Research Article

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Assessment of the effects of novel insecticides on green peach aphid (*Myzus persicae*) feeding and transmission of Turnip mosaic virus (TuMV)

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Abstract

BACKGROUND: Laboratory bioassays using treated leaf disks of peach were conducted to determine the efficacy of nine insecticides against the green peach aphid (GPA), *Myzus persicae* (Sulzer). The effects of these insecticides on aphid feeding behaviors and rates of transmission of Turnip mosaic virus (TuMV) to potted rutabaga plants were also determined.

RESULTS: Median lethal concentration (LC_{50}) values after 48 h feeding varied considerably, ranging from lows of 1.5 and 4.6 μg a.i./L for sulfoxaflor and λ-cyhalothrin, respectively, to 97.2 and 167.9 μg a.i./L for flonicamid and spirotetramat. LC_{50} values were lowest and roughly equivalent for λ-cyhalothrin (1.2) acetamiprid (2.1), sulfoxaflor (0.23) and flupyradifurone (2.3) after 72 h feeding. Electrical penetration graph (EPG) recordings showed modest effects on feeding behaviors for certain insecticides, with sulfoxaflor, spirotetramat, and acetamiprid non-significant reduction in feeding duration and number of pathway and potential drop phases occurring during the first 5 min compared with the control. However, greenhouse experiments carried out to investigate the effect of these insecticides on rates of transmission of TuMV, which is transmitted non-persistently by GPA, resulted in only modest non-significant reductions in infection rates for acetamiprid, pymetrozine, λ-cyhalothrin, and flonicamid of 27%, 23%, 20%, and 17%, respectively.

CONCLUSION: All test materials were efficacious to GPA at differing levels, and some such as sulfoxaflor and acetamiprid non-significantly reduced the duration and number of pathways and potential drop phases of feeding within the first 5 min. None, however, resulted in significant reductions in rates of transmission of TuMV.

Keywords: lethal concentration; toxicity; green peach aphid; insecticide; electrical penetration graph

1 INTRODUCTION

Insecticide applications to control aphids that vector plant diseases have been widely investigated.[1] Circulative or persistent plant viruses that require a prolonged period of feeding by their aphid vectors for both acquisition and successful inoculation of plants have been effectively controlled with traditional synthetic pyrethroid, organophosphorus and carbamate insecticides.[1–3] By contrast, insecticides generally act too slowly to inhibit the spread of non-persistent or stylet-borne plant viruses that are transmitted by migrant winged aphids following short periods of feeding lasting from several seconds to minutes.[1,4,5] Behavioral laboratory studies of the effects of fast-acting pyrethroid insecticides on aphid probing and feeding behaviors have shown significant decreases in the duration and number of probes.[6–8] Although a reduction in transmission rates of non-persistent viruses with sprays of pyrethroids has been reported under laboratory conditions,[5,9] control in the field has been unsuccessful or inconsistent,[5,9,10] often resulting in higher levels of disease. Higher levels of infection following application of pyrethroids have been attributed to enhanced agitation and dispersal of viruliferous aphids which increases transmission to other plants.[5,7,11]

A number of new aphicides belonging to new chemical classes have recently been registered in Canada for the control of hemipteran pests; the mode of action for some, such as flonicamid and

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pymetrozine, includes rapid inhibition of aphid feeding. These or closely related compounds have been evaluated for their utility managing piercing–sucking pests of many crops,[12–17] but there is a need to compare the relative toxicities of these new insecticides in the green peach aphid (GPA), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), one of the most economically important aphid pests in Canada, and determine their impact suppressing aphid-borne non-persistent plant viruses. For an insecticide to effectively control a disease caused by a non-persistent virus it must kill the vector very rapidly, repel the vector, or modify the feeding behavior to prevent probing.[1] The electrical penetration graph (EPG) system incorporates the insect and its feeding substrate into an electrical circuit and produces waveform signals that correspond to certain penetration and feeding behaviours.[18–20] This technique has improved our ability to record and monitor changes in the feeding behaviors of aphid vectors of plant diseases caused by insecticide application.[21–23] The onset and duration of feeding probes and the plant tissues contacted during feeding (leaf epidermis, mesophyll, phloem, etc.) influence the likelihood of successful acquisition or inoculation of infectious virus particles.

The GPA is an economically important pest of vegetables and fruit trees that causes direct feeding damage, leaf curling, malformation of fruits and shoots, and secretion of honeydew that fosters sooty mold. Of equal importance, it is considered one of the most important vectors of a wide range of persistent and non-persistent plant viruses.[24] Because of its abundance, widespread distribution, wide host range, and frequent occurrence on greenhouse crops, strains or biotypes of GPA have developed resistance to a greater number of insecticides than perhaps any other crop pest. Screening novel products against GPA that have greater specificity to aphids is a desirable contribution to the management of these pests that could minimize insecticide resistance and prolong the effectiveness of existing products.

Turnip mosaic virus (TuMV) (family Potyviridae: genus *Potyvirus*), which causes major losses to crops mostly belonging to the Cruciferae family,[25] was chosen as a model system for our laboratory studies. Four strains of TuMV have been identified in southern Ontario,[26] with the highest incidence recorded on rutabaga, *Brassica napobrassicae* (L.), and winter canola, *B. napus* (L.). During epidemic years, infection rates for rutabaga in some areas of southwestern Ontario have reached 80–100% with complete crop loss.[27] Rutabaga was chosen as the experimental host due to ease of production, distinctive symptomology, suitability as a host for GPA, and our prior experience working with it in our TuMV research.[5,28]

The aims of this study were: to investigate the relative toxicities of nine insecticides, five of which have only recently been registered for the control of GPA in Canada; monitor their effects on GPA probing and feeding activity using the EPG system; and evaluate their effectiveness for reducing transmission of TuMV to rutabaga.

2 MATERIALS AND METHODS

2.1 Aphid culture

GPA from an existing colony maintained at AAFC-Vineland were reared in ventilated Plexiglass cages on Bok choy [*Brassica rapa*, subspecies *pekinensis* var. Heavy (422E), Stokes Seeds, St. Catharines, ON, Canada] under fluorescent lighting on a 16:8 h light/dark photoperiod. Ceramic plant-watering spikes (Lee Valley Tools, Burlington, ON, Canada) were inserted in each pot to minimize exposure of the plants to outside aphid contamination through hand-watering. Prior to use, plants were grown in plastic pots in a glasshouse at 22 ± 2°C under a 16:8 h light/dark photoperiod supplied by artificial lighting, and were fertilized weekly with a 20:20:20 (NPK) soluble fertilizer.

2.2 Insecticide efficacy bioassays

Nine insecticides from five classes, as outlined in Table 1, were tested in a leaf-disk dip-bioassay to obtain baseline dose–response relationships and determine diagnostic lethal concentrations to GPA. The same insecticides were used in the EPG feeding behavior studies and TuMV transmission trials. λ-Cyhalothrin (Matador™) was included to provide a comparison with previous research. Because of their frequent use and demonstrated effectiveness for the control of persistent plant viruses, we included three neonicotinoids with slightly differing characteristics and registration dates. Five novel insecticides belonging to four classes registered recently in Canada for the control of aphids were also evaluated for their toxicity to GPA. Stock solutions (10×) of the commercially formulated insecticides were prepared according to the labels in a 0.025% solution of Agral 90™ (Syngenta Canada) in deionized water. Further serial dilutions were prepared with water.

Preliminary tests including an Agral 90 only control were first carried out for each insecticide to determine effective concentration ranges for later evaluation. Bioassays were conducted using leaf disks of peach dipped in test solutions as outlined by Lowery and Simirle.[29] Disks were cut from leaves of peach using a 10-mm diameter cylindrical cork borer and submerged for ∼ 5 s in 20 ml of the different concentrations of each tested insecticide or the control solution. After the treated leaf disks had been allowed to dry in a fume hood for ∼ 1 h, they were transferred on a damp filter papers, four per dish, to 5-cm self-sealing Petri dishes (VWR Scientific), with ten small holes burnt through the lids of the dishes for ventilation. Dishes were placed upside-down on moistened Kim Wipe within a clear plastic container, and 10 fourth-instar GPA per leaf disk were placed on the treated leaf disks in each dish using a fine, moistened paintbrush. Containers with each replicate of an insecticide bioassay were maintained in a growth chamber at 22 ± 1.0°C, 65 ± 5% relative humidity and 16:8 h light/dark photoperiod. Numbers of live aphids were assessed after 48 and 72 h. Each insecticide was tested at five concentrations and ten replicates, resulting in 100 aphids per concentration. Aphid mortality was based on the inability of the aphid to withdraw their stylet or walk in a coordinated manner after being touched gently with a fine brush.

2.3 Electrical penetration graph experiments

An EPG was used to monitor plant penetration feeding activities by single apterous adult GPA on 5-week-old peach seedlings treated with the abovementioned insecticides at the field application rate. The copper insect input electrode consist of the brass insect nail, copper wire and gold wire. The insect electrode was prepared by attaching the aphid dorsum to a 2–4 cm-long, thin gold wire (20 µm in diameter) using water-soluble conductive silver glue. The opposite end of the gold wire was attached to a copper electrode (2–3 cm), long head of a 3-mm diameter brass nail, 1 mm in diameter.[19] The plant output electrode was a 10 cm copper post (2 mm in diameter) inserted into the plant pot. Aphids attached to the gold wire were left to starve for 2 h and then connected to the DC-EPG device (Giga-4; EPG System).[20] The EPG acquisition procedure was performed inside a Faraday cage to prevent

Table 1. List of insecticides included in the outlined efficacy, feeding and virus transmission trials; their chemical classes (Insecticide Resistance Action Committee), insecticidal modes-of-action, and application activity

Insecticide (product)	Chemical class	Mode-of-action	Activity
λ-Cyhalothrin (Madator 120 EC®)	3 pyrethroid	Disrupts nerve sodium channel activation gate	Non-systemic contact and oral activity; some repellency
Acetamiprid (Assail 70 WP®)	4A neonicotinoid	CNS nicotinic acetylcholine receptor agonist	Contact and oral activity; systemic, translaminar
Flupyradifurone (Sivanto-200®)	4A neonicotinoid	CNS nicotinic acetylcholine receptor agonist	Contact and oral activity; systemic, translaminar
Sulfoxaflor (Closer 240 SC®)	4C neonicotinoid	CNS nicotinic acetylcholine receptor agonist	Contact and oral activity; systemic, translaminar
Pymetrozine (Endeavour 50 WG®)	9B pyridine azomethines	Acts on chordotonal mechanoreceptors (stretch receptors) to inhibit feeding	Contact and oral systemic activity against piercing–sucking insects
Flonicamid (Beleaf 50 SG®)	9C pyridinecarboxamide	Targets insect potassium A-type channel; rapidly inhibits feeding	Systemic, translaminar persistent oral activity
Tolenpyrad (Torac-15 EC®)	21A Pyrazole-5-carboxamide derivative	Mitochondrial respiration inhibitor	Foliar contact
Spirotetramat (Movento 24SC®)	23 tetramic acid derivative (ketoenole)	Interferes with lipid biosynthesis.	Systemic, persistent foliar or root drench.
Cyantraniliprole (Cyazypyr 200SC®)	28 anthranilic diamide	Activates ryanodine receptor modulators	Systemic, translaminar broad spectrum activity

Suppliers: Madator 120 EC® and Endeavour 50 WG®, Syngenta Crop Protection Canada Inc., Guelph, ON, Canada; Sivanto-200® and Movento 24SC®, Bayer Crop Science, Triangle Park, NC, USA; Closer 240 SC®, Dow Agroscience, Indianapolis, IN, USA; Beleaf 50 SG®, FMC Corp., Agr. Products Group, Concord, OH, USA; Torac-15 EC®, Nichino America, Wilmington, DE, USA; Assail 70 WP® and Cyazypyr 200SC®, E.I. DuPont Canada Co., Mississauga, ON, Canada.

electrical background interference. EPG signals were recorded for 1 h immediately after aphids were placed on a peach leaf, as this is the maximum time that Plum pox virus (PPV) is retained on aphid stylets after they start feeding.[30,31] All experiments were carried out under laboratory conditions (22 ± 1°C), with 20 replicates for each treatment. Data acquisition was performed using STYLET$^+$ software (Giga-4; EPG System) and processed in an Excel® spreadsheet (Microsoft). The EPG waveform was defined as follows: non-probing (np), intercellular apoplastic stylet pathway (C), and intracellular stylet puncture potential drops (pd).[19,20]

2.4 Effects of insecticides on TuMV transmission

Transmission of TuMV by GPA to rutabaga plants treated with commercial formulations of tolfenpyrad, flupyradifurone, sulfoxaflor and the other insecticides was evaluated under greenhouse conditions. Rutabagas, cv. Laurentian 370 (Stokes Seeds), were grown in a greenhouse in plastic pots at 22 ± 2°C under a 16:8 h light/dark photoperiod and fertilized weekly with a 20:20:20 (NPK) soluble fertilizer. At the age of 4 weeks, plants were sprayed with an aqueous solution of the insecticides at the recommended field application rate using a small hand-held atomizer, or with water for the control.

The procedure used to transmit TuMV with GPA was that outlined by Lowery *et al.*[5] Fourth-instar nymphs and apterous adult GPA were transferred using a fine artist's brush to 5-cm Petri dishes (VWR Scientific) with tight-fitting lids, and were starved at ambient temperature (22 ± 2°C) for a minimum of 2 h prior to connection of the virus-acquisition probes. Aphids were then allowed to feed on rutabaga leaf pieces from plants infected with the virus for 5 min. Aphids, 20 per plant, were then transferred onto plants sprayed previously with one of the insecticides in Table 1. Seedlings were sprayed with a selective aphicide, Pirimor™ (pirimicarb), 24 h prior to insecticide treatment. Following transfer of the viruliferous aphids, seedlings were sealed in 9 kg polyethylene bags and stored in the dark in plastic lidded Rubbermaid™ tubs for 48 h, after which time the bags were removed. Seedlings were then transferred to containment rooms and grown for an additional 3 weeks (22 ± 2° C, 4100 lux halide lighting × halide lighting, 16:8 h light/dark photoperiod). When rutabaga plants were visually assessed for foliar symptoms of TuMV infection, fully expanded leaves were macerated in enzyme-linked immunosorbent assay (ELISA) extraction buffer (0.5 g tissue plus 3 ml extraction buffer)[32] in 12 × 14-cm sample extraction bags (Bioreba AG). Samples were tested in duplicate wells using ELISA as previously described by Stobbs and Shattuck.[26]

2.5 Data analysis

Insecticide bioassays with control mortalities > 10% were discarded and repeated. Aphid natural mortality was corrected using Abbott's formula[33] for each insecticide. Median and 90% lethal concentration (LC_{50} and LC_{90}) values and their 95% confidence limits (95% CL) were calculated from probit regressions using SAS software (SAS Institute, Inc.). EPG parameter values were established for each individual aphid, and the mean and standard deviation of the mean (SEM) of the total duration and frequency of occurrence were calculated, as were the times needed to initiate the first pathway, intracellular punctures potential drop (pd), and phloem phases. Parametric differences were analyzed using Duncan's multiple range test, whereas non-parametric recordings were tested by Fisher's exact test.[34] All inoculation data from the TuMV transmission trials were analyzed by analysis of variance (ANOVA) using the General Linear Models (PROC GLM) procedure,[35] with Duncan's multiple range test used to separate between the means.

Table 2. LC_{50} and LC_{90} values for green peach aphid adults after 48 h exposure to peach leaf disks dipped in different concentration of insecticides

	Aphid mortality after 48 h exposure									
Insecticide	No. of GPA used	Regression equations[1]	χ^2 (df)	Slope ± SE	LC_{50}* (µg a.i./L)	95% CL	LC_{90}* (µg a.i./L)	95% CL	RFR (µg a.i./L)	Ratio[2]
Acetamiprid	328	$y = -0.67 + 0.11x$	30.50 (6)	2.24 ± 0.34	17.67^{d}	14.78–21.58	163.82^{b}	108.90–287.16	60.2	2.72
Tolfenpyrad	398	$y = -0.19 + 0.09x$	23.45 (3)	0.86 ± 0.16	27.84^{c}	23.39–32.84	186.82^{ab}	146.08–252.78	296	0.63
Flupyradifurone	384	$y = -0.89 + 0.10x$	13.16 (3)	0.98 ± 0.17	7.99^{c}	6.10–10.31	64.12^{ab}	43.91–107.60	75	1.34
Sulfoxaflor	331	$y = -3.49 + 0.20x$	6.03 (5)	0.76 ± 0.51	1.45^{a}	0.76–2.34	101.7^{ab}	41.19–514.48	48	0.68
Flonicamid	386	$y = -0.85 + 0.06x$	24.78 (3)	3.46 ± 0.49	97.24^{f}	79.66–137.97	367.21^{c}	221.73–1033	80	4.59
Pymetrozine	394	$y = -3.44 + 0.28x$	103.66 (8)	1.17 ± 0.12	30.1^{e}	22.6–40.6	603.3^{c}	245.7–2288	10	6.03
λ-Cyhalothrin	384	$y = -1.28 + 0.11x$	99.77 (9)	1.48 ± 0.09	4.57^{b}	4.04–5.18	43.81^{a}	34.48–58.22	12.5	3.51
Cyantraniliprole	303	$y = -4.45 + 0.29x$	16.24 (6)	0.61 ± 0.16	5.26^{bc}	2.77–8.35	212.4^{dc}	93.83–730.4	150	1.42
Spirotetramat	356	$y = -0.39 + 0.14x$	9.70 (6)	3.81 ± 0.32	167.87^{g}	131.12–231.24	322.1^{bc}	166.0–851.6	104.4	3.09

Mortality in all control treatments was always < 10%. Results are presented with corresponding 95% confidence limits (CL), Pearson chi-square results, degree of freedom (df) and regression equations.
GPA, green peach aphid; RFR, recommended field rate in (µg a.i./L).
[1] Regression equations were estimated by probit regression.
[2] Ratio = LC_{90} value (µg a.i./L) divided by RFR. Lower ratio indicates that the pesticides are more toxic at LC_{90} value.
*LC_{50} and LC_{90} values in (µg a.i./L) having different letters are significantly different (95% CL did not overlap).

3 RESULTS

3.1 Insecticide efficacy bioassays

Lethal concentrations, i.e., LC_{50} and LC_{90} values, calculated from log-dose probit mortality regression are given for each insecticide in Table 2. GPA control mortalities from these leaf disk bioassays were always < 10% after 48 h and < 20% after 72 h. Diagnostic LC_{50} values after 48 h differed significantly among insecticides ($\alpha = 0.05$), with the highest mortalities recorded for sulfoxaflor (1.45 µg a.i./L), λ-cyhalothrin (4.57 µg a.i./L), cyantraniliprole (5.26 µg a.i./L) and flupyradifurone (7.99 µg a.i./L). The highest LC_{50} values were recorded for spirotetramat (167.87 µg a.i./L) and flonicamid (97.24 µg a.i./L) (Table 2). After 48 h, LC_{90} values were lowest for λ-cyhalothrin (43.81 µg a.i./L), flupyradifurone (64.12 µg a.i./L) and sulfoxaflor (101.7 µg a.i./L), and highest for pymetrozine (603.3 µg a.i./L) and flonicamid (367.2 µg a.i./L) (Table 2). Slopes of the probit regression lines for the 48 h bioassays (Table 2) ranged from 0.61 and 0.76 for cyantraniliprole and sulfoxaflor, respectively, to 3.46 for flonicamid and 3.81 for spirotetramat.

There were significant differences in mortality of GPA among insecticides (non-overlapping 95% LC) for the 72 h bioassays. The lowest LC_{50} values (highest mortality) were recorded for sulfoxaflor (0.23 µg a.i./L) and cyantraniliprole (0.63 µg a.i./L); similarly after 72 h, the highest were recorded for spirotetramat (34.53 µg a.i./L) and flonicamid (41.97 µg a.i./L) (Table 3). Based on LC_{90} values after 72 h, sulfoxaflor, cyantraniliprole and acetamiprid were again among the most toxic, but toxicity was now highest for flupyradifurone (LC_{90} = 10.26 µg a.i./L). As recorded after 48 h, the lowest mortality after 72 h occurred for spirotetramat, pymetrozine and flonicamid, with all three LC_{90} values exceeding 420 µg a.i./L (Table 3). Slopes of the regression lines for the tested insecticides after 3 days in increasing order were: λ-cyhalothrin (0.12), sulfoxaflor (0.15), cyantraniliprole (0.30), flupyradifurone (0.50), acetamiprid (0.51), pymetrozine (0.87), spirotetramat (1.55), tolfenpyrad (1.87), and flonicamid (3.51) (Table 3).

Comparison of the ratios of the 48 h LC_{50} (µg a.i./L) values of the tested insecticides to their recommended field rates (RRR) (Table 2) showed the lowest ratios of < 1.0 for tolfenpyrad (0.63) and sulfoxaflor (0.68), and the highest ratios for flonicamid (4.59) and pymetrozine (6.03). After 72 h, ratios were lowest for cyantraniliprole (0.13), sulfoxaflor (0.19) and flupyradifurone (0.21), and highest for pymetrozine (4.21), spirotetramat (4.52) and flonicamid (5.43) (Table 3).

3.2 Electrical penetration graph experiments

The EPG results with GPA are shown in Table 4. Different waveforms and their correlations with aphid behavior were described according to Tjallingii and Gabrys[19] as follows: non-probing (waveform np); pathway phase (waveform A–C) and potential drop (waveform pd) reflecting an intercellular stylet pathway with intracellular punctures and phloem activities (waveform E1 and E2) reflecting salivation into sieve elements and phloem ingestion respectively. There were significant differences in the time required by GPA to initiate the first pathway phase ($F = 1.67$; $P = 0.169$), phloem phase ($F = 8.53$; $P = <0.0001$), and potential drop phases ($F = 1.49$; $P = 0.218$) on peach seedlings sprayed with the insecticides compared with the control treatment. For the time to initiate the pathway phase, the control treatment differed significantly ($P < 0.05$) from all other treatments (Table 4). Other than λ-cyhalothrin that reduced the average time to the start of pathway phase feeding by ~ 21 s (13.3 vs. 34.1 s for the control), foliar sprays of all the other insecticides delayed the onset of pathway phase feeding from a modest 23 and 29.8 s for flonicamid and pymetrozine, respectively, to as much as 189 s for sulfoxaflor (Table 4). Time to initiate potential drop feeding did not differ significantly ($P > 0.05$) between the control and pymetrozine, flonicamid and cyantraniliprole treatments, whereas λ-cyhalothrin resulted in a significantly shorter ($P < 0.05$) initiation time. The remaining treatments significantly delayed initiation of feeding from ~50 s for pymetrozine to as much as 212.5 s for sulfoxaflor. Time to the initiation of phloem feeding for flupyradifurone, sulfoxaflor, and spirotetramat did not differ significantly ($P > 0.05$) from the control. Times to first phloem feeding for the remaining six test insecticides were significantly shorter than for the control treatment; λ-cyhalothrin had the shortest time, 570.5 s, compared with 1350.0 s for GPA on the control plants (Table 4).

Potential drop of the three sub-phases related to intracellular punctures associated with acquisition and inoculation of non-

Table 3. LC_{50} and LC_{90} values for green peach aphid adults after 72 h exposure to peach leaf disks dipped in different concentration of insecticides

	Aphid mortality after 72 h exposure									
Insecticide	No. of GPA used	Regression equations[1]	χ^2 (df)	Slope ± SE	LC_{50}* (µg a.i./L)	95% CL	LC_{90}* (µg a.i./L)	95% CL	RFR (µg a.i./L)	Ratio[2]
Acetamiprid	328	$y = -1.59 + 0.11x$	3.52 (6)	0.51 ± 0.28	2.09 ^{c}	1.67–2.56	19.19 ^{ab}	14.44–27.23	60.2	0.32
Tolfenpyrad	398	$y = -0.18 + 0.08x$	18.57 (3)	1.87 ± 0.19	20.51 ^{e}	17.31–24.06	116.29 ^{c}	92.53–154.12	295.95	0.39
Flupyradifurone	384	$y = -0.85 + 0.07x$	15.30 (3)	0.50 ± 0.20	2.33 ^{c}	1.80–2.94	10.26 ^{a}	7.65–15.26	48	0.21
Sulfoxaflor	331	$y = -4.76 + 0.23x$	1.72 (5)	0.15 ± 1.12	0.23 ^{a}	0.05–0.51	28.2 ^{ab}	11.98–150.60	150	0.19
Flonicamid	386	$y = -0.39 + 0.11x$	100.62 (3)	3.51 ± 0.46	41.97 ^{f}	33.09–54.31	434.4 ^{d}	250.35–1075	80	5.43
Pymetrozine	394	$y = -2.98 + 0.21x$	4.64 (4)	0.87 ± 0.12	5.53 ^{d}	3.86–7.46	420.9 ^{d}	227.1–1011	100	4.21
λ-Cyhalothrin	384	$y = -2.87 + 0.16x$	63.01 (7)	0.12 ± 0.07	1.2 ^{b}	0.97–1.46	37.12 ^{b}	26.77–55.30	12.48	2.97
Cyantraniliprole	303	$y = -3.16 + 0.16x$	62.58 (6)	0.30 ± 0.11	0.63 ^{a}	0.40–0.88	19.47 ^{ab}	14.71–27.55	150	0.13
S pirotetramat	356	$y = -0.72 + 0.13x$	16.4 (6)	1.55 ± 0.15	34.53 ^{f}	28.74–41.44	471.71 ^{e}	328.84–750.9	104.4	4.52

Mortality in all control treatments was always below 10%. Results are presented with corresponding 95% confidence limits (CL), Pearson chi-square results, degree of freedom (df) and regression equations.
GPA, green peach aphid; RFR, recommended field rate in (µg a.i./L).
[1] Regression equations estimated by probit regression.
[2] Ratio = LC_{90} value in µg a.i./L divided by RFR. A lower ratio indicates that the pesticides are more toxic at the LC_{90} value.
*LC_{50} and LC_{90} values in (µg a.i./L) having different letters are significantly different (95% CL did not overlap).

persistent viruses was recorded over 1 h for GPA feeding on peach seedlings treated with the nine insecticides (Figure 1). Total probing times in sub-phases II-1 and II-2 did not differ between the control and any of the insecticides ($P > 0.05$), whereas the amount of time in sub-phase II-1 was significantly shorter for cyantraniliprole compared with spirotetramat. GPA spent considerably less time feeding in sub-phase II-3 on peach seedlings sprayed with tolfenpyrad, flupyradifurone, and cyantraniliprole than on the control ($P < 0.05$), and there was no difference between those three insecticides (Figure 1). Total time during a 1-h recording ($F = 1.34$; $P = 0.263$) showed no statistical reduction for the other insecticides compared with controls. Number of occurrences in sub-phases II-1, II-2 and II-3 did not differ significantly between the control and all insecticides ($P > 0.05$).

Table 4. Electrical penetration graph feeding and probing parameters recorded over a 1-h period for apterous green peach aphid fed on water- or insecticide-treated peach seedlings. Mean ± SD of total duration (s) and total number of occurrences of the various feeding behaviors

	Time (s) required to initiate the first		
	Pathway	Potential drop	Phloem
Control	34.1 ± 6.1 c	65.8 ± 39.4 b	1350.5 ± 206.5 a
Acetamiprid	148.5 ± 52.8 a	235.3 ± 60.7 a	1096.9 ± 200.9 b
Tolfenpyrad	81.7 ± 21.9 b	273.3 ± 70.7 a	1085.5 ± 215.6 b
Flupyradifurone	117.7 ± 38.8 ab	214.9 ± 66.5 a	1591.1 ± 311.3 a
Sulfoxaflor	223.9 ± 140.8 a	278.3 ± 136.8 a	1336.1 ± 314.2 ab
Flonicamid	57.0 ± 13.5 b	126.8 ± 24.5 b	940.2 ± 234.3 b
Pymetrozine	63.9 ± 12.8 ab	115.7 ± 27.5 b	1040.4 ± 389.6 b
λ-Cyhalothrin	13.3 ± 4.43 b	56.6 ± 23.6 c	570.5 ± 221.1 c
Cyantraniliprole	102.3 ± 35.8 ab	138.3 ± 35.18 ab	1270.6 ± 177.9 b
Spirotetramat	164.3 ± 58.83 a	204.8 ± 79.7 a	2353.9 ± 505.6 a

Within a column, means followed by the same letter are not significantly different ($P > 0.05$) using Duncan's multiple range test.

A 5-min recording was used in this study to examine the impact of insecticide applications on aphid feeding and settling behavior and their potential to transmit non-persistent viruses that only need short and frequent epidermal probes to acquire or inoculate the virus.[1,21,36] During the first 5 min of the recording, GPA non-probing time on peach plants treated with λ-cyhalothrin was significantly prolonged compared with the control; for the other insecticides it was not enhanced statistically ($F = 2.47$; $P = 0.055$) compared with the control. Total duration of the pathway phase ($F = 4.94$; $P = 0.002$) was also non-significantly reduced by half for the aspirotetramat and acetamiprid treatments compared with the control. The pd time ($F = 1.44$; $P = 0.233$) was shortest for flupyradifurone, sulfoxaflor and acetamiprid, but this was not statistically significant. Numbers of occurrences in the non-probing, pathway and pd phases did not differ significantly between the control and all insecticides ($P > 0.05$) (Figure 2). There were no significant differences ($P > 0.05$) in the length of pd salivation feeding or the number of feeding events for sub-phases II-1, II-2, and II-3 ($F = 1.83$; $P = 0.138$) between any of the insecticide treatments and the control (Figure 3).

3.3 Effects of insecticides on TuMV transmission

GPA efficiency transmitting TuMV assessed in the greenhouse with rutabaga plants treated with insecticides at the recommended field application rates showed modest but not statistically significant ($P > 0.05$) reductions in infection rates for acetamiprid, pymetrozine and λ-cyhalothrin of 20–27% compared with the control plants (Table 5). Applications of any of the tested insecticides did not result in a significant reduction in transmission of TuMV by GPA compared with the water only control.

4 DISCUSSION

Insecticide efficacy research has mostly focused on the ability of insecticides to control insect populations and the damage they

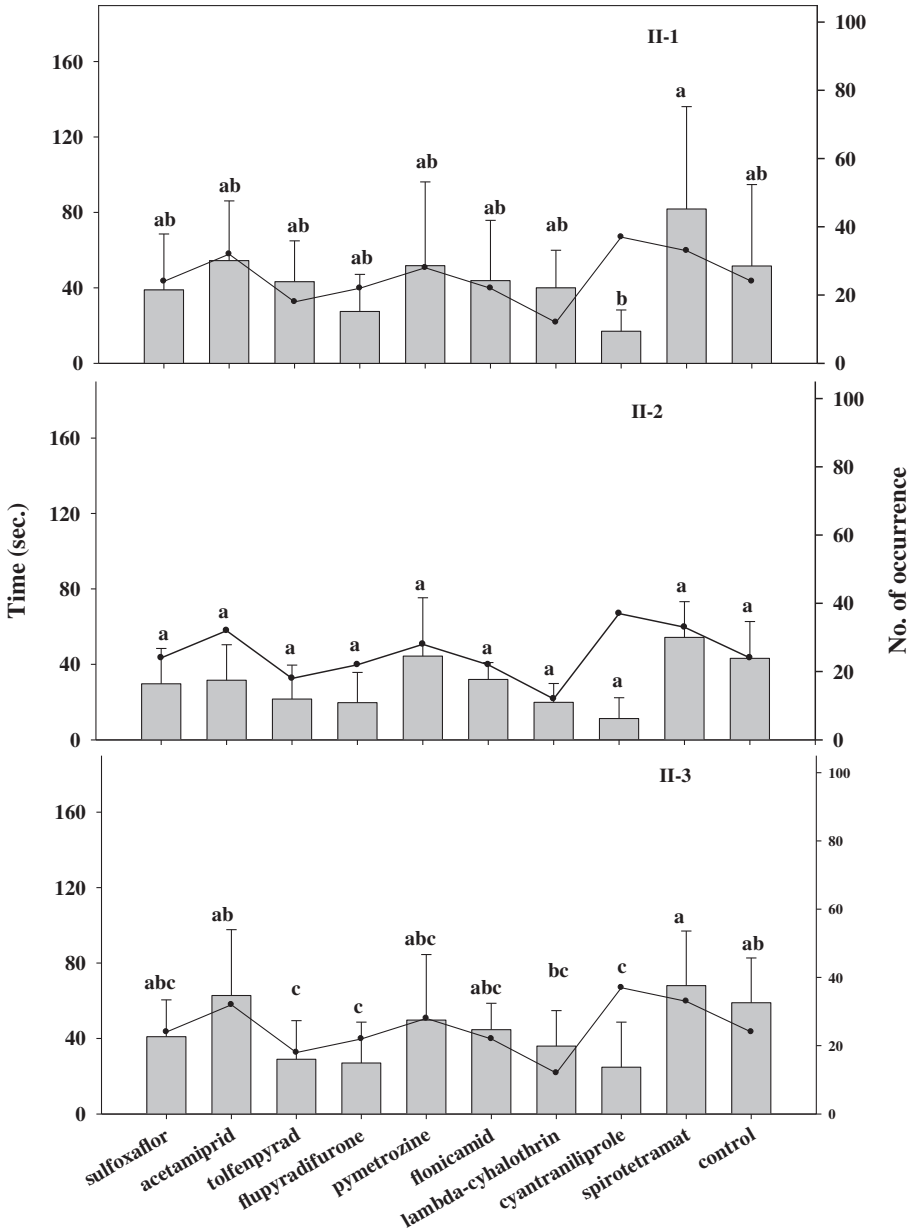

Figure 1. Electrical penetration graph analyses of green peach aphid, *Myzus persicae*, probing and feeding behaviors on peach seedlings treated with insecticides or water as a control. Total duration (s) (bars) and mean number of occurrences (line) of the various intracellular puncture activities of the three sub-phase patterns during 1 h of recording. The three sub-phases related to intracellular punctures associated with acquisition and inoculation of non-persistent viruses. Means followed by the same letter on the bars are not significantly different ($P < 0.05$) based on Duncan's multiple range test. Vertical lines represent the standard deviations of the means. Letters for number of occurrences are not presented owing to no statistical differences.

cause. The latter includes the spread of persistent viruses rather than non-persistent viruses that are generally transmitted too rapidly for insecticides to have any effect. Reduced transmission of non-persistent viruses by GPA primarily depends on how rapidly and actively the insecticide prevents repeated short intracellular punctures of the epidermal and mesophyll cells within the first 5 min of feeding. Five intracellular stylet punctures were found to lead to higher Plum pox virus infection rates,[37] whereas 1–2 s punctures during the intracellular phase gave higher infection rates of Cucumber mosaic virus (CMV) and Potato virus Y.[38] The optimal duration for the acquisition and inoculation of non-persistent viruses through artificial membranes has been reported to be 15–60 s.[38] Recent development of insecticides with different modes of action, including those that act to disrupt feeding by insects having piercing–sucking mouthparts (e.g., pymetrozine, flonicamid), has led to interest in these materials as possible controls for non-persistent viruses. Understanding the toxicity of insecticides to aphids and their effects on their feeding behaviors are both important indicators of their potential to disrupt the transmission of non-persistent viruses.

In agreement with their targeted use against hemipteran crop pests, all of the tested materials were toxic to a greater or lesser extent to GPA based on laboratory bioassays. λ-Cyhalothrin, flupyradifurone and sulfoxaflor were most toxic to GPA after 48 h

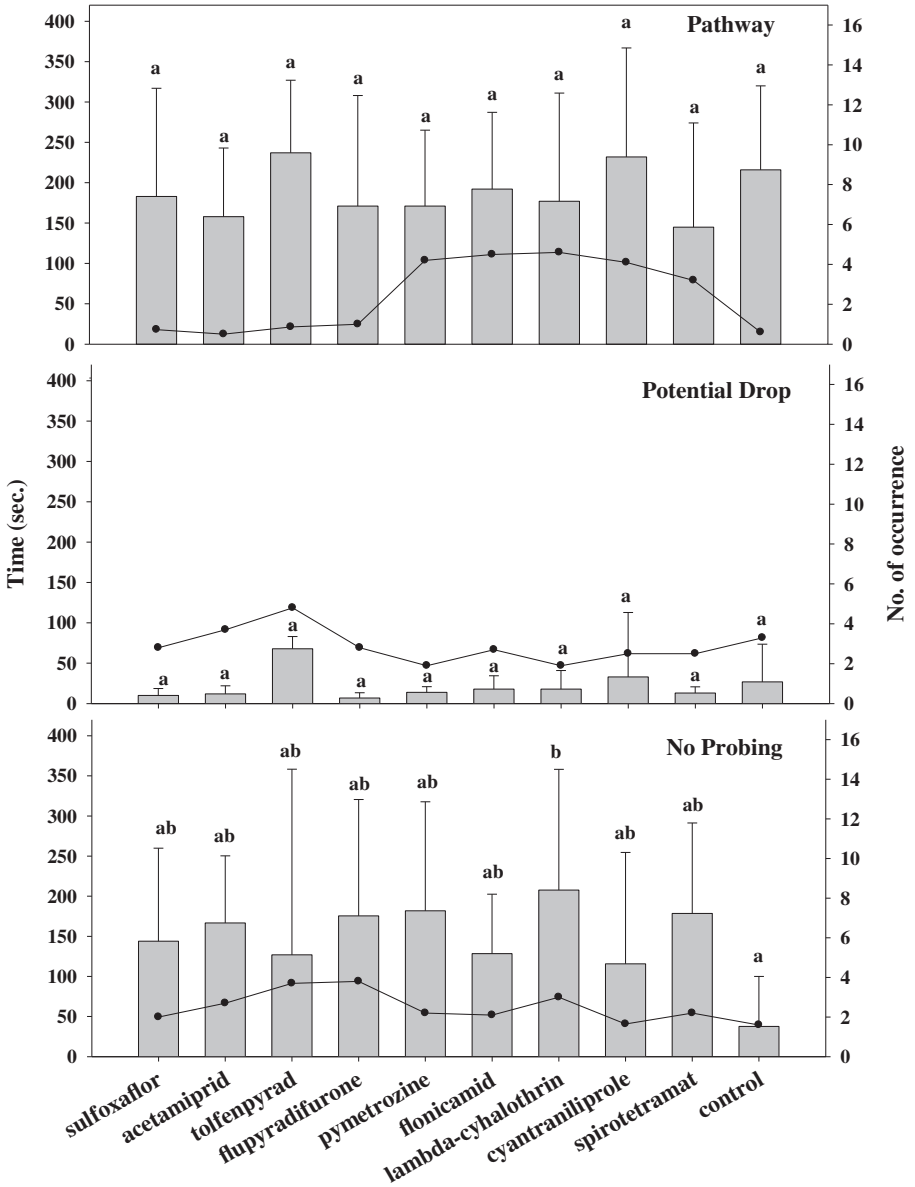

Figure 2. Electrical penetration graph analyses of green peach aphid, *Myzus persicae*, probing and feeding behavior on peach seedlings treated with insecticides or water as a control. Total duration (s) (bars) and mean number of occurrences (line) of the various pathway, pd and np patterns during the first 5 min of recording. Means followed by the same letter on the bars are not significantly different ($P < 0.05$) based on Duncan's multiple range test. Vertical bars represent the standard error of the means. Letters for number of occurrences are not presented owing to no statistical differences.

(Table 2), and sulfoxaflor and tolfenpyrad had the most favorable LC_{90} to RFR ratios. As expected, LC_{90} values were consistently lower for all insecticides after 72 h exposure, with flupyradifurone (LC_{90} = 10.3 µg a.i./L), acetamiprid (LC_{90} = 19.2 µg a.i./L), cyantraniliprole (LC_{90} = 19.5 µg a.i./L) and sulfoxaflor (LC_{90} = 28.2 µg a.i./L) the lowest (Table 3). The lowest LC_{90} to RFR ratios were for flupyradifurone, sulfoxaflor and cyantraniliprole. The intermediate ratio for λ-cyhalothrin (~3) might suggest a low level of resistance development for this laboratory strain of GPA. Evaluation and registration of these insecticides, some having novel modes of action, will aid insecticide resistance management and offer greater selectivity for the integrated management of hemipteran pests.

Our results demonstrate the dichotomy between effective insecticidal control of aphid vectors and their utility in reducing transmission of non-persistent plant viruses. Based on our laboratory bioassay results (Tables 2 and 3), the nine insecticides from five different classes used in this study would provide effective control of the GPA and contribute to the management of persistent viruses that require a lengthy period of aphid feeding for virus acquisition and inoculation. By contrast, our greenhouse spray trials showed only modest non-significant reductions in numbers of rutabaga plants infected with the non-persistent virus TuMV (Table 5).

Although inconsistent in effectiveness, some studies have shown a modest reduction in the spread of non-persistent viruses with applications of fast-acting pyrethroid insecticides.[5,9] Our results with the pyrethroid λ-cyhalothrin showed it to be one of the most toxic to GPA after 48 and 72 h exposure (Tables 2 and 3). During the 1-h EPG monitoring, times to initiate pathway, potential drop and phloem feeding were significantly shorter

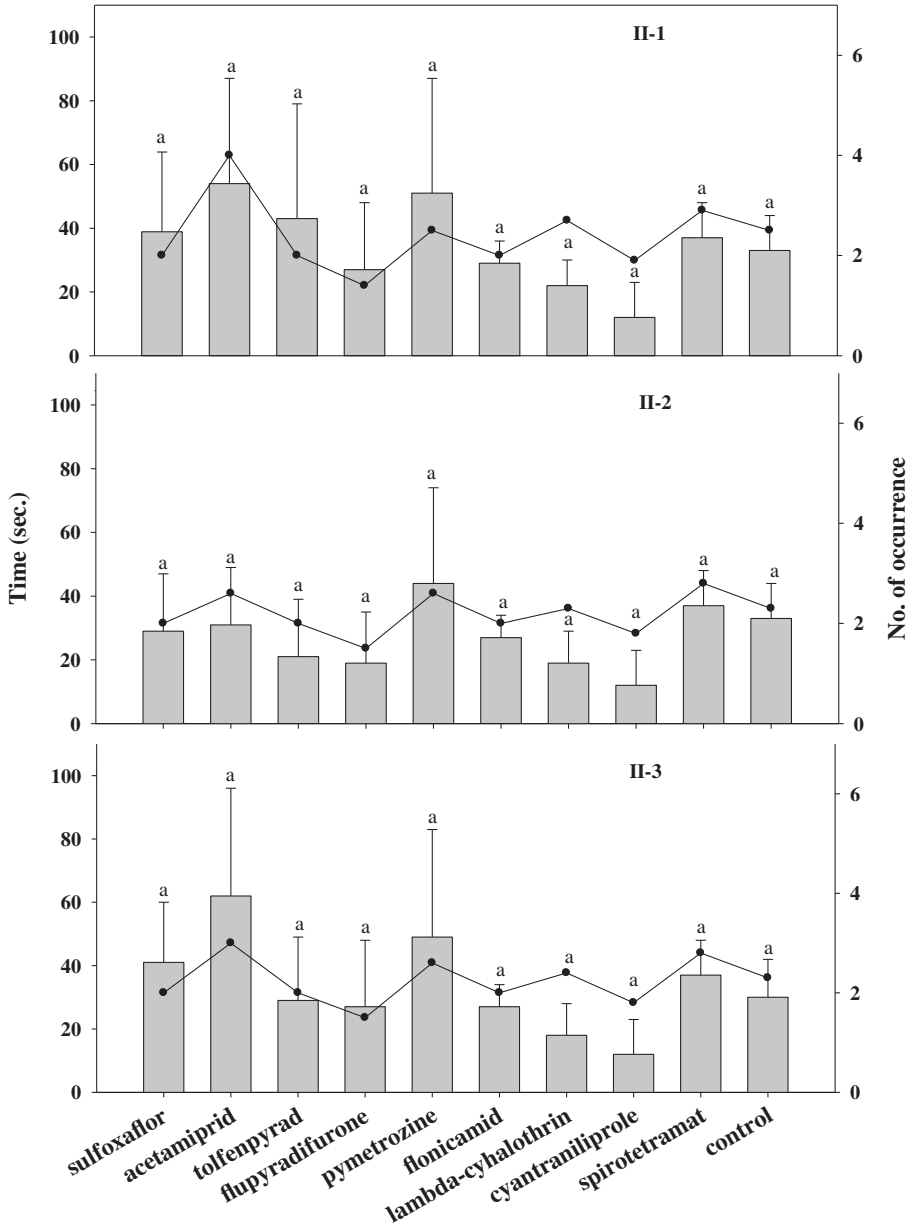

Figure 3. Electrical penetration graph analyses of green peach aphid, *Myzus persicae*, probing and feeding behavior on peach seedlings treated with insecticides or water as a control. Total duration (s) (bars) and mean number of occurrences (line) of the various intracellular puncture activities of the three sub-phase patterns during the first 5 min of recording. Means followed by the same letter on the bars are not significantly different ($P < 0.05$) based on Duncan's multiple range test. Vertical bars represent the standard error of the means. Letters for number of occurrences are not presented owing to no statistical differences.

($P < 0.05$) on peach sprayed with λ- cyhalothrin compared with the control (Table 4), but GPA spent significantly less time probing on the λ-cyhalothrin-treated plants than on the control during the first 5 min (Figure 2). λ- Cyhalothrin reduced TuMV infection of rutabaga by a modest and non-significant 20.2% relative to the control (Table 5), which is similar to the 29.8% reduction in TuMV infection rates achieved in a laboratory trial using GPA and sprays of cypermethrin.[5]

Research by Qureshi *et al.*[39] showed a greater reduction in numbers of adult Asian citrus psyllid, *Diaphorina citri*, with tolfenpyrad and sulfoxaflor compared with flupyradifurone and acetamiprid.[39,40] Tolfenpyrad was reportedly a highly selective stomach poison against cotton bollworm, *Helicoverpa armigera*, and diamondback moth, *Plutella xylostella*, and also antifeedant to bean aphid, *Aphis craccivora*, mosquito, *Culex pipiens pallens*, and spider mite, *Tetranychus cinnabarinus*.[41] Our results show tolfenpyrad to be moderately toxic to GPA but with a favorable LC_{90} to RFR ratio (Table 3); it delayed the onset of pathway, potential drop and phloem feeding over a 1-h EPG bioassay (Table 4), but had no significant effect on GPA feeding during the first 5 min (Figure 2). Sprays of tolfenpyrad to rutabaga did not reduce the transmission of TuMV (Table 5). Reflecting the widespread use of neonicotinoid insecticides, research with hemipteran pests has shown sulfoxaflor to be highly toxic to GPA,[42] cotton aphids, *Aphis gossypii*, whiteflies, and leafhoppers,[43] Asian citrus psyllid,[44] and tarnished plant bug[45]; whereas flupyradifurone was highly

Table 5. Effect of foliar applications of insecticides or water as a control on percent infection of rutabagas with Turnip mosaic virus following inoculation by the GPA, *Myzus persicae*

Treatment	No. platns tested	Infected plants	Mean % infection*	Std	% Reduction
Control	111	76	68.4 a	22.28	
Acetamiprid	79	39	49.8 a	30.94	27.2
Sulfoxaflor	83	50	60.6 a	29.73	11.4
Flupyradifurone	71	47	66.5 a	25.28	2.8
Tolfenpyrad	61	34	55.0 a	21.01	13.4
Flonicamid	64	36	56.7 a	24.8	17.1
Cyantraniliprole	70	49	70.3 a	23.6	(−2.7)
Pymetrozine	68	35	52.4 a	22.1	23.4
λ-Cyhalothrin	68	37	54.6 a	24.7	20.2
Spirotetramat	66	50	75.7a	20.0	(−10.7)

*Means followed by the same letter are not significantly different ($P > 0.05$; using Duncan's multiple range test).

effective against aphids,[46,47] whiteflies,[48] and Asian citrus psyllid.[39] Acetamiprid was shown to have both toxic and reproductive effects on the greenbug, *Schizaphis graminum*.[49]

EPG studies of GPA feeding on pepper plants treated with cyantraniliprole and imidacloprid by Jacobson and Kennedy[23] showed both materials significantly reduced the total amount of time probing, mean time phloem feeding, and mean number of intracellular probes over a 4-h period compared with a water-treated control. The authors conclude, however, that the alteration in feeding over a longer time frame would not prevent the transmission of non-persistent viruses, as has been shown for Potato virus Y infection of potato sprayed with imidacloprid.[50] Our results showed acetamiprid and cyantraniliprole are highly toxic to GPA (Tables 2 and 3), and delay the onset of pathway and potential drop feeding (Table 4), but there were no significant differences in probing behaviors during the critical first 5 min of feeding (Figures 2 and 3). Sprays of acetamiprid and cyantraniliprole to rutabaga did not reduce transmission of TuMV to a significant degree (Table 5). Flupyradifurone and sulfoxaflor were highly toxic to GPA (Tables 2 and 3) and EPG recordings showed they reduced the mean time and occurrences of the pathway and pd phases, but did not reduce transmission of TuMV (Table 5). Acetamiprid and tolfenpyrad were less toxic to GPA, and EPG recording showed longer mean time and a higher occurrence of pathway and pd feeding, but the slight reduction in TuMV infection rates was not significant (Table 5). Sprays causing increased agitation of aphids and frequent shorter probes may stimulate movement to neighboring plants and increase virus transmission and infection rates.[5,7,9]

5 CONCLUSION

This research provides baseline data on the relative toxicities of several established and novel insecticides to the GPA. Utilization of the EPG system showed effects on feeding behaviors over a 1-h period, but no significant reduction in intracellular probing of the epidermis and mesophyll during the critical first 5 min of probing when non-persistent viruses are acquired and inoculated. The nine insecticides from five insecticide classes would help manage the spread of persistent plant viruses that require longer periods of feeding, but our results showed only modest and non-significant reductions in transmission of non-persistent TuMV by the GPA.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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