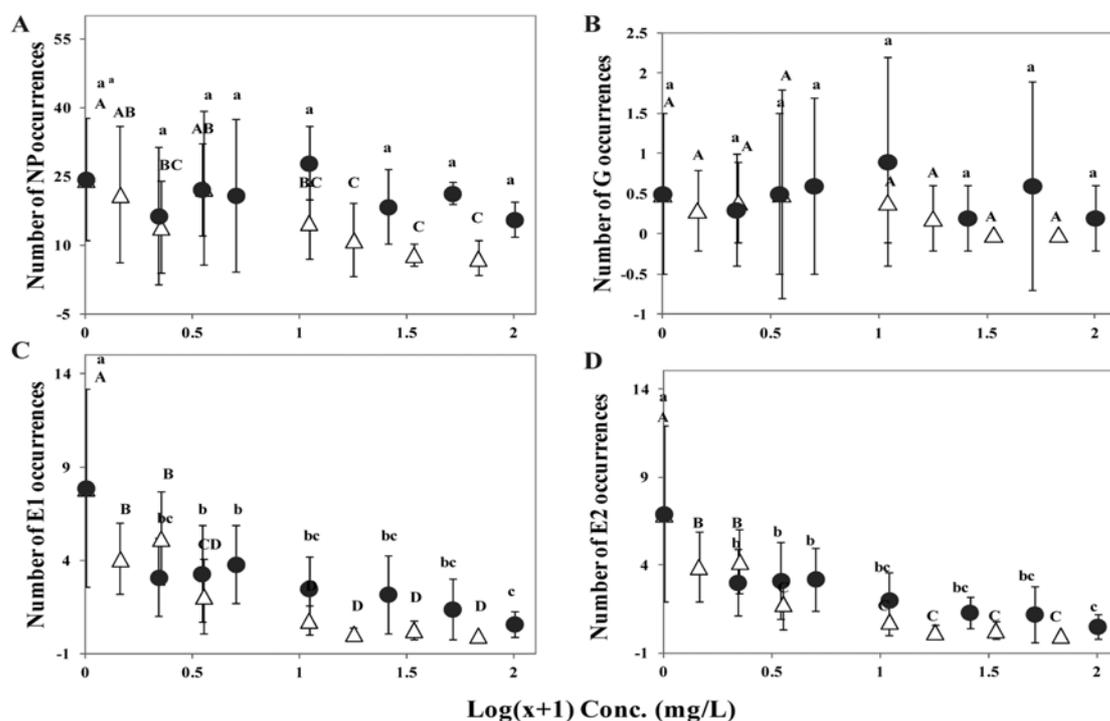


Fig. 6. Relationship between each insecticide concentration and the numbers of occurrences of each EPG waveform, including the number of NP waveform (A), G waveform (B), E1 waveform (C), and E2 waveform (D). Open triangles are flonicamid-treated groups (significance: capital letter) and closed circles are thiamethoxam-treated group (significance: small letter). Error bars indicate \pm SE.

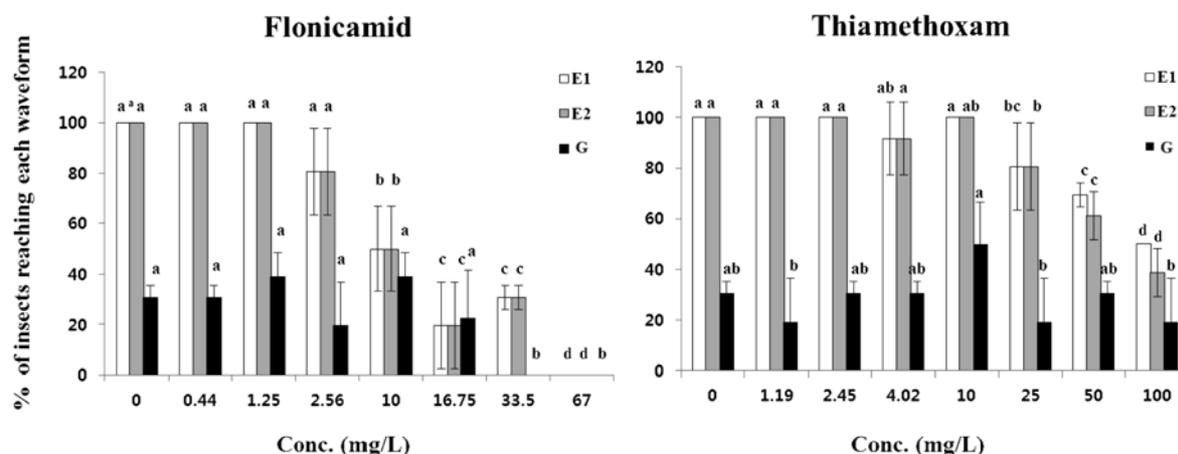


Fig. 7. The proportions of insects reaching the phloem and xylem phases after being treated with different concentrations of flonicamid and thiamethoxam. The numbers in parentheses by each data point denote the number of insects reaching E1, E2, or G waveforms/the number of total valid replicates.

LC₃₀, 2.2 \pm 1.7 min) were shorter than those of control group (6.2 \pm 7.9 min) and rapidly begins to decrease at more than 50 mg/L (1.0 \pm 1.1 min). The numbers of occurrences of the E1 waveform produced by aphids feeding on Chinese cabbage treated with sublethal concentration of thiamethoxam was also lower than those of control group.

When aphids start phloem ingestion, the EPG exhibits the typical E2 waveform. Aphids feeding on Chinese

cabbage treated with LC₁₀ and LC₃₀ flonicamid spent 206.7 \pm 137.5 and 222.1 \pm 140.8 min, respectively, performing phloem feeding, which was significantly more time than aphids feeding on Chinese cabbage treated with distilled water (195.2 \pm 85.4 min) (Fig. 5D). In addition, the number of occurrences of the E2 waveform produced by aphids feeding on Chinese cabbage treated with sublethal concentrations of flonicamid was significantly lower than the frequency of the waveform produced by aphids

feeding on Chinese cabbage treated with distilled water (Fig. 6D). However, at more than LC_{50} flonicamid, the total duration of the E2 waveform and the number of occurrences rapidly decreased. The total duration of the E2 waveform of LC_{10} thiamethoxam (214.2 ± 115.1 min) was longer than those of the control group (195.2 ± 85.4 min), but there was no significant difference. At more than LC_{30} thiamethoxam, the total duration of the E2 waveform rapidly decreases. The numbers of E2 occurrences increased as thiamethoxam concentrations increased.

Figure 7 shows the proportions of *M. persicae* reaching the phloem (E1 and E2) and xylem (G) phases after being treated with flonicamid or thiamethoxam. *M. persicae* treated with sublethal concentration of flonicamid reached the phloem region. The percentage begins to decrease at 2.56 mg/L and aphids failed to show both phloem and xylem phases after being treated with 67 mg/L, the highest concentration tested in this study. In contrast, *M. persicae* treated with thiamethoxam reached the phloem and xylem region even at the highest concentration of 100 mg/L.

Discussion

In the present study, we compared the effect of flonicamid and thiamethoxam on *M. persicae* physiology and feeding behavior. Flonicamid is a feeding inhibitor that causes high mortality due to starvation [Morita *et al.*, 2007]. Thiamethoxam is a neonicotinoid insecticide having both gut and contact activities [Maienfisch *et al.*, 2001]. Our research shows that flonicamid and thiamethoxam caused a range of sublethal effects, including developmental period of nymph, adult longevity, and fecundity of *M. persicae*. Also, aphid-feeding data obtained from EPG analysis revealed that flonicamid and thiamethoxam produce different feeding behaviors in *M. persicae*.

Upon exposure to sublethal concentrations of flonicamid and thiamethoxam, the mean nymphal period of adult aphids was delayed compared with control, but was not statistically significant. Boina *et al.* [2009] found that sublethal doses of imidacloprid significantly delayed the developmental time of *Diaphorina citri* nymphs. IPP-10, a recently developed neonicotinoid insecticide, also delayed the development of *Rhopalosiphum humpadi* [Cui *et al.*, 2010]. Similar results have also been found in other studies [Ruii *et al.*, 2006; Pineda *et al.*, 2007]. Effects of exposure to sublethal concentrations of pesticides on longevity seem to be highly dependent on the type of pesticide and the exposed insect species. Some studies show that sublethal concentrations of pesticides reduce

the longevity [Ibrahim and Yee, 2000; Desneux *et al.*, 2007; Boina *et al.*, 2009], whereas others indicate no sublethal effects on insect longevity [Yokoyama and Pritchard, 1984; Pons *et al.*, 1999]. Occasionally, exposure to sublethal doses of pesticides can increase the lifespan of certain insects [Ball and Su, 1979]. In our study, the longevity of adult *M. persicae* increased significantly when they were exposed to sublethal concentrations (LC_{10}) of thiamethoxam. The results suggest that a LC_{10} thiamethoxam cause physiological resurgence. However, LC_{30} thiamethoxam was not significantly different from the control. Both sublethal rates (LC_{10} and LC_{30}) of flonicamid only slightly affected adult longevity. Our research also showed that LC_{10} and LC_{30} flonicamid and LC_{10} of thiamethoxam increased significantly fecundity. As shown in EPG data, phloem-feeding duration at the above doses of the two insecticides also increased significantly. Effects of exposure to sublethal pesticide doses on fecundity may be ascribed to physiological and behavioral effects [Desneux *et al.*, 2007]. Daniels *et al.* [2009] reported that a sublethal dose of thiamethoxam decreased fecundity in *R. padi*. In addition, imidacloprid and dinotefuran reduced the fecundity of *Nilaparvata lugens* in macropterous families and in brachypterous families when compared with untreated controls. By contrast, triazophos and fenvalerate increased fecundity [Bao *et al.*, 2009]. These results suggest that fecundity seems to be highly dependent on the type of pesticide and the exposed insect species.

In the present study, feeding data obtained from EPG analysis indicated that the phloem-feeding behavior of *M. persicae* on fed plants treated with sublethal doses of flonicamid changed significantly. However, LC_{50} flonicamid caused a decrease in the phloem-feeding period and an increase in the NP period. In contrast, phloem-feeding behavior of *M. persicae* fed plants treated with sublethal doses of thiamethoxam was significantly changed only at LC_{10} . The phloem-feeding period decreased and the NP period also increased at LC_{50} thiamethoxam. Interestingly, at more than LC_{50} flonicamid, the total time of phloem-feeding as decreased with an increase in the concentration and completely stopped the feeding behavior at two-fold higher than the recommended concentration. At more than LC_{50} thiamethoxam, the total time of phloem-feeding decreased with an increase in the concentration. However, feeding behavior was not inhibited even at a concentration two-fold higher than the recommended concentration. Anti-feedant effects of flonicamid leading to weight loss and subsequent death from starvation have already been reported for *M. persicae*, [Morita *et al.*, 2007]. However, this paper presents the first report on the sublethal effect of flonicamid on the feeding behavior of

M. persicae.

In conclusion, our work shows that sublethal concentrations of flonicamid and thiamethoxam increase fecundity and phloem-feeding behavior on *M. persicae*. However, to fully assess the sublethal effects of these pesticides on *M. persicae* under field conditions, additional research are needed. In addition, the side effects of flonicamid and thiamethoxam on natural enemies should also be studied.

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