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FEEDING SITE-DEPENDENT PROBING BEHAVIOUR OF THE PEA APHID *ACYRTHOSIPHON PISUM* HARRIS ON THREE SPECIES OF *LUPINS LUPINUS* L.

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Abstract: Feeding site selection by the pea aphid *Acyrthosiphon pisum* Harris was studied using the technique of electronic registration of stylet penetration in plant tissues (EPG) on leaves and stems of white lupin (*Lupinus albus* L.), yellow lupin (*L. luteus* L.), and narrow-leaved lupin (*L. angustifolius* L.). The EPG revealed waveform C (probing in parenchyma) and waveforms E1 and E2 (salivation in phloem vessels and ingestion of sap, respectively). The differences in the duration, frequency, and proportion of various behavioural activities between aphids on stems and on leaves depended on the lupin variety. *A. pisum* was the most feeding site-sensitive on white lupin, var. Butan and the leaves were the preferred site. The preference was manifested mainly in the longer duration of probing and phloem sap ingestion, and shorter time to reach phloem vessels.

Key words: *Acyrthosiphon pisum, Lupinus albus, L. luteus, L. angustifolius,* EPG, aphid probing behaviour, plant resistance

INTRODUCTION

There are approximately 200 species of lupins in the world flora. In Poland, there occurs one wild species, the big-leaf lupin *Lupinus polyphyllus* Lindl. and many varieties of cultivated lupins that belong to three species: white lupin *L. albus* L., yellow lupin *L. luteus* L., and narrow-leaved lupin *L. angustifolius* L. In Poland as well as in Europe and all over the world, the area of cultivation of lupins is growing due to their features that are desirable in agronomy and animal nutrition. At present, sitona weevils (*Sitona* spp., Coleoptera: Curculionidae) and the pea aphid *Acyrthosiphon pisum* Harris are the major insect pests of wild and cultivated lupins in Poland and direct yield losses in lupin seed caused by aphid feeding alone may reach 100% (Kordan 1999). Losses are the highest when aphids infest lupins in early vegetative stages of development, especially when they settle on developing flower buds and young pods (Zehnder *et al.* 2001; Kordan 2006). There is a great variation in susceptibility to aphid infestation of different species and varieties of lupins (Kordan 2006; Kordan *et al.* 2008). The behaviour of aphids and the susceptibility of plants to aphids may vary significantly depending on the feeding site: e.g., the cabbage aphid *Brevicoryne brassicae* (L.) rarely interrupted probing and phloem sap ingestion while on stems, whereas on leaves, it had difficulty in finding and accepting the phloem and the probes were relatively short and rarely reaching beyond the outer layers of mesophyll (Gabryś *et al.* 1997). The pea aphid behaviour has never been analysed in relation to the feeding site. The aim of the present work was to investigate the hitherto unknown aspects of trophic relationships of the *A. pisum*, i.e., the behavioural background of feeding site selection on lupins. The pea aphid probing behaviour was studied on stems and leaves of *L. albus, L. luteus* and *L. angustifolius*.

MATERIALS AND METHODS

Cultures of aphids and plants

The pea aphid *A. pisum* Harris was reared on *L. luteus* var. Mister under laboratory conditions at 20°C and 16L : 8D photoperiod. In a previous study (Kordan 2006), Mister was found the most suitable variety of lupins for the pea aphid. Aphid-free lupins for experimental purposes were maintained in a growing chamber (21±2°C, 75% r.h., and 16L : 8D). For experiments, the following varieties of lupins were used: *L. luteus*: Dukat, and Lord; *L. albus*: Butan and Boros; *L. angustifolius*: Kalif. The probing behaviour of the pea aphid was monitored on 4-week old plants with 4–6 fully expanded leaves.

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Bioassays

Feeding site selection by the pea aphid was studied using the technique of electronic registration of stylet penetration in plant tissues referred to as EPG. In this experimental set-up, aphid and 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. This technique is frequently employed in insect–plant relationship studies (Tjallingii 1995). In the present study, aphids were attached to a golden wire electrode with silver paint and starved for 1 h prior to the experiment. Probing behaviour of 15 apterous adult females per studied aphid/lupin combination was monitored for 8 h continuously with a four-channel DC EPG recording equipment. Each aphid was given access to the top leaf blades or apical parts of the stem of experimental plants. Signals were saved on the computer disc and analysed using the PROBE 2.1 software provided by Tjallingii. The following EPG patterns were distinguished: np (no penetration – aphid stylets outside the plant), A, B, C, F (pathway phase – penetration of non-phloem tissues), E1 (salivation into sieve elements), E2 (ingestion of phloem sap), and G (ingestion of xylem sap). One-way analysis of variance (ANOVA) was carried out using the experimental results for comparison among lupin varieties. Following ANOVA, a Mann–Whitney two-sample test was used to compare values of EPG parameters at species level. All calculations were performed using the STATISTICA 6.1 package (StatSoft, Tulsa, OK, USA).

RESULTS

The electronic registration (EPG) of the pea aphid probing behaviour on white, yellow, and narrow-leaved lupins revealed waveform C that represents probing in mesophyll and waveforms E1 and E2 that indicate salivation in phloem vessels and ingestion of sap, respectively (Tables 1, 2). Occasionally, patterns F and G occurred, which reflected difficulties in stylet penetration and ingestion of xylem sap, respectively (data not shown). On *L. albus* var. Boros, there were no significant differences in the duration and frequency of almost all of analysed parameters related to the pea aphid probing on leaves and stems, except of the frequency and the duration of individual probes. The probes on leaves were numerous and short (ca. 12 min on average) while on stems there were few probes of longer duration (ca. 1.1 h). On *L. albus* var. Butan, the total probing time and the duration of pathway and phloem sap ingestion were much longer on leaves than on stems, the time to reach phloem vessels was more than three hours shorter on leaves than on stems, and the proportion of activities in sieve elements in total probing was higher on leaves than on stems. The frequency and mean duration of probes were similar on leaves and on stems (Tables 1, 2). The number of probes, the mean probing time, and the duration of sap ingestion were much greater in aphids on stems of var. Boros than on stems of var. Butan. The time to reach phloem vessels was twice shorter on var. Boros than on Butan. There were no differences in aphid behaviour on leaves of the two varieties of white lupin (Fig. 1). On *L. angustifolius* var. Kalif, the probing behaviour of the pea aphid was similar on leaves and on stems, except that the proportion of the phloem phase in all probing activities was five times greater on leaves than on stems (Tables 1, 2). On *L. luteus*

Table 1. Duration of probing activities of the pea aphid *A. pisum* on stems and leaves of different varieties of lupins *Lupinus* spp.

	Total probing time*	Total pathway time*	Total phloem phase time*	Time to 1st phloem phase*	Mean probing time*
L. albus					
Boros leaf	4.1±0.6	2.5±0.3	1.6±0.6	5.1±0.9	0.2±0.1
Boros stem	3.9±0.8	3.4±0.8	0.5±0.1	3.7±1.0	1.1±0.3
p	0.9292	0.3743	0.2481	0.4772	0.0209
Butan leaf	3.6±0.6	2.8±0.4	0.8±0.3	4.2±0.9	0.1±0.0
Butan stem	1.7±0.6	1.7±0.6	0.0±0.0	7.5±0.5	0.1±0.0
p	0.0233	0.0494	0.0041	0.0126	0.0567
L. angustifolius					
Kalif leaf	5.6±0.5	4.3±0.4	1.3±0.3	2.4±0.9	0.8±0.6
Kalif stem	5.2±0.6	4.7±0.6	0.5±0.4	5.5±0.8	0.7±0.3
p	0.8798	0.2899	0.0539	0.1041	0.4057
L. luteus					
Dukat leaf	5.4±0.5	4.9±0.5	0.5±0.3	5.6±0.9	0.6±0.3
Dukat stem	4.5±0.6	3.4±0.4	1.2±0.5	5.6±0.9	0.3±0.1
p	0.1736	0.0494	0.3643	0.8798	0.8206
Lord leaf	5.2±0.5	4.2±0.6	1.1±0.4	3.4±0.8	0.4±0.1
Lord stem	2.7±0.5	2.0±0.4	0.7±0.4	6.6±0.6	0.3±0.1
p	0.0041	0.0082	0.2123	0.0140	0.3258

*time given in hours ±SE; p – value according to Mann-Whitney U-test

Table 2. Frequency and proportion of probing activities of the pea aphid *A. pisum* on stems and leaves of different varieties of lupins *Lupinus* spp.

	Number of probes [#]	Phloem salivation periods [#]	Phloem sap ingestion periods [#]	E/E + C [%]	E1/E1 + E2 [%]
L. albus					
Boros leaf	32.8±5.1	3.2±0.9	2.1±0.5	29.7±8.9	25.4±10.2
Boros stem	5.3±1.0	5.9±2.0	5.9±2.0	12.9±5.1	6.9±2.1
p	0.0006	0.2863	0.0832	0.2135	0.4772
Butan leaf	34.7±3.7	8.2±2.8	5.3±2.1	18.3±5.1	25.5±5.4
Butan stem	24.8±7.4	0.2±0.1	0.0±0.0	1.4±1.3	20.0±13.3
p	0.0963	0.0052	0.0025	0.0102	0.0963
L. angustifolius					
Kalif leaf	28.8±4.8	7.4±1.6	6.0±1.5	20.8±5.1	25.2±8.8
Kalif stem	16.1±3.1	3.2±1.8	2.6±1.4	4.4±3.4	26.5±8.4
p	0.0413	0.0696	0.1212	0.0312	0.9698
L. luteus					
Dukat leaf	20.0±3.6	1.2±0.5	0.6±0.3	9.2±5.7	19.1±10.2
Dukat stem	19.7±4.1	1.4±0.5	0.8±0.3	19.9±7.9	2.4±1.2
p	0.7913	0.7337	0.5967	0.2899	0.4727
Lord leaf	21.9±4.0	3.1±0.9	1.1±0.3	19.8±6.6	41.3±13.9
Lord stem	17.6±4.1	0.9±0.4	0.5±0.2	17.9±8.5	11.4±9.9
p	0.2899	0.0343	0.1405	0.3075	0.0211

*values are means ± SE; p – values according to Mann-Whitney U-test
C – probing in peripheral tissues ('pathway'), E – phloem phase (salivation E1 and ingestion E2)

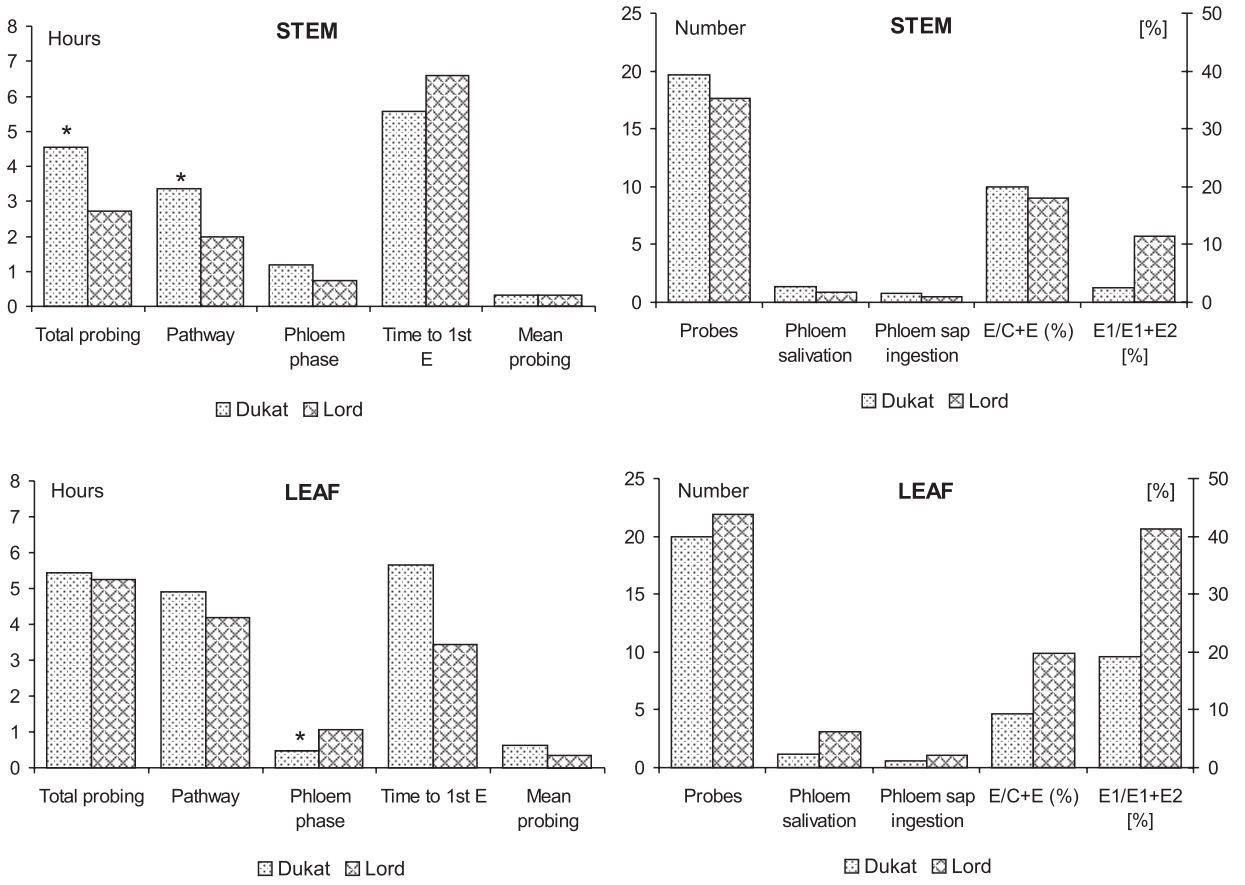

Fig. 1. Variation of probing activities of the pea aphid *A. pisum* on white lupin *L. albus*. Mean duration of total probing, pathway, phloem phase, time to 1st phloem phase, and mean probing time is given in hours (n = 15). Number of probes, number of phloem salivation and phloem sap ingestion periods are means of 15n. Proportion of phloem phase in total probing (E/E+C) and proportion of phloem salivation in total phloem phase is given in % (n = 15). Asterisks indicate statistically significant differences in aphid behaviour on white lupin varities at $p < 0.05$ according to Mann-Whitney U-test

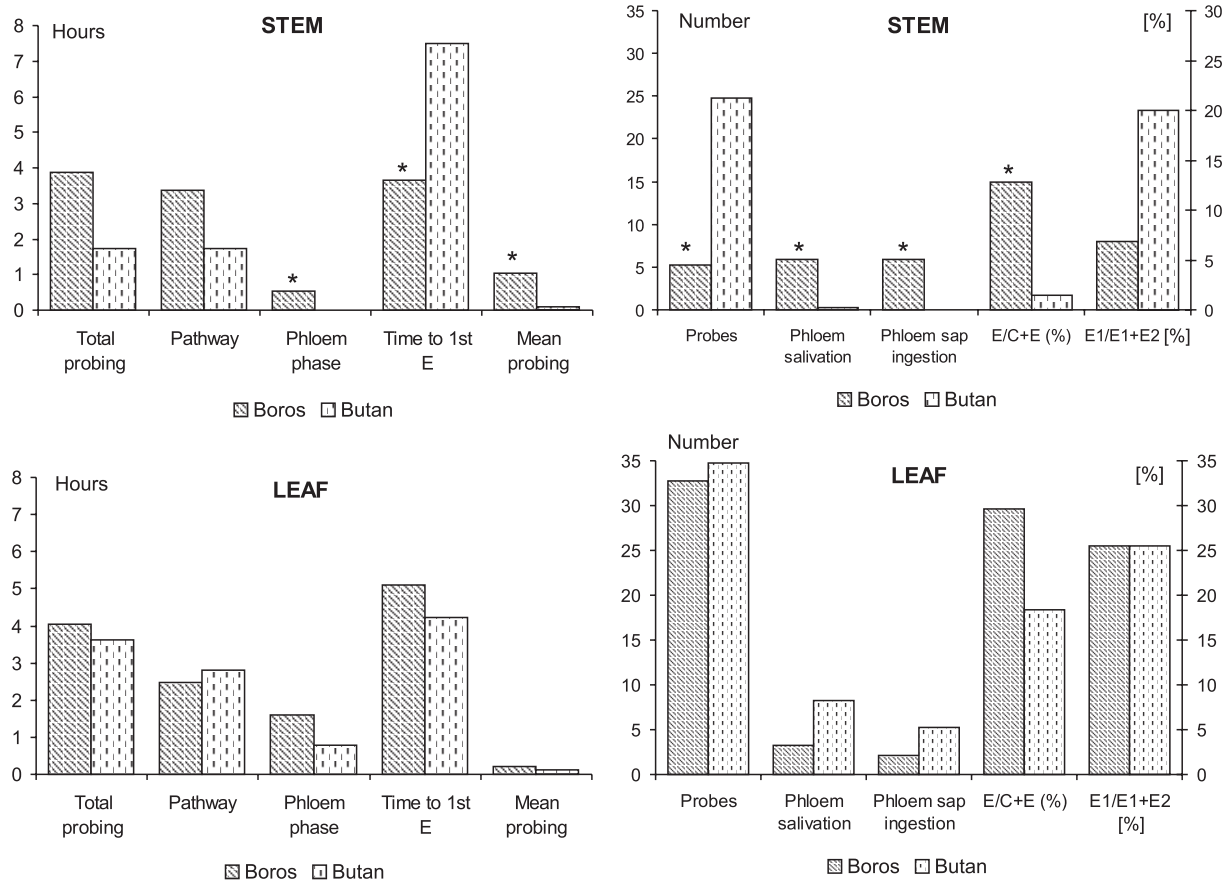

Fig. 2. Variation of probing activities of the pea aphid *A. pisum* on yellow lupin *L. luteus*. Mean duration of total probing, pathway, phloem phase, time to 1st phloem phase, and mean probing time is given in hours (n = 15). Number of probes, number of phloem salivation and phloem sap ingestion periods are means of 15n. Proportion of phloem phase in total probing (E/E+C) and proportion of phloem salivation in total phloem phase is given in % (n = 15). Asterisks indicate statistically significant differences in aphid behaviour on yellow lupin varieties at $p < 0.05$ according to Mann-Whitney U-test

var. Dukat, the behaviour of aphids was similar on leaves and on stems, except for a slightly reduced pathway duration on stems. On the var. Lord, the total probing time and the total duration of aphid stylet penetration in peripheral tissues was longer on leaves than on stems. The time to reach phloem elements was shorter on leaves than on stems but the proportion of salivation to all activities in sieve elements was four times higher on leaves than on stems (Tables 1, 2). The total probing and pathway time were longer in aphids on stems of var. Dukat than on stems of var. Lord. There were no differences in aphid behaviour on leaves of the two varieties of yellow lupin, except of a slightly shorter total duration of the phloem phase on var. Dukat as compared to Lord (Fig. 2).

DISCUSSION

The electronic monitoring of aphid probing behaviour (EPG) provides useful information on the resistance factors and their localisation in plant tissues (Tjallingii and Mayoral 1992). The analysis of parameters derived from EPG (frequency, duration, and sequence of different waveforms) are good indicators of plant suitability or interference of probing by chemical or physical factors in plant tissues (Mayoral *et al*. 1996).

In this study, differences in aphid behaviour related generally to frequency and duration of probes, total duration of phloem sap ingestion, a proportion of sap ingestion in all probing activities, and a ratio of salivation in all aphid activities in sieve elements. The duration of all probing activities and the proportion of sap ingestion in all probing activities were usually longer on leaves than on stems, although not in all cases significantly longer. Differences were highly dependent on a lupin variety. The pea aphid was the most feeding site-sensitive on *L. albus* var. Butan: leaves were a preferred site and stems were clearly rejected. The preference was manifested mainly in a longer duration of probing, shorter time to reach phloem vessels, and a longer duration of phloem sap ingestion. Nevertheless, acceptability of the phloem sap of all studied lupin species and varieties for the pea aphid seems to be very low, which is marked in a high ratio of salivation in all activities in phloem. In our experiments, a prolonged salivation (19–41% of all activities in phloem) was found almost in all of the studied plant/aphid combinations. Short salivation on leaves vs. stems in relation to all activities in phloem (2–7%) was found only in aphids on leaves of Boros (*L. albus*) and Dukat (*L. luteus*). However, these leaf/stem differences were not significant. A substantial proportion of phloem salivation may indicate a deterrent factor in the phloem elements of these plants (Tjallingii 1994), which may impede aphid settling. Prolonged E1 salivation has often been reported characteristic of aphid behaviour on resistant plant cultivars or

non-host plants (Van Helden and Tjallingii 1993; Wilkinson and Douglas 1998; Gabryś and Pawluk 1999). It was established that aphids saliva might be used for neutralising plant defence mechanisms located in sieve elements (Miles 1990). Aphid salivary enzymes (e.g., polyphenol oxidase and peroxidase) are involved in detoxification of plant xenobiotics (Leszczynski *et al*. 1992).

A high number of short probes on one plant in contrast to few long probes on another plant is usually an indication of deterrent factors present in peripheral tissues, such as epidermis and mesophyll. On the other hand, frequently repeated insertion and withdrawal of the stylets may partly be due to the effect of tethering: aphid movement on a plant is limited by a golden wire electrode in the EPG experimental setup. It is possible that aphids would walk away from an unsuitable host after a short probe if they were free to move (Powell *et al*. 1993; Gabryś *et al*. 1997). Aphids are able to recognize a suitable host plant long before their stylets reach the phloem sap (Gabryś and Tjallingii 2002).

Generally, the differences in duration, frequency, and proportion of various behavioural activities between aphids on stems and on leaves occurred on every studied variety of white, yellow, and narrow-leaved lupins. These differences were variety-related and might have reflected the presence of probing and feeding deterrent factors in parenchymatous as well as in vascular tissues. Further study, especially on a chemical composition of the phloem sap is needed to uncover the background of the pea aphid – lupin relationships as it may appear a valuable information on plant resistance.

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POLISH SUMMARY

WPŁYW MIEJSCA ŻEROWANIA NA ZACHOWANIE SIĘ MSZYCY GROCHOWEJ *ACYRTHOSIPHON PISUM* HARRIS NA TRZECH GATUNKACH ŁUBINÓW *LUPINUS* L.

Badano wybór miejsca żerowania na roślinie przez mszycę grochową *Acyrthosiphon pisum* Harris, z wykorzystaniem techniki elektronicznej rejestracji penetracji kłujki mszyc w tkankach roślinnych (EPG), na liściach i pędach łubinu białego (*Lupinus albus* L.), łubinu żółtego (*L. luteus* L.) i łubinu wąskolistnego (*L. angustifolius* L.). Stwierdzono występowanie modelu 'C' (penetracja tkanki miękiszowej) oraz modeli E1 (wydzielanie śliny do elementów floemu) i E2 (pobieranie soku floemowego). Różnice w czasie trwania, częstotliwości występowania i względnych proporcjach poszczególnych modeli, które wykazano u mszyc żerujących na liściach i pędach roślin, uzależnione były od gatunku i odmiany łubinu. Miejsce żerowania na roślinie miało największy wpływ na zachowanie się *A. pisum* na łubinie białym odmianie – Butan: mszyce wyraźnie preferowały liście w stosunku do pędów. Na liściach, mszyce między innymi dłużej penetrowały tkanki roślinne, dłużej pobierały sok floemowy i szybciej docierały do elementów floemu.