https://doi.org/10.26651/allelo.j/2020-49-1-1258 0971-4693/94 Euro 20.00
Allelopathy Journal 49 (1): 113-124 (January, 2020) *International Allelopathy Foundation* 2020
Table: 2, Figs : 4

Comparison of probing/ feeding behavior for diet analysis to control strategy: A case study on aphids

S. Goławska*, I. Łukasik, I. Sprawka, H. Sytykiewicz and A. Chojnacki
Siedlce University of Natural Sciences and Humanities,
Faculty of Exact and Natural Sciences, Institute of Biological Sciences,
Prusa 14, 08-110 Siedlce, Poland
E.Mail: sylwia.golawska@uph.edu.pl

(Received in revised form: December 11, 2019)

ABSTRACT

We investigated the aphid probing behavior, on liquid artificial diets and agarose-sucrose gels by electrical penetration graphs (DC EPG) to monitor the probing/feeding behavior of the *Rhopalosiphum padi* L. and *Acyrthosiphon pisum* Harris. One alfalfa saponin, zanhic acid tridesmoside (a compound with wide range of biological properties), was tested for its effects on feeding behavior of aphids. The EPG patterns generated by aphids feeding on plants were used to interpret the patterns observed on the diets and gels. To investigate the effects of two factors: (i). Diet type (liquid artificial diets/agarose sucrose gels) and (ii). Aphid (*R. padi*/*A. pisum*) species, on number of waveforms and average time of waveforms, generalized linear mixed model (GLMM) with a Gaussian error structure were used. On artificial diets and gels, number of penetrations and average time of waveforms depended on studied factors. The essential factors in these analyses were : Diet type and Aphid species. These results suggested that liquid agarose diet and sucrose- agarose gels could be used to study the aphid probing/feeding behavior and in biotechnological projects for resistant plant breeding to sucking-piercing herbivores.

Keywords: *Acyrthosiphon pisum*, aphid diet, feeding behavior, *in vitro*, *Rhopalosiphum padi*, sucrose-agarose gels.

INTRODUCTION

Aphids are the most serious agricultural insect pests and cause major economic losses in several crops worldwide, directly due to feeding on crops and indirectly by inflicting plant debility. *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) aphid is worldwide pest of legume crops (pea and broad bean, the red clover and alfalfa races), while *Rhopalosiphum padi* L. is highly polyphagous with innumerable hosts from the Poaceae family including all major cereals (15,42). These two aphids are major pests mainly due to (i). direct probing damage to crop plants and (ii). their ability to act as efficient vector of plant pathogens (20). Aphids transmit about 50 % of insect-borne plant viruses (7). *A. pisum* is vector of > 30 viruses, including bean yellow mosaic virus, red clover vein mosaic virus and pea streak virus (22) and *R. padi*, especially barley yellow dwarf virus (9), all of which reduces the crops yields (11).

The aphids are controlled by harmful chemical pesticides. However, many aphid species have become resistant to various classes of chemical compounds (25,28) and many insecticides are ineffective to control the aphid-borne non-persistent viruses (34). Furthermore, insecticides may contribute to the spread of virus transmission (3). The anti-metabolites offers a future alternative control strategy for aphids (16,18,19) and some exhibit antifeedant activity (12,15). Plant lectins are potential control agents for aphids in

*Correspondence author

sucrose-agarose gel studies (40,41). Therefore, there is need to develop non-chemical management strategies to control aphids. Researchers have been investigating alternatives to conventional chemical control of aphids (21,37), including the use of resistant cultivars, transgenic plants containing novel genes that confer resistance to phloem-feeding insects, and chemicals that are compatible with integrated pest management. It is vital to find new molecules which are less deleterious to the environment. One of the most promising sources of such compounds are plants, which fight against insects in many ways and one of these is the use of insecticidal molecules found in plants. The search for insecticidal compounds in plants and specifically fodder plants consumed by mammals, could be valuable to develop biopesticides for sustainable and healthy agriculture (6). To understand how these alternative methods affects the aphid feeding requires a method to monitor aphid feeding, and this is difficult because the aphids feed on phloem sap. Although aphid stylet activity, saliva excretion, and food ingestion cannot be directly observed because the probing and feeding site is internal to the host, but these activities can be monitored with the electrical penetration graph (EPG) method. This method is widely used to study the probing and feeding behavior of aphids (17,16,19,33,35,36).

Numerous molecules with insecticidal activity against aphids have been attracting the attention of researchers. Some studies have been done on plants on chemical factors (8,26) and on artificial diets (2,10,36). The EPG technique has been used to monitor the effects of individual compounds on probing and feeding behavior *in vitro*, i.e., on agarose-sucrose gels (12,15,18,40,41). The use of agarose-sucrose gels with EPG is good method to investigate the mechanism of action of new chemicals. These methods are useful to better understand the aphids' probing and feeding behavior of various chemicals. There are reports of electrophysiological approach of aphids (24,30,45). There is no research with electrophysiological method, the DC (direct current) EPG system (system based on direct current DC, that registers voltage variations caused by resistance (R) and electromotive forces (emf)), was used to compare the insects probing and feeding behavior on agarose-sucrose gels and on artificial diet. Such studies using the liquid artificial diet and agarose-sucrose gel substrates may assist to assess the potential insecticides/aphicides on the insects probing and feeding behavior. EPG monitoring provides real time information on probing by insects in the form of waveforms, which can be correlated to specific activities and stylet tip locations. This method has been widely used on aphids (45,46,50), but information is lacking about how EPG data can be used to characterize and interpret waveforms produced by aphids feeding on agarose-sucrose gels and artificial diet.

This study aimed to (i). determine the probing/feeding behavior of aphids on liquid artificial diet and sucrose-agarose gel (with addition of zanhic acid tridesmoside, a natural compound of alfalfa, with wide range of biological properties), (ii). compare the probing/feeding behavior piercing-sucking insects on liquid artificial diet and sucrose-agarose gel and (iii). to interpret the waveforms observed on liquid artificial diet and sucrose-agarose gel with waveforms observed on plants. No previous study has compared the use of EPG method on the liquid artificial diet and sucrose-agarose gel. Investigation on new chemicals precise modes of activity and biological effects are needed, if we want to use new compounds for creating transgenic plants that are resistant to herbivores and safe for humans. EPG is a good method, as it provides information on probing/feeding events and detects different waveforms patterns related to aphid activities and the effects of new compounds on aphid feeding can be more easily studied with EPG in diets and gels

than in plants and analysis of penetration activities could help in the incorporation of novel chemicals in plant breeding' programmes.

MATERIALS AND METHODS

I. Aphids

Females of the bird cherry-oat aphids, *Rhopalosiphum padi* L. were brought from the wild population on primary host in Municipal Park "Aleksandria" Siedlce, Poland. The *Acyrthosiphon pisum* Harris aphid were collected from the broad bean (*Vicia faba* L.) field, Siedlce district (52°09′54″N, 22°16′17″E). Adult apterous females were used in all experiments.

II. Chemicals

Zanhic acid tridesmoside, was obtained from the Institute of Soil Science and Plant Cultivation, Pulawy, Poland, where it was isolated as under from the alfalfa tops (leaves and stems) by Oleszek *et al.* (31,32). Crude compound was extracted from plant material with 30 % methanol and the extract was purified by using a C_{18} column (3 cm × 30 cm, 50 μm, Waters, Milford, Massachusetts, USA). The saponin fraction was thereafter separated on steel columns (1.2 cm × 30 cm). For separation, the Eurospher 80 C_{18} column was used at 50°C. The mobile phase consisted of a linear gradient of 1% $H_3PO_4 \rightarrow$AcN (10-90%) and a flow rate of 1ml/min.

III. Liquid artificial diets and sucrose-agarose gels

Liquid artificial diet [containing optimal contents of essential ingredient for feeding: 30 % aqueous sucrose solution] (1) and zanhic acid tridesmoside 50 μg × cm^{-3} concentration were used. The diets were sterilized by filtration through 0.45 μm Millipore filters. A total of 0.5 ml solution was added to each aphid feeding chamber, consisting of plastic rings (35 mm dia; 15 mm height) covered with 2 layers of stretched Parafilm M®; between which the diet was sandwiched (Fig. 1).

Figure 1. The experimental EPG system used to monitor the feeding behavior of insects on agarose-sucrose gels (A) and liquid artificial diets (B).

Sucrose-agarose gels were prepared by adding 1.25 % agarose (Sigma A-0169) into 30 % sucrose solution. Experimental gels were prepared by adding the zanhic acid tridesmoside 0.005 % concentration. The mixtures were stirred for 5-10 min and thereafter, heated in water bath (75°C for 30 min) and then poured into plastic rings (10 mm high; 15 mm dia) covered with stretched Parafilm M® membrane, waited for them to freeze and formed transparent gels. 1-2 minutes after the formation of Transparent gels these were given to aphids for probing (Fig. 1).

IV. Electrical monitoring of aphids probing and feeding behavior

The feeding behavior of adult aphids was recorded *in vitro* using DC EPGs (46). The EPG records the different waveform patterns formed related to aphid activities and stylet locations during penetration of plant tissue or other substrates. EPG waveforms were recorded in laboratory in Faraday cage [21±1 °C, L16 : Dark8 photoperiod and 70 % Relative humidity]. Apterous adults were collected between 6 and 7 a.m. and then dorsally tethered by the abdomen with a 20-µm-diameter gold wire and water-based, conductive, silver paint (Demetron, L2027, Darmstadt, Germany). After the insects were starved for 2 h to recover from tethering, EPGs were started (between 9 and 10 a.m.) by carefully transferring the aphids to liquid artificial diet/sucrose-agarose gel and individually placed in the centre of membrane on the liquid artificial diet/sucrose-agarose gel (one aphid per diet/gel). A second electrode (a copper wire 9 cm long; 1 mm dia) was introduced into the diet/gel. Aphids were connected to Giga-4 EPG amplifiers (Wageningen, Agricultural University, Entomology Department, The Netherlands) coupled to an IBM-compatible computer through a DAS 8 SCSI acquisition card (Keithley, USA). The EPGs were recorded under continuous laboratory lighting and all EPG recordings were made for 10 aphids on 10 separate diets and also on 10 separate gels. Aphid probing and feeding behavior was monitored for 4 h.

EPGs were acquired and analyzed with STYLET 2.2 software provided by W.F. Tjallingii. Based on terms previously used to describe EPG waveforms generated by aphids probing/feeding on plants, we propose using a “l-” and “g-” prefixes to indicate liquid artificial diet and agarose-sucrose gel EPG waveforms, respectively. Five waveforms were identified (15,18,19) similar to those for plants (46,47,48,49): pattern l-np/g-np (non-penetration), pattern l-C/g-C (stylet activity in the diet/gel), pattern l-E1/g-E1 (salivation into the diet/gel), pattern l-E2 (ingestion from the diet) and pattern l-G/g-G (ingestion from the diet/gel)(Table 1).

Statistical analysis: To determine the effects of diets types and species of aphid on number of waveforms and average time of waveforms, we used generalized linear mixed model (GLMM) with a Gaussian error structure (27). In the first analysis response, variable was the number of waveforms and in second analysis, it was average time of waveforms. The two explanatory variables (fixed effects) were: (a) Diet type (liquid artificial diet vs. sucrose-agarose gel, (b) Aphid species (*R. padi, A. pisum*). The kind of the waveform was included as random effects. The interactions between the factors in all GLMMs was introduced in the initial parametrization. Values reported are mean ± 1 SE. Statistical analyses were done in SPSS v.21.0 (IBM Corp, 2012). P values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical control has many disadvantages and there is a growing use of alternative strategies for insect control (37). EPG is the only method to provide detailed information on the probing and feeding behavior of sucking-piercing insects. It has been used to study aphid probing and feeding on plants (46-49) and sucrose-agarose gels (12,15,40,41), but there is no work presented the comparison the probing and feeding behavior of aphids on agarose-sucrose gels and liquid artificial diet. This is the first study to compare the probing and feeding behavior of sucking-piercing insects on liquid artificial diet and sucrose-agarose gels.

Table 1. Recorded EPG waveforms during the feeding of *R. padi* and *A. pisum* on liquid artificial diet and sucrose-agarose gels and representative waveforms for aphids feeding on plants.

	Waveform		Activities in plant			Proposed correlations*	
Plant	Liquid artificial diet	Sucrose -agarose gel	Position (Plant)	Phase (Plant)	Aphid activity (Plant)	Aphid activity (Diet)	Aphid activity (Gel)
np	l-np	g-np			Aphid was not probing the plant	Aphid was not probing the diet	Aphid was not probing the gel
C	l-C	g-C	Epidermis mesophyll any tissue	Path	Contact with plant, excretion of gelling sheath saliva, path activity	Contact with diet, excretion of gelling sheath saliva, path activity	Contact with gel, excretion of gelling sheath saliva, path activity
E1	l-E1	g-E1	Phloem	Phloem	Saliva excretion	Possibly saliva excretion	Possibly saliva excretion
E2	l-E2	g-E2	Phloem	Phloem	Water saliva excretion and ingestion	Possibly water saliva excretion and ingestion	Not recorded
G	l-G	g-G	Xylem	Xylem	Ingestion	Ingestion	Ingestion

*Activities in liquid artificial diet and sucrose-agarose gel assigned for similar waveforms in aphids on plant.
Position (plant): Plant tissues with insects stylets;
Phase (plant): Phases of insects activities in plant tissues;
np (l-np, g-np): Non-probing phase: The aphid's stylet is outside the plant (the diet/ gel);
C (l-C, g-C): Stylet penetration phase, stylet activity in plant tissues, epidermis and mesophyll (stylet activity in diet/ gel);
E1 (l-E1, g-E1): Phloem phase: Salivation into phloem sieve tubes (salivation into the diet/gel);
E2 (l-E2, g-E2): Phloem phase: Passive ingestion of phloem sap (passive ingestion of the diet/gel);
G (l-G, g-G): Xylem phase: Ingestion of xylem sap (active ingestion of the diet/gel).

In this study, waveforms generated by aphids on liquid artificial diet were very similar to those generated on plants and gels, and we therefore propose that the same designations developed by Tjallingii (45) for plants and by Goławska *et al*. (18) for gels be used for liquid artificial diet (with addition of the prefix l-) and for sucrose-agarose gel (with addition of the prefix g-). The EPGs were generally similar for *R. padi* and *A. pisum*

probing and feeding on liquid artificial diet and on sucrose-agarose gels with waveforms on plants. In this study on liquid artificial diet, we observed five main waveforms (l-np, l-C, l-E1, l-E2 and l-G) and on sucrose-agarose gels four (g-np, g-C, g-E1 and g-G). Goławska *et al*. (18) on sucrose-agarose gels observed five main waveforms and Sauvion *et al*. (39) on artificial diets three. The graphical representation of each waveforms on liquid artificial diet, sucrose-agarose gels and plants were similar. Data for waveforms amplitude, shape or frequency were similar, so we infer that the probing and feeding behavior was also similar. All EPG patterns observed by us on diets and gels and representative waveforms for aphids feeding on plants are presented in Table 1. The first observed waveform was non-probing phase (l-np, g-np). In this waveform, the aphid's stylet is outside the diet/ gel, analogous to the stylet being outside the plant. The non-probing phase was followed by stylet penetration (l-C, g-C). Pattern l-C/g-C indicated stylet activity in the diet/ gel, analogous to the stylet penetrating the epidermis and mesophyll. The next were phloem phase (l-E1, g-E1) and xylem phase (l-G, g-G). Pattern l-E1/g-E1 indicated salivation into the diet/gel, analogous to the salivation into phloem sieve tubes and pattern l-G/g-G indicated active ingestion of the diet/gel, analogous to ingestion of xylem sap. Pattern l-E2 indicated passive ingestion of the diet, analogous to passive ingestion of phloem sap. Waveform analogous to passive ingestion on plants was not recorded on sucrose-agarose gel. In our study EPG waveforms, their order and duration, observed for aphids on liquid artificial diet and sucrose-agarose gel were very similar for aphids and other homopterans on plants and artificial diets (4,5,13,14,15,16,23,39,44).

Table 2. General Linear Mixed Model showing the effects of number of waveforms and average time of waveforms for two aphids species.

Variable	Parameter	SE	T-test	P
Number of waveforms				
Species: *Acyrthosiphon pisum*	4.967	2.646	1.877	0.063
Species: *Rhopalosiphum padi*	0.000	-	-	
Diet: Liquid artificial diet	35.633	2.226	13.468	<0.001
Diet: Sucrose-agarose gel	0.000		-	-
Average time of waveforms (s)				
Species: *Acyrthosiphon pisum*	-41.352	27.984	-5.103	<0.001
Species: *Rhopalosiphum padi*	0.000	-	-	-
Diet: Liquid artificial diet	-50.006	38.886	-5.484	<0.001
Diet: Sucrose-agarose gel	0.000	-	-	
Species*diet	38.015	8.880	3.564	0.001

*: Interaction between variables
The model was used as random factors and these were non-significant in both analyses.

We found that on liquid artificial diet and sucrose-agarose gel, the number of penetrations and average time of waveforms depended on the studied factors. The both studied factors (the species of aphid and the kind of diet) were essential (Table 2). The number of waveforms was affected by the diet type (GLMM, $F_{1,116} = 310.84$, $P < 0.001$, Table 2). Frequency of these waveforms differed in the liquid artificial diet and on

sucrose-agarose gel (Fig. 2). Higher number of waveforms were observed for aphids on liquid artificial diet. Average time of waveforms was affected by the diet type (GLMM, $F_{1,116} = 17.57$, $P < 0.001$, Table 2). The difference in average time of waveforms on studied diets and gels was variable, too (Fig. 3).

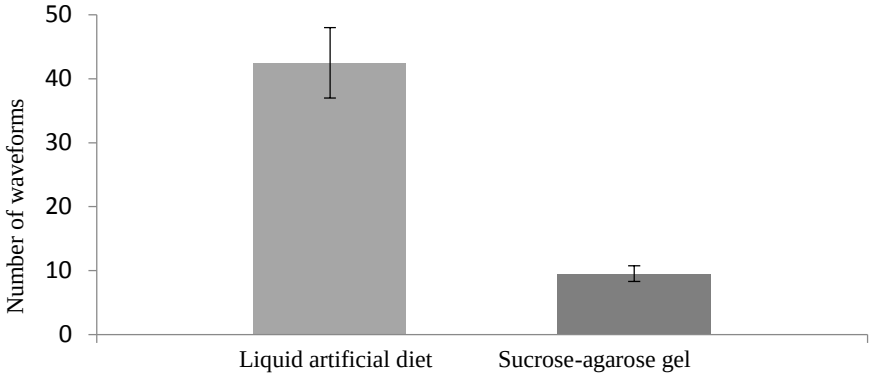

Figure 2. Number of waveforms on liquid artificial diet and sucrose-agarose gel.

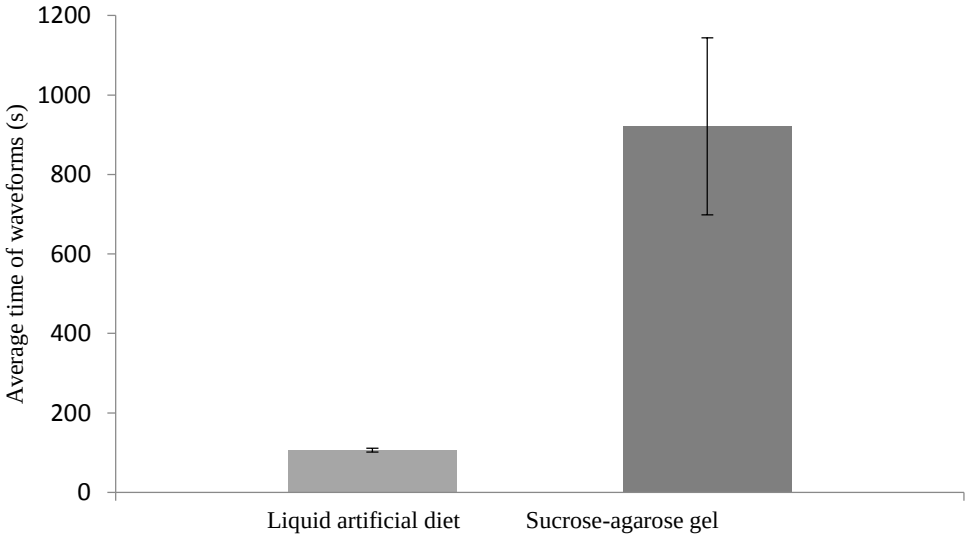

Figure 3. Average time of waveforms on liquid artificial diet and sucrose-agarose gel.

Aphids spent more time penetrating the sucrose-agarose gel. The difference in average time of waveforms between two species of aphids was also significant (GLMM, $F_{1,116} = 13.35$, $P < 0.001$, Table 2). The highest time of waveforms was shown for *R. padi* (Fig. 4). Interactions between two studied factors were significant (GLMM, $F_{1,116} = 12.70$, $P = 0.001$, Table 2).

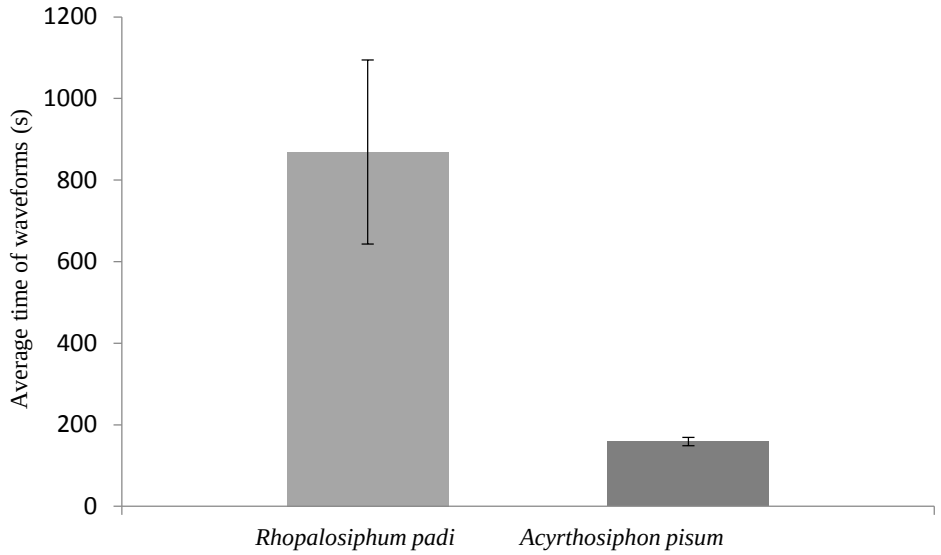

Figure 4. Average time of waveforms at species: *R. padi* and *A. pisum*.

Aphid feeding behavior depended on species and diet type. It showed that there must be a relationship between studied species of aphids and diets/gels. Aphid probing/feeding behavior depended on its hosts, their qualitative and quantitative composition (29). Chemical compounds interfered with aphids behavior (13,16,43). The EPG recordings of pea aphid probing behavior of alfalfa tissues containing different levels of saponins corresponded to differences in its feeding behavior. The apterous adults spent more time on ingestion of phloem sap on the better host alfalfa with low level of saponin, while aphids feeding on alfalfa cultivar Radius, rich in saponin content, reduced the phloem sap ingestion. The short phloem salivation on Radius sieve elements indicates the presence of deterrent factors (17). The higher concentration of these allelochemicals reduced the aphid feeding activities corresponding to the duration of ingestion of phloem sap. In our study we found that the number of waveforms and average time of waveforms was affected by diet type. The difference in average time of waveforms between two species of aphids was also significant. Our data showed that studied diets and gels are useful to investigate the influence of chemicals on aphids.

CONCLUSIONS

The Diets types fed to Aphids had variable effects on the aphids species. Investigation of effects of new compounds on aphid feeding can be more easily studied with EPG in diets and gels. The liquid artificial diet and sucrose agarose gels are good for feeding behavior research, but liquid artificial diet seems was better because on this diet all EPG waveforms were observed on plants. The liquid artificial diet and agarose-sucrose gel combed with EPG helped to study the aphid probig/feeding behavior, but comparison with

data obtained by other techniques (light and electron microscopy, radioactive traces or electromyography) is required to provide more detailed information on probing and feeding behavior of aphids and to determine the differences between the effects of various Diets' on aphids.

ACKNOWLEDGEMENTS

The study was done with the financial support of National Science Centre, Poland (Grant No. 2016/21/B/NZ9/00612) and the Ministry of Science and Higher Education (245/08/S).

REFERENCES

1. Ackey, D.L. and Beck, S.D. (1972). Nutrition of the pea aphid *Acyrthosiphon pisum*: Requirements for trace metals, sulfur, and cholesterol. *Journal of Insect Physiology* **18:** 1901-1904.
2. Auclair, J.L. (1965). Feeding and nutrition of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae), on chemically defined diets a various pH and nutrient level. *Annals of the Entomological Society of America* **58:** 855-875.
3. Budnik, K., Laing, M.D. and da Graca, J.V. (1996). Reduction of yield losses in pepper crops caused by potato virus Y in KwaZula-Natal, South Africa, using plastic mulch and yellow sticky traps. *Phytoparasitica* **24:** 119-124.
4. Calatayud, P.A., Seligmann, C.D., Polania, M.A. and Bellotti, A.C. (2001). Influence of parasitism by encyrtid parasitoids on the feeding behaviour of the cassava mealybug *Phanacoccus herreni*. *Entomologia Experimentalis et Applicata* **98:** 271-278.
5. Cid, M. and Fereres, A. (2010). Characterization of the probing and feeding behavior of *Planococcus citri* (Hemiptera: Pseudococcidae) on Grapevine. *Annals of the Entomological Society of America* **103:** 404-417.
6. Da Silva, P., Eyraud, V., Carre-Pierrat, M., Sivignon, C., Rahioui, I., Royer, C. and Gressent, F. (2012). High toxicity and specificity of the saponin 3GlcA-28-AraRhaXyl-medicagenate from the *Medicago truncatula* seeds, for *Sitophilus oryzae*. *BMC Chemical Biology* **12:** 3.
7. Dedryver, C.H.A, La Ralec, A. and Fabre, F. (2010). The conflicting relationships between aphids and men: A review of aphid damage and control strategies. *Comptes Rendus Biologies* **333**: 539-553.
8. Denholm, I., Horowitz, M., Cahill, M. and Ishaaya, I. (1998). Management of resistance to novel insecticide. In: *Insecticides with Novel Modes of Action; Mechanisms and Application* (Eds., I. Ishaaya and D. Degheele), pp. 260-282. Springer - Verlag, Berlin Haidelberg.
9. Descamps, L.R. and Chopa, C.S. (2011). Population growth of *Rhopalosophum padi* L. (Homoptera: Aphididae) on different cereal crops from the semiarid Pampas of Argentina under laboratory conditions. *Chilean Journal of Agricultural Research* **71**: 390-394.
10. Febvay, G., Delobel, B. and Rahbé, Y. (1988). Influence of amino acid balance on the improvement of an artificial diet for a biotype of *Acyrthosiphon pisum* (Homoptera: Aphididae). *Canadian Journal of Zoology* **66:** 2449-2453.
11. Garlinge, J. and Robartson, D. (1988). Crop variety sowing guide for Western Australia. *Bulletin of Agriculture for Western Australia* **4341**: 214-250.
12. Goławska, S. (2007). Deterrence and toxicity of plant saponins for the pea aphid *Acyrthosiphon pisum* Harris. *Journal of Chemical Ecology* **33:** 1598-1606.
13. Goławska, S., Leszczyński, B. and Oleszek, W. (2006). Effects of low and high-saponin lines of alfalfa on pea aphid. *Journal of Insect Physiology* **52:** 737-743.
14. Goławska, S. and Łukasik I. (2009). Acceptance of low-saponin lines of alfalfa with varied phenolic concentrations by pea aphid (Homoptera: Aphididae). *Biologia* **64:** 377-382.
15. Goławska, S. and Łukasik, I. (2012). Antifeedant activity of luteolin and genistein against the pea aphid, *Acyrthosiphon pisum*. *Journal of Pest Science* **85:** 443-450.
16. Goławska, S., Łukasik, I., Goławski, A., Kapusta, I. and Janda, B. (2010). Alfalfa (*Medicago sativa* L.) apigenin glycosides and their effect on the pea aphid (*Acyrthosiphon pisum*). *Polish Journal of Environmental Studies* **19:** 913-919.
17. Goławska, S., Łukasik, I. and Leszczyński, B. (2008). Effects of alfalfa saponins and flavonoids on pea aphid. *Entomologia Experimentalis et Applicata* **128:** 147-153.

18. Goławska, S., Sprawka, I., Goławski, A. and Matok, H. (2014a). Are agarose-sucrose gels useful for studying the probing and feeding behavior of aphids? *Australian Journal of Crop Science* **8:** 263-270.
19. Goławska, S., Sprawka, I., Łukasik, I. and Goławski, A. (2014b). Are naringenin and quercetin useful chemicals in pest-management strategies? *Journal of Pest Science* **87:** 173-180.
20. Hooks, C.R.R. and Fereres, A. (2006). Protecting crops from non-persistently aphid-transmitted viruses: A review on the use of barrier plants as a management tool. *Virus Research* **120:** 1-16.
21. Horowitz, A.R. and Ishaaya, I. (2004). Biorational Insecticides - Mechanisms, selectivity and importance in pest management programs. In: *Insect Pest Management* (Eds., A.R. Horowitz. and I. Ishaaya), pp.1-28. Springer - Verlag, Berlin, Heidelberg.
22. Jones, R.A.C. and Proudlove, W. (1991). Further studies on cucumber mosaic virus infection of narrow-leafed lupin (*Lupinus angustifolius*): Seed-borne infection, aphid transmission, spread and effects on grain yield. *Annals of Applied Biology* **118**: 319-329.
23. Kordan, B., Gabryś, B., Dancewicz, K., Lahuta, L.B., Piotrowicz-Cieślak, A. and Rowińska, E. (2008). European yellow lupine, *Lupinus luteus* and narrow-leaf lupine, *Lupinus angustifolius*, as hosts for the pea aphid, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* **128:** 139-146.
24. Lett, J.M., Granier, M., Grondin, M., Turpin, Molinaro, F., Chiroleu, F., Peterschmitt, M. and Reynaud, B. (2001). Electrical penetration graphs from *Cicadulina mbila* on maize, the fine structure of its stylet pathways and consequences for virus transmission efficiency. *Entomologia Experimentalis et Applicata* **101:** 93-109.
25. Li, F. and Han, Z. (2004). Mutations in acetylcholinesterase associated within secticide resistance in the cotton aphid *Aphis gossypii* Glover. *Insect Biochemistry and Molecular Biology* **34:** 397-405.
26. Morita, M., Ueda, T., Moneda, T., Koyanagi, T. and Haga, T. (2007). Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. *Pest Management Science* **49:** 252-258.
27. Nakagawa, S. and Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews* **85**: 935-956.
28. Nebeshima, T., Kozaki, T., Tomita, T. and Kono, Y. (2003). An amino acid substitution on the second acetylcholinesterase in the pirimicarb-resistant strains of the peach potato aphid, *Myzus persicae*. *Biochemical and Biophysical Research* **307:** 15-22.
29. Nespolo, R.F., Figureoa, C.C., Plantegenst, M. and Simon, J.C. (2008). Short-term population differences in the genetic architecture of life history traits related to sexuality in an aphid specie. *Heredity* **100:** 374-381.
30. Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. and Woodcock, Ch.M. (1991). Behavioral and electrophysiological responses of aphids to host and non host plant volatiles. *Journal of Chemical Ecology* **17**: 1231-1242.
31. Oleszek, W., Jurzysta, M., Płoszynski, M., Colquhoun, I.J., Price, K.R. and Fenwick, G.R. (1992). Zanhic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. *Journal of Agricultural and Food Chemistry* **40:** 191-196.
32. Oleszek, W., Price, K.R., Colquhoun, I.J., Jurzysta, M., Płoszynski, M. and Fenwick, G.R. (1990). Isolation and identification of alfalfa (*Medicago sativa* L.) root saponin: Their activity in relation to a fungal bioassay. *Journal of Agricultural and Food Chemistry* **38:** 1810-1817.
33. Pelletier, Y. and Giguere, M.A. (2009). Effect of manipulations on the host selections behavior of *Sitobion avenae* (Homoptera: Aphididae). *Journal of Insect Behavior* **22:** 165-171.
34. Perring, T.M., Gruenhagen, N.M. and Farrar, C.A. (1999). Management of plant viral diseases through chemical control of insect vectors. *Annual Review of Entomology* **44:** 457-481.
35. Powell, G. (1993). The effect of pre-acquisition on aphid transmission of potyviruses during observed and electrically recorded stylet penetrations. *Entomologia Experimentalis et Applicata* **66:** 255-260.
36. Prado, E. and Tjallingii, W.F. (1999). Effects of experimental stress factors on probing behaviour by aphids. *Entomologia Experimentalis et Applicata* **90**: 289-300.
37. Rharrabe, K., Bakrim, A., Ghailani, N. and Sayah, F. (2007). Bioinsecticidal effect of harmaline on *Plodia interpunctella* development (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology* **89:** 137-145.
38. Sadeghi, A., Van Damme, E.M. and Smagghe, G. (2009). Evaluation of the susceptibility of the pea aphid, *Acyrthosiphon pisum*, to a selection of novel biorational insecticides using an artificial diet. *Journal of Insect Science* **9**: 65, available online: insectscience.org/**9**: 65
39. Sauvion, N., Charles, H., Febvay, G. and Rahbe, Y. (2004). Effects of jackbean lectin (ConA) on the feeding behaviour and kinetics of intoxication of the pea aphid, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* **110:** 31-44.
40. Sprawka, I. and Goławska, S. (2010). Effects of the lectin PHA on the feeding behavior of the grain aphid. *Journal of Pest Science* **83:** 149-155.

41. Sprawka, I., Goławska, S., Czerniewicz, P. and Sytykiewicz, H. (2011). Insecticidal action of phytohemagglutinin (PHA) against the grain aphid, *Sitobion avenae*. *Pesticide Biochemistry and Physiology* **100:** 64-69.
42. Sprawka, I., Goławska, S. , Parzych, T., Goławski, A., Czerniewicz, P. and Sytykiewicz, H. (2014). Mechanism of entomotoxicity of the cancanavalin A in *Rhopalosiphum padi* (Hemiptera: Aphididae). *Journal of Insect Science* **14**: 1-6.
43. Sytykiewicz, H., Goławska, S. and Chrzanowski, G. (2011). Effect of the bird cherry-oat aphid *Rhopalosiphum padi* L. feeding on phytochemical responses within the bird cherry *Prunus padus* L. *Polish Journal of Ecology* **59:** 329-338.
44. Stafford, C.A. and Walker, G.P. (2009). Characterization and correlation of DC electrical penetration graph waveforms with feeding behavior of beet leaf hopper, *Circuli fertenellus*. *Entomologia Experimentalis et Applicata* **130:** 113-129.
45. Tjallingii, W.F. (1978). Electronic recording of penetration behavior by aphids. *Entomologia Experimentalis et Applicata* **24:** 721-730.
46. Tjallingii, W.F. (1988). Electrical recording of stylet penetration activities. In: *Aphids: Their Biology, Natural Enemies and Control* (Eds. A.K. Minks and P. Harrewijn), vol 2B, pp. 95-108. Elsevier, Amsterdam.
47. Tjallingii, W.F. (1995a). Regulation of phloem sap feeding by aphids. In: *Regulatory Mechanisms in Insect Feeding* (Eds., R.F. Chapman and G. de Boer), pp. 190-209. Chapman & Hall.
48. Tjallingii, W.F. (1995b). Electrical signals from the depths of the plant tissues: The electrical penetration graph (EPG). *Techniques in Plant-Insects Interactions and Biopesticides. Proceedings IFS Workshop* (Ed., H. Niemeyer), pp. 49-58. Santiago, Chile.
49. Tjallingii, W.F. (2001). Plant penetration by aphids as revealed by electrical penetration graphs. *Aphid and Other Homopterous Insects* **8:** 105-120.
50. Tjallingii, W.F. and HogenEsch, T.H. (1993). Fine structure of aphid stylet routes in plant tissue in correlation with EPG signals. *Physiological Entomology* **18:** 317-328.