APHIDS AND OTHER HEMIPTEROUS INSECTS | VOL.17 (2011) 129–136

Effect of garlic (*Allium sativum* L.) and tansy (*Tanaceum vulgare* L.) extracts and potassic horticultural soap on the probing and feeding behaviour of *Myzus persicae* (Sulzer, 1776)

Katarzyna Dancewicz[1], Beata Gabryś[2], Marta Przybylska

Department of Botany and Ecology, University of Zielona Góra
Szafrana 1, 65-516 Zielona Góra, Poland
[1] k.dancewicz@wnb.uz.zgora.pl; [2] b.gabrys@wnb.uz.zgora.pl

ABSTRACT

The probing and feeding activity of the peach potato aphid - *Myzus persicae* (Sulzer, 1776) on plants treated with preparations of potassic horticultural soap, alone and in combination with extracts of garlic (*Allium sativum* L.) and tansy (*Tanaceum vulgare* L.) was monitored using the EPG (Electrical Penetration Graph) technique. The total probing time in peripheral tissues and the proportion of sap ingestion activity among all aphid activities in plant tissues were similar in all aphids irrespective of an applied treatment. Statistically significant differences occurred in aphid activities in sieve elements. The lowest proportion of probes that contained phloem phase, the longest duration of the first phloem salivation period, and the highest proportion of salivation in aphid activities in the phloem occurred in aphids on garlic+1% soap- and garlic+tansy-treated leaves. The results of the EPG experiments demonstrate that these preparations show the postingestional deterrent activity.

KEY WORDS: antifeedants, probing behaviour, EPG, aphids

INTRODUCTION

Phytochemicals and plant extracts have long been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impact. Plant extracts obtained from garlic (*Allium sativum* L.) and tansy (*Tanaceum vulgare* L.) have a broad spectrum of biological activity.

They have anti-inflammatory, antibacterial and antifungal activity (Yin & Tsao, 1999; Samuel *et al*., 2000; Harris *et al*., 2001; Keskitalo *et al*., 2001; Curtis *et al*., 2004; Lahlou *et al*., 2008). Water and methanolic extracts of garlic are larvicidal against red flour beetle *Tribolium castaneum* (Herbst, 1797), maize weevil *Sitophilus zeamais* Motschulsky, 1855, several species of mosquitoes, cluster caterpillar *Spodoptera litura* (Fabricius, 1775), and the lymantriid *Euproctis* spp., (Ho *et al*., 1996). Water extracts of tansy have antifeedant and insecticidal effect on larvae and adults of some species of Lepidoptera and Coleoptera (Larocque *et al*., 1999). In addition, garlic extracts have been reported to exhibit insecticidal activity against peach potato aphid *Myzus persicae* (Sulzer, 1776) and cabbage aphid *Brevicoryne brassicae* (Linneus, 1758) (Achremowicz & Cież, 1988).

The peach potato aphid *Myzus persicae* is a polyphagous aphid species that feeds on secondary hosts of over 40 different plant families. It is also the most important vector of more than 50% of insect-borne plant viruses (Katis *et al*., 2007). At present, aphid control depends mainly on the use of insecticides. Due to the repeating applications, many aphid species, especially the peach potato aphid, have developed resistance to several chemical aphicides. The use of targeted chemicals that would repel aphids or deter their feeding is one of the most promising approaches to population control.

In the previous study (Dancewicz & Gabryś, 2008), it had been found that the settling of *M. persicae* on plants was strongly deterred by certain preparations of potassic horticultural soap, alone and in combination with extracts of garlic and tansy. The deterrent effect was observed for at least 24 hours after application. The aim of the present study was to investigate the behavioural backround of the settling deterrent activity of those products. The probing and feeding activity of aphids was monitored using the EPG (Electrical Penetration Graph) technique that provides a possibility to trace aphid stylet activities in plant tissues. The parameters describing aphid behaviour during probing and feeding, such as the total time of probing, number of probes, duration of phloem sap ingestion, duration of sap ingestion from one sieve element, etc., are good indicators of plant suitability to or interference of probing by chemical or physical factors in particular plant tissues (Mayoral *et al*., 1996).

MATERIAL AND METHODS

Aphids and plants

Aphids (*M. persicae*) and plants (Chinese cabbage *Brassica pekinensis* (Lour.)) were reared in a laboratory at 20°C, 65% r.h., and L16:8D photoperiod. Young, 2-3 days old viviparous apterous females were selected for experiments. Cabbage plants used in the bioassays were 5-6 weeks old.

Extracts: The activity of the following preparations was evaluated:
E1 - Potassic horticultural soap 1%
E2 - Potassic horticultural soap 4%
E3 - Bioczos (garlic extract) FORTE (2%) and potassic horticultural soap 1% (1:1)
E4 - Bioczos (garlic extract) 1% and tansy extract 1% (1:1)

The preparations were kindly provided by HIMAL PPH (Łódź) company and were used as water solutions. Water was used as a control in all assays. Test leaves were dipped in the experimental solutions for 10 seconds and allowed to dry before being offered to aphids.

Aphid probing and feeding behaviour

Aphid probing and especially the phloem sap uptake was monitored using the technique of electronic registration of aphid probing in plant tissues, known as EPG, that is frequently employed in insect–plant relationship studies (Tjallingii, 1995). In this experimental set-up, an aphid and a plant are made parts of an electric circuit, which is completed when the aphid inserts its stylets into the plant. Weak voltage is supplied in the circuit, and all changing electric properties are recorded as EPG waveforms that can be correlated with aphid activities and stylet position in plant tissues. Aphids were attached to a golden wire electrode with conductive silver paint and starved for 1h prior to the experiment. Probing behaviour of 12 apterous females per studied plant/aphid combination was monitored for 8h continuously with a four-channel DC EPG recording equipment. Each aphid was given access to a freshly prepared plant. Signals were saved on the computer and analysed using the PROBE 3.1 software provided by W. F. Tjallingii (www.epg-systems.eu). The following aphid behaviours were distinguished: non penetration (baseline 'np' in EPG recording – aphid stylets outside the plant), pathway phase – penetration of non-phloem tissues: epidermis and mesophyll (waveforms 'ABC'), watery salivation into sieve elements (waveform 'E1'), ingestion of phloem sap (waveform 'E2'), and ingestion of xylem sap (waveform 'G') (Fig. 1). The parameters derived from EPGs were analysed according to their frequency and duration in the configuration related to activities in peripheral and vascular tissues.

The EPG parameters were analysed using Mann-Whitney U test, available in STATISTICA 8 package (StatSoft, USA), at $p < 0.05$.

RESULTS AND DISCUSSION

The electronic registration (EPG) of the peach potato aphid behaviour on leaves treated with plant extracts and horticultural soap revealed waveform 'C' (pathway activities) that represented probing in mesophyll and waveforms 'E1' and 'E2' that indicated salivation in phloem vessels and ingestion of sap, respec-

tively. Occasionally, waveforms 'F' and 'G' occurred, which reflected difficulties in stylet penetration and ingestion of xylem sap, respectively. Due to their sporadic appearance, the waveforms 'F' and 'G' were included in pathway activities ('C') in all calculations (Tab. 1).

The total time that aphids spent on the probing of peripheral tissues (i.e., so-called pathway activity) was similar in all aphids irrespective of the treatment and in comparison with the control, and ranged from 69 to 85 percent of experimental time on garlic+soap- and 1% soap-treated leaves, respectively. Likewise, there was no significant difference in the proportion of sap ingestion activity among all aphid activities in plant tissues. The total time preceding the first phloem phase was similar in all aphids (2-3 hours on average) and the non-probing acitivities occcupied 0.3 (on 1% soap treated plants) to 0.8 (on soap+garlic treated plants) hour, i.e., 13 to 26 percent of that time. Aphid probing was rarely interrupted (there was a similar number of probes in all aphids) and the probes were relatively long (Tab. 1). Among the probes, probes longer than 2 minutes predominated, which meant that they reached beyond epidermis and in the case of probes longer than 10 minutes, they might have reached sieve elements (Gabryś *et al*., 1997).

The aphid behaviour during and after the first phloem phase was generally similar. All aphids, except some individuals on soap+garlic-treated leaves, reached phloem vessels but some of them failed to ingest sap in a long-term and continuous manner, i.e., any sap ingestion phase in those aphids was shorter than 10 minutes (Fig. 1). On all plants, except 4% soap- and soap+garlic-treated ones, more than 25% of aphids showed sustained phloem sap ingestion during the first phloem phase.

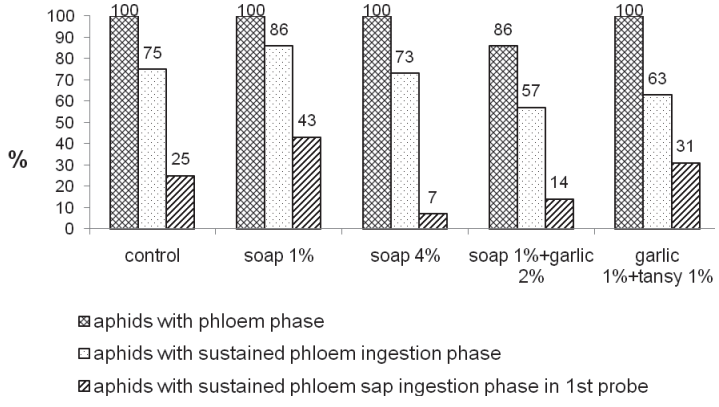

Figure 1. Proportion of aphids that reached phloem vessels (i.e., showed at least waveform E1 – phloem salivation), ingested sap in a sustained manner (i.e., showed waveform E2 – sap ingestion – longer than 10 minutes), and started sustained sap ingestion in the first probe, on plants treated with horticultural soap and garlic and tansy extracts

Table 1. Probing activities of peach potato aphid (*Myzus persicae*) after the application of garlic (*Allium sativum*) and tansy (*Tanacetum vulgare*) extracts and potassic horicultural soap

EPG parameters		Control	PHS 1%	PHS 4%	PHS 1% + garlic 2%	garlic 1% + tansy 1%
General aspects of aphid probing behaviour						
Total duration of probing	min	359.7 (±23.0)	401.3 (±14.2)	357.3 (±20.5)	328.8 (±21.4)	379.9 (±12.8)
Total duration of pathway	min	282.3 (±20.0)	249.3 (±28.0)	250.6 (±21.3)	243.4 (±18.9)	288.6 (±21.5)
Total duration of phloem phase	min	94.3 (±19.4)	152.1 (±37.8)	116.7 (±26.9)	85.5 (±29.9)	91.3 (±26.1)
Total duration of phloem sap ingestion phase	min	81.3 (±18.5)	146.8 (±38.3)	103.6 (±27.2)	76.3 (±29.8)	83.2 (±25.8)
Proportion of phloem phase in total probing	%	24.3 (±4.7)	35.0 (±8.0)	30.0 (±6.2)	21.8 (±6.6)	22.6 (±6.1)
Number of probes	#	34.8 (±3.5)	25.1 (±4.3)	28.9 (±3.0)	33.4 (±4.7)	35.3 (±3.4)
Number of probes with phloem phase	#	3.1 (±0.4)	2.2 (±0.4)	2.6 (±0.5)	*1.9 (±0.3)	2.4 (±0.2)
Activities in peripheral tissues before phloem phase[1]						
Time from 1st probe to 1st phloem phase	min	114.6 (±28.4)	117.9 (±43.5)	137.3 (±22.3)	194.2 (±41.0)	127.9 (±16.6)
Duration of non-probing before 1st phloem phase	min	27.6 (±7.9)	16.4 (±5.7)	36.6 (±11.1)	51.0 (±12.5)	33.0 (±8.5)
Number of probes before 1st phloem phase	#	10.9 (±3.0)	6.7 (±2.3)	10.5 (±2.3)	11.4 (±2.9)	13.1 (±2,0)
Probes <2 min.	#	6.1 (±1.9)	1.9 (±0.6)	4.2 (±1.2)	4.8 (±1.6)	5.1 (±1.2)
Probes 2-10 min.	#	3.9 (±1.2)	2.9 (±1.2)	4.5 (±1.0)	5.2 (±1.2)	5.8 (±1.2)
Probes >10 min.	#	0.9 (±0.4)	1.9 (±0.6)	1.7 (±0.6)	1.4 (±0.4)	2.2 (±0.5)
Activities in sieve elements[1]						
Duration of 1st phloem phase	min	11.3 (±5.5)	49.6 (±28.1)	20.2 (±14.7)	28.1 (±15.2)	24.3 (±13.7)
Duration of 1st phloem sap ingestion phase	min	10.7 (±5.5)	49.5 (±28.0)	46.3 (±29.7)	20.4 (±16.1)	22.8 (±14.6)
Duration of 1st salivation phase	min	0.7 (±0.1)	0.8 (±0.1)	0.9 (±0.1)	*1.8 (±0.5)	*1.0 (±0.1)
Time from 1st phloem phase to 1st sustained phloem sap ingestion phase	min	120.8 (±43.7)	117.9 (±43.5)	110.5 (±34.4)	86.9 (±34.5)	80.1 (±32.0)
Proportion of salivation in phloem phase	%	21.0 (±4.0)	*12.9 (±5.8)	24.0 (±6.9)	27.5 (±8.8)	30.6 (±8.1)
Number of probes after 1st sustained phloem sap ingestion phase	#	13.5 (±4.0)	6.0 (±2.9)	6.4 (±3.3)	5.8 (±1.8)	14.5 (±4.7)

Values represent means ±SE, [1]only aphids that showed a phloem phase (E1), phloem sap ingestion phase (E2) or sustained sap ingestion phase (E2>10 min.) were included in statistical analysis and if an aphid did not show one or all of these phases, the value of a given parameter for such an individual was zero; * statistically significant difference in comparison to control ($p<0.05$; Mann-Whitney U-test)

Statistically significant differences occurred in aphid activities in sieve elements. On garlic+soap- and garlic+tansy-treated leaves, there was observed the lowest proportion of probes that contained phloem phase (6 and 7 percent, respectively) as opposed to probes on the control and 1% and 4% soap treated leaves (8.6, 8.0, and 9.0 percent, respectively). At the same time, the duration of the first phloem salivation period was the longest and the proportion of salivation in aphid activities in the phloem was the highest in aphids on garlic+soap- and garlic+ tansy-treated leaves. The increased duration of salivation was often reported in aphids feeding on resistant varieties of crop plants (van Helden & Tjallingii, 1993; Mayoral *et al*., 1996; Wilkinson & Douglas, 1998) probably because the enzymes present in the saliva might metabolize toxic constituents of allelochemicals during the ingestion of food (Leszczyński, 2001).

Frazier & Chyb (1995) suggested that insect feeding can be inhibited at three levels: preingestional (immediate effect associated with host finding and host selection processes involving gustatory receptors), ingestional (related to food transport and production, release, and digestion by salivary enzymes), and postingestional (long-term effects involving various aspects of digestion and absorption of food). The results of the present study show that neither the potassic horticultural soap nor any of the studied plant extracts had immediate effect on *M. persicae* probing and feeding. This is also true for other aspects of aphid behaviour, such as finding the sieve elements and accepting the phloem sap for sustained ingestion. At the same time, there was a slight increase in proportion of salivation, especially during the first contact with phloem sap and many aphids refused to ingest sap when they reached sieve elements for the first time on the leaves treated with preparations that contained garlic. Nevertheless, all aphids, irrespective of the treatment, consumed considerable amounts of the sap, considering the duration of ingestion periods in the 8-hour experiment. On the other hand, all preparations used in the present study had a deterrent effect on aphid settling (Dancewicz & Gabryś, 2008). It must be stressed that the EPG experiment is a non-choice test used to monitor stylet penetration in plant tissues. The aphid is connected to the electrode, so its movement is limited to a restricted area. In the settling assay, the aphids are free to choose between the control and the treated substrate. On the basis of the EPG experiments, it may be concluded that horticultural soap and extracts of garlic and tansy are neither preingestional nor ingestional feeding deterrents. However, considering the results of the aphid settling experiments and prolonged salivation in some individuals, the postingestional deterrent activity of the studied preparations, especially those containing garlic, is very likely.

REFERENCES

Achremowicz J., Cież W. 1988. Doświadczenia nad skutecznością działania wyciągów z roślin stosowanych jako aficydy. Zesz. Probl. PNR. 353: 53-65.

CURTIS H., NOLL U., STÖRMANN J., SLUSARENKO A.J. 2004. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiol. Mol. Plant P., 65: 79-89.

DANCEWICZ K., GABRYŚ B. 2008. Effect of extracts of garlic (*Allium sativum* L.), wormwood (*Artemisia absynthium* L.) and tansy (*Tanacetum vulgare* L.) on the behaviour of the peach potato aphid *Myzus persicae* (Sulz.) during the settling on plants. Pesticides, 3-4: 93-99.

FRAZIER J.L., CHYB S. 1995. Use of feeding inhibitors in insect control. [In:] Chapman R.F., de Boer G.G. (eds.). Regulatory Mechanisms in Insect Feeding. Chapman & Hall New York, 364-381.

GABRYŚ B., TJALLINGII W.F., VAN BEEK T.A. 1997. Analysis of EPG recorded probing by cabbage aphid on host plant parts with different glucosinolate contents. J. Chem Ecol. 23: 1661-1673.

HARRIS J.C., COTTRELL S.L., PLUMMER S., LLOYD D. 2001. Antimicrobial properties of *Allium sativum* (garlic). Appl. Microbiol. Biotechnol., 57: 282-286.

HO S.H., KOH L., MA Y., HUANG Y., SIM K.Y. 1996. The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch Postharvest Biol. Technol., 9: 41-48.

KATIS N.I., TSITSIPIS J.A., STEVENS M., POWELL G. 2007. Transmission of plant viruses. [In:] van Emden H.F., Harrington R. (eds). Aphids as crop pests. CAB International, 353-390.

KESKITALO M., PEHU E., SIMON J.E. 2001. Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. Biochem. Syst. Ecol., 29: 267-285.

LAHLOU S., ISRAILI Z.H., LYOUSSI B. 2008. Acute and chronic toxicity of a lyophilised aqueous extract of *Tanacetum vulgare* leaves in rodents. J. Ethnopharmacol., 117: 221-227.

LAROCQUE N., VINCENT C., BÉLANGER A., BOURASSA J.-P., 1999. Effects of Tansy Essential Oil from *Tanacetum vulgare* on Biology of Oblique-Banded Leafroller, *Choristoneura rosaceana*. J. Chem. Ecol., 25: 1319-1330.

LESZCZYŃSKI, B. 2001. Rola allelozwiązków w oddziaływaniach owady – rośliny. [In:] Oleszek W., Głowniak K., Leszczyński B. (eds.). Biochemiczne oddziaływania środowiskowe. Akademia Medyczna. Lublin, 61-86.

MAYORAL A.M., TJALLINGII W.F., CASTANERA. P. 1996. Probing behaviour of *Diuraphis noxia* on five cereal species with different hydroxyamic acid levels. Ent. Exp. Appl., 78: 341-348

SAMUEL J.K., ANDREWS B., JEBASHREE H.S. 2000. In vitro evaluation of the antifungal activity of *Allium sativum* bulb extract against *Trichophyton rubrum*, a human skin pathogen. World J. Microbiol. Biotechnol., 16: 617-620.

TJALLINGII W.F. 1995. Electrical signals from the depths of plant tissues: the electrical penetration graph (EPG). [In:] Niemeyer H. (ed.) Proc. IFS Workshop in Chemical Ecology, Santiago, Chile, 48-58.

VAN HELDEN M., TJALLINGII W.F. 1993. Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. Entomol. Exp. Appl. 68: 269–278.

WILKINSON T.L. DOUGLAS A.E. 1998. Plant penetration by pea aphids (*Acyrthosiphon pisum*) of different plant range. Ent. Exp. Appl. 87: 43-50.
YIN M., TSAO S. 1999. Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. Int. J. Food Microbiol., 49: 49-56.

Wpływ ekstraktów z czosnku (*Allium sativum* L.) i wrotyczu (*Tanaceum vulgare* L.) oraz mydła ogrodniczego na zachowanie mszycy brzoskwiniowej - *Myzus persicae* (Sulzer, 1776) podczas żerowania

STRESZCZENIE

Zachowanie mszycy brzoskwiniowej – *Myzus persicae* (Sulzer, 1776) podczas penetracji i żerowania na roślinach traktowanych preparatami mydła ogrodniczego potasowego oraz mydła w połączeniu z ekstraktami z czosnku i wrotyczu badano z wykorzystaniem techniki elektronicznej rejestracji żerowania (EPG). Całkowity czas penetracji w tkankach pozafloemowych oraz udział pobierania soku floemowego we wszystkich aktywnościach mszyc podczas penetracji był podobny u wszystkich mszyc bez względu na zastosowany preparat. Statystycznie istotne różnice wystąpiły podczas penetracji rurek sitowych. Najmniejszy procent penetracji zawierających fazę floemową, najdłuższy czas trwania wydzielania śliny do floemu oraz największy udział wydzielania śliny podczas aktywności mszyc we floemie występowały u mszyc na roślinach traktowanych preparatami zawierającymi ekstrakt z czosnku i 1% roztwór mydła oraz ekstrakt z czosnku i wrotyczu. Wyniki rejestracji żerowania mszyc (EPG) wskazują na to, że wymienione preparaty wykazują aktywność deterentną na etapie pobierania pokarmu przez mszyce z elementów floemu.