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Acyrthosiphon pisum (Harris, 1776) feeding behaviour on various host – plants

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Introduction

The leguminous plants are very common and play an important role as hosts for phytophagous insects. Aphids are important pests of forage crops and among the aphid species, the pea aphid *Acyrthosiphon pisum* (Harris) is generally considered to be one of the most common and injurious on Fabaceae. Pea aphid color clones normally vary from light to dark green and are widely distributed all over the world. Usually they prefer different host plants. Hitherto most of the studies concerning the effect of hosts on the pea aphid have been conducted on experimental plots and demonstrated a reduction of development of the pea aphid depending on the content of secondary metabolites (Szynkarczyk *et al.*, 2001; Goławska *et al.*, 2005; 2006; Goławska, 2006). Aphids have sucking-piercing mouthparts (stylets) and are able to feed by taking sap directly from sieve tubes of plants. The acceptability of the host plants for the aphids is often measured by its quality as a food source (Montllor & Tjallingii, 1989). Valuable information on chemical factors and their localization within plant tissues that may affect feeding is provided by monitoring the aphid probing behaviour using the electrical penetration graph (EPG) technique (Tjallingii & Mayoral, 1992). Analysis of several parameters derived from the EPG (frequency, duration, and sequence of different waveforms) may reflect behavioural responses of the aphids to differences in plant suitability (Mayoral *et al.*, 1996). The pea

aphid is a dominant species on leguminous plants. So far, the reason of such host-plant preference of the pea aphid has been unknown. The aim of the present work is to compare the feeding activity of the pea aphid on selected leguminous plant species.

Material and methods

Plants

The experiments were carried out on five *Fabaceae* species: pea (Tulipan cv.), broad bean (Start cv.), alfalfa (Radius cv.), clover (Bona cv.), and phaseolus (Laguna cv.). Seeds of the plants were germinated in a climatic chamber (20-25°C, 70% relative humidity, 16 hrs : 8 hrs L:D photoperiod). Plants were grown in a medium nutrient fine structure compost with sand, in 7 cm x 7 cm x 9 cm plastic pots, one plant per pot and were regularly watered and no extra fertilizer was added. Ten-day-old seedlings of the studied plant species were used for the experiments.

Aphids

Adult apterae individuals of the pea aphid were used in the experiments. The aphids came from a stock culture kept at the University of Podlasie at Siedlce on broad bean seedlings (*Vicia faba* L. var. Start) in an environmental chamber (21°C, 70% relative humidity, 16 hrs : 8 hrs L:D photoperiod).

EPG recordings

The EPG recordings were performed on 10-day-old plants for 8 hrs as described earlier (Tjallingii, 1990). One electrode in form of a 2 cm gold wire (diameter = 20μm) attached to the dorsum of the aphids by conductive silver paint (Demetron) was connected to the EPG amplifier. The other electrode was put in the soil of potted plants. The whole set-up was placed in the Faraday cage and all signals were recorded on a hard disk and analyzed using the Stylet (DOS PCs) software. Monitoring was carried out, for each of the cultivars on 10 different plants and 10 different adult apterous aphids of *A. pisum*. The considered aphid activities while probing the plant tissues were: non-probing (Np-pattern), penetration of peripheral plant tissues (ABC-patterns), salivation into sieve elements (E1-pattern), phloem sap ingestion (E2-pattern) and xylem sap ingestion (G-pattern).

Statistics

The differences in the pea aphid feeding behaviour on the studied *Fabaceae* were subjected to one-way ANOVA followed by the post-hoc Newman-Keuls test.

Results

The EPG recordings revealed clear differences in the pea aphid feeding behaviour on the studied *Fabaceae* plant species. During 8-hrs of the EPG recordings, the aphids mostly penetrated pea and broad bean seedlings and spent only little time on penetration of the alfalfa, bean, clover and phaseolus tissues. The total number of the plant penetrations varied from 6.0 on the pea plants, to 26.0 for the phaseolus seedlings (Tab. 1.).

Table 1. Feeding behaviour of the pea aphid on the studied *Fabaceae* plants

Aphid activity	**Plant species**				
	Pea	Broad bean	Alfalfa	Clover	Phaseolus
Duration of total penetration (s)	27998.7^{a}	26168.3^{ab}	23020.5^{b}	24631.0^{b}	24410.0^{b}
Number of penetrations	6.0^{b}	9.1^{ab}	25.2^{a}	24.4^{a}	26.0^{a}
Duration of peripheral tissues penetration (s)	2116.0^{c}	2909.5^{c}	14600.7^{b}	21596.1^{a}	17450.5^{ab}
Number of peripheral tissues penetrations	1.9^{b}	4.6^{ab}	15.8^{a}	16.10^{a}	14.8^{a}
Duration of phloem salivation (s)	4174.4^{ab}	3846.5^{ab}	945.8^{b}	1405.4^{ab}	4818.6^{a}
Number of phloem salivations	1.7^{a}	3.2^{a}	5.4^{a}	3.0^{a}	6.1^{a}
Duration of phloem ingestion (s)	13660.7^{a}	11281.0^{b}	2174.0^{c}	247.7^{d}	51.1^{d}
Number of phloem ingestions	1.4^{a}	2.1^{a}	2.1^{a}	3.0^{a}	3.2^{a}
›Duration of xylem ingestion (s)	8047.6^{a}	7930.8^{a}	5299.9^{a}	1381.8^{b}	2090.3^{b}
Number of xylem ingestions	1.0^{a}	1.9^{a}	1.9^{a}	2.3^{a}	1.9^{a}

Values in rows followed by various letters are significantly different at $p \leq 0.05$ (Newman-Keuls test)

The total duration of peripheral tissues penetration was the longest for the aphids which fed on clover, phaseolus and alfalfa and the shortest for those on pea and broad bean, and the differences were statistically significant. The longest aphid salivation into sieve elements was recorded for the individuals which fed on pea, broad bean and phaseolus and there were no clear differences in the number of the E1 pattern for the all the studied plant species (Tab. 1.). During 8 hrs of the EPG recordings, the pea aphid adults mostly ingested phloem sap from pea and broad bean plants. The xylem sap ingestion from the studied leguminous species was the most intensive on pea and broad bean, and significant variation in such aphid activities was found (Tab. 1.).

The analysis of the pea aphid feeding behaviour on the *Fabaceae* species showed that the apterous adult spent most of the time on penetration of peripheral tissues on alfalfa, clover and phaseolus. Such activity took them about 50,70% on alfalfa to 74,99% on clover of total time of the recordings. Aphids on pea and broad bean spent most time of penetration on the ingestion of phloem sap from sieve elements. This activity took them about 39,17% on broad bean to 47,43% on pea of total time of the EPG recordings (Fig. 1.).

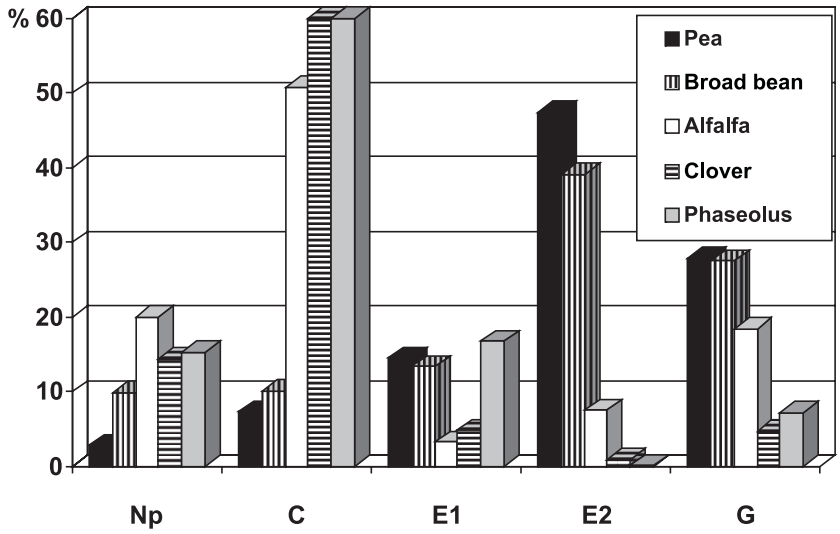

Fig. 1. The average percentage of particular EPG patterns of the pea aphid on the studied *Fabaceae* plants.

When details of the E2 pattern were analyzed, such as the number of penetrations to the first E2, duration to appearance of the first E2, duration of the first E2, total number of E2, and total duration of the E2 showed that among the studied hosts, the pea and broad bean seedlings were the best for *A. pisum*. (Tab. 1. and 2.).

Table 2. Phloem sap ingestion parameters of the pea aphids which fed on studied *Fabaceae* plants

Aphid activity	Plant species				
	Pea	Broad bean	Alfalfa	Clover	Phaseolus
Number of penetration to the first E2	1.5[b]	2.5[a]	0.8b[c]	0.2[c]	0.7b[c]
Duration to the first E2 (s)	5560.3[b]	4942.5[b]	6018.4[b]	7008.0[b]	11344.67[a]
Duration of the first E2 (s)	13478.4[a]	6038.9[b]	583.5[c]	248.4[c]	25.6[c]

Values in rows followed by different letter are significantly different at $p \leq 0.05$ (Newman-Keuls test)

Discussion

The aphids on all studied leguminous plants showed distinguishable patterns of probing activity. The waveforms representing pathway activities (C), phloem salivation (E1) and phloem sap ingestion (E2) were recognized. However, the electronic monitoring of the feeding behaviour showed differences in acceptance of the leguminous plants by the pea aphid.

The obtained results clearly suggest that the pea aphid fed much better on pea and broad bean than on alfalfa, clover and phaseolus. On these hosts the apterous adults ingested quite much phloem sap. The little percentage in participation of the phloem salivation on alfalfa, clover and phaseolus, indicate a deterrent factor in the phloem elements of these plants (*Tjallingii*, 1994), which may impede aphids settling (Gadomski, 1992; Gabryś & Pawluk, 1999). On alfalfa, clover and phaseolus, aphid feeding was clearly deterred, apparently by factors present in the phloem. For most of the study time the sap ingestion periods were short and the percentage of time spent on phloem activities was consistently lower. Such reduction of the feeding activity caused the severely reduced fecundity of *A. pisum* on these plants during laboratory tests (Goławska, not published).

Aphid distribution is quite often associated with the quality of their host-plants (Holmes, 1988; Honek *et al.*, 1998). Chemical and physical plant characteristics can greatly influence herbivorous insects and their enemies. Selection and acceptance of the host plants, and feeding sites are a result of testing mainly chemical properties of the nutrient value of plant tissues. The data presented here imply that the pea and broad bean are better hosts to the pea aphid, since it fed on them more extensively. Earlier investigations showed that on proper host-plants, aphids usually fed much longer than on the inap-

propriate ones (Nielson & Don, 1974; Kennedy *et al.*, 1978; Montlor *et al.*, 1990; Leszczyński & Wójcicka, 1998; Cichocka & Leszczyński, 1999).

There are examples of aphid feeding behaviour that was strictly related to the chemical composition of their host-plant tissues (Caillaud *et al.*, 1995; Leszczyński, 1999). Since aphids, especially adults, are phloem feeders, nutritional quality of the phloem sap is crucial (Todd *et al.*, 1971). The content of sugars and its relation to free aminoacids in the host plant tissues is significant for the aphid development and its feeding (Chapman *et al.*, 1981; Mittler, 1988). In case of the *Medicago sativa* species resistant to *A. pisum* this proportion was 3.6 and in case of the non-resistant one about 0.6 (Febvay *et al.*, 1988). Also, allelochemicals are limiting factors of the feeding of aphids (Argandona *et al.*, 1983; Dreyer & Jones, 1981; Sandstrom, 1998). They might influence the stylet penetration and affect the aphid feeding behaviour as well (Leszczyński *et al.*, 1996; Schoonhoven *et al.*, 1998; Leszczyński, 1999; Ponder *et al.*, 2000). Such relation was also observed for the grain aphid fed on cultivars of winter wheat that were either rich or poor in cereal allelochemicals (Leszczyński, 1999). Similar results were obtained for the black bean aphid, *Aphis fabae* (Scop.), which fed on broad bean cultivars (Cichocka *et al.*, 1999). The adverse effect of plant phenolics on aphid species was registered. It was showed that phenolic and flavonoid compounds may repel or deter the aphids from feeding and may exert a negative influence on growth and reproduction of aphids (Todd *et al.*, 1971; Montgomery & Arn, 1974; Miles, 1978; Jordens-Rotger, 1979; Dreyer & Jones, 1981; Cole, 1984; Kjaer *et al.*, 2001; Wurms *et al.*, 2003).

The mode of plant allelochemicals on the *A. pisum* aphid has been not extensively studied. Goławska (2006) showed that the adverse effect of alfalfa might be associated with phenolics, since abundance of the pea aphid on the studied alfalfa lines was negatively correlated with content of the total phenols. Sapnins were also reported to be important in varietal resistance of alfalfa to the pea aphid and they are known allelochemicals that form a chemical barrier for feeding of the phytophagous insects (Oleszek *et al.*, 1992). Goławska *et al.* (2006) showed differences in pea aphid feeding behaviour on alfalfa with low and high saponin content. Aphids fed on high-saponin lines had prolonged penetration of the epidermis and mesophyll (pattern C) and showed a significant reduction in phloem sap ingestion. On the other hand, a negative effect of saponins on herbivore performance – e.g., reduction of growth and pupal mass – could be a consequence of the shortening or suppressing of the aphid feeding activity. Szynkarczyk *et al.* (2001) and Goławska *et al.* (2005) also found a negative relationship between saponin concentration and development of the pea aphid on alfalfa lines. Antifeedant activity of the saponins has been also shown by Applebaum & Birk (1979), Sutherland *et al.* (1982), Potter & Kimmerer (1989), Nozzolillo *et al.* (1997), Matsuda *et al.* (1998), Adel *et al.* (2000), Herlet *et al.* (2002), Waligóra (2005), Agrell *et al.* (2006). Goławska

(2007) found that saponin compounds may in vitro repel or deter the pea aphid from feeding. Generally, higher concentrations of these compounds caused reduction of aphid activities that corresponded to the ingestion of phloem and xylem sap. Similar results have been reported previously for saponins isolated from seeds of *Barringtonia asiatica* in relation to *Epilachna* sp. larvae (Herlet *et al.*, 2002).

Thus, the results presented here suggest that abundance of the pea aphid on various host-plants is strictly associated with its feeding behaviour. *A. pisum* recognises its host plants by using chemical cues that are present within plant tissues. Feeding stimuli and secondary compounds occurring in *Fabaceae* plant tissues might be responsible for variation in acceptability of different species by the pea aphid. The obtained results showed also that the EPG is a good method for testing various plants species as a potential pea aphid hosts. Our data suggests that if allelochemicals are involved they differ in the deterrent activity and may impede stylet penetration at the level of epidermis, parenchymatous tissues, as well as in the phloem elements, depending on the plant species.

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Zachowanie *Acyrthosiphum pisum* (Harris, 1776) podczas żerowania na różnych roślinach żywicielskich

Streszczenie

W prezentowanej pracy metodą EPG zbadano zachowanie się dorosłych bezskrzydłych samic mszycy grochowej, *A. pisum* podczas żerowania na wybranych roślinach żywicielskich.

W trakcie 8-godzinnych rejestracji EPG samice mszycy grochowej najwięcej czasu spędzały penetrując tkanki grochu i bobiku. Mszyce występujące na grochu i bobiku znacznie krócej nakłuwały tkanki peryferyjne, natomiast obserwowano na nich dłuższe wydzielanie śliny do elementów sitowych oraz długotrwałe intensywne pobieranie soku floemowego. Mszyce występujące na pozostałych żywicielach większość czasu spędzały na penetracji tkanek peryferyjnych. Spośród badanych roślin żywicielskich mszycy grochowej, *A. pisum*, groch i bobik okazały się znacznie lepiej akceptowanymi przez tego szkodnika, w porównaniu z pozostałymi.