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EFFECT OF LUPIN CYCLITOLS ON PEA APHID PROBING BEHAVIOUR

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Abstract: The cyclitols: D-pinitol, D-*chiro*-inositol are naturally present in the tissues of *Lupinus angustifolius*. The effect of these cyclitols on the behaviour of the pea associated clone of *Acyrthosiphon pisum* during various stages of probing was studied. The main stage of probing studied was the stylet penetration in mesphyll and vascular bundle. D-pinitol, D-*chiro*-inositol and their mixture were exogenously applied to pea *Pisum sativum* explants and the aphid probing behaviour was evaluated using the Electrical Penetration Graph technique (EPG). Feeding of peas with cyclitols at a concentration of 10 mM, caused a selective accumulation of D-pinitol and D-*chiro*-inositol in stems, leaf petioles, and leaf blades. In aphid bodies, both cyclitols were traced, respectively, to the host plant treatment. The new cyclitols in pea tissues did not significantly affect the total duration and frequency of aphid activities during probing in peripheral as well as vascular tissues. However, the aphid behaviour on cyclitol-treated plants as compared to their behaviour on the control was slightly altered. Non-probing and probing in mesophyl prevailed among aphid activities during the initial period of stylet penetration. Aphids on D-pinitol+D-*chiro*-inositol-treated plants reached phloem vessels relatively later than aphids on the control and D-*chiro*-inositol plants. There were recurrent switches between E1 (salivation) and E2 (sap ingestion) patterns in some aphids during the phloem phase on D-pinitol and D-pinitol+D-*chiro*-inositol – treated plants. This may reflect difficulties in the uptake of the phloem sap, and point to lupin cyclitols as being responsible, at least in part, for the rejection of *L. angustifolius* as a host plant by the pea clone of *A. pisum*.

Key words: D-pinitol, D-*chiro*-inositol, *Pisum sativum*, *Lupinus angustifolius*, *Acyrthosiphon pisum*, chemical ecology, Electrical Penetration Graph Technique (EPG)

INTRODUCTION

Cyclitols, the hydroxylated cycloalkanes biosynthetically derived from glucose, occur in all living cells and express a broad spectrum of biological activity. They participate in many cellular processes including membrane biogenesis and dynamics, signal transduction, ion channel physiology, osmoregulation, and antioxidation (Bieleski 1994; Loewus and Murthy 2000; Merchant *et al.* 2006; Michell 2008) as well as in environmental interactions, *e.g.*, in conferring salt tolerance in plants and plant response to water stress (Arndt *et al.* 2004; Das-Chatterjee *et al.* 2006; Merchant *et al.* 2006). Cyclitols may also be involved in plant-insect relationships. However, this aspect of their biological activity has rarely been studied. Glendinning *et al.* (2000) found that *myo*-inositol was vital in regulating feeding in tobacco hornworm *Manduca sexta* caterpillars by counteracting the inhibitory effects of caffeine. Dreyer *et al.* (1979) demonstrated that pinitol was a larval growth inhibitor for *Helicoverpa zea* (Boddie) in soybeans *Glycine max* (L.) Merr.

Leguminous plants (Fabaceae) contain an exceptionally high amount of cyclitols compared to the majority of plant species: in some seeds, these sugar alcohols may make up to 30% of the total content of soluble carbohydrates (Szczeciński *et al.* 2000). Among a variety of herbivores that feed on legumes, the pea aphid *Acyrthosiphon pisum* (Harris 1776) (Homoptera: Aphididae) is of special character and importance. It is a well-known almost worldwide oligophagous species that infests leguminous plants and transmits over 30 virus diseases (Blackman and Eastop 1985). Interestingly, *A. pisum* is a complex of subspecies, races, and clones with different host preferences and different abilities to transmit viruses (Blackman and Eastop 2007; Katis *et al.* 2007). Moreover, the pea aphid races that live sympatrically on closely related host plants are reproductively isolated from one another (Via 1999). In Poland, the pea aphid populations are the most abundant on the pea *Pisum sativum* L., broad bean *Vicia faba* L., lentils *Lens culinaris* Medik., lucerne *Medicago sativa* L. and clover *Trifolium* spp. Due to the rising economic importance of lupins *Lupinus* spp., *A. pisum* is becoming a threat

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to this crop, especially the sweet low-alkaloid lupin varieties (Kordan 2006). Rahbe *et al.* (1988) and Sandström and Pettersson (1994) found that nutritional factors, *i.e.*, primary plant substances, mainly amino acids and sugars were not responsible for host specialization in *A. pisum*. According to del Campo *et al.* (2003), pea aphids accept plants if they contain host-specific chemical compounds. In our previous study on the capability of the pea-associated clone of *A. pisum* to infest lupins, we compared the pea aphid's development, probing behaviour, and plant acceptance on six varieties of two species of lupins: narrow leaf lupin *Lupinus angustifolius* L. and yellow lupin *Lupinus luteus* L. The EPG (Electrical Penetration Graph = electrical registration of aphid probing behaviour) experiments showed that all varieties of *L. angustifolius* were rejected by aphids during an early stage of probing in peripheral tissues, epidermis, and/or mesophyll. Aphids did not ingest phloem sap while probing on *L. angustifolius*, and the probes were very short (Kordan *et al.* 2008). *L. luteus* and *L. angustifolius* that we studied, differed in qualitative and quantitative content of cyclitols: the amount of *myo*-inositol and D-ononitol was similar in both lupin species, D-pinitol was 2–3 times more abundant in *L. angustifolis* than in *L. luteus*, and D-*chiro*-inositol occurred only in *L. angustifolius*. We speculated that at the species level, the distinct differences in plant chemistry detected by *A. pisum* at the epidermis and mesophyll level might have caused the acceptance of *L. luteus* and the rejection of *L. angustifolius* (Kordan *et al.* 2008). Campbell and Binder (1984) demonstrated that *A. pisum* ingested cyclitols with the phloem sap. However, the influence of these compounds on host plant selection in general and the probing behaviour in particular by the pea aphid has not been studied in detail, yet.

The aim of the present study was to evaluate the effect of individual cyclitols and their mixture naturally present in tissues of *L. angustifolius*, on the behaviour of *A. pisum* during various stages of probing, mainly the stylet penetration in mesphyll and vascular bundle.

MATERIALS AND METHODS

Cultures of aphids and plants

Pea aphid *A. pisum* was reared on peas *P. sativum* var. Oregon under laboratory conditions at 20°C and 16L:8D photoperiod. Aphid-free *P. sativum* var. Oregon for experimental purposes was maintained in a growing chamber [21(±2)°C, 75% RH, and 16L : 8D].

Plant – aphid transfer of polyols

The following cyclitols were used: D-pinitol (Sigma) and D-*chiro*-inositol (Fine Chemicals; Industrial Research Ltd, Lower Hutt, New Zealand). The cyclitols were applied to peas *P. sativum* L. that naturally contains only *myo*-inositol. The apical parts (8–10 cm long) with two leaves of 3-week old peas were cut. The stem was immediately placed in water (the control) or a water solution of D-pinitol, D-*chiro*-inositol, and their mixture (at 10 mM concentration each), for 3 h prior to the experiments. The concentration of cyclitols was chosen according to the results of previous experiments performed on vetch explants (Lahuta *et al.* 2005a, 2005b). The plant parts remained in water or the cyclitol solution for a further 8 h during the EPG (monitoring of aphid feeding) experiment. All together, plant parts remained in the watering medium a total of 11 hours. The cyclitol solution – plant – aphid transfer was confirmed by chemical analysis of plants and aphids used for the experiments. EPG-recorded aphids were placed on the leaf blades. Given the length of the golden wire electrode (*ca.* 3 cm), aphids had a limited possibility of movement on the plant. However, it cannot be excluded that at some point of the 8-hour experiment they changed the feeding site from leaf blade to a leaf petiole or the apical part of the stem. Therefore, after the EPG recording, plants were divided into three parts: leaf blades, leaf petioles, and stems. Fresh plant tissue was put into a microwave at 300 W for 2 minutes (for enzymes inhibition), dried at 80°C for 24 hours, and stored in a freezer until the analysis. The aphids were removed from the plants and put into tubes containing 200 µl of 80% ethanol, then heated in a water bath at 80°C for 2 minutes, and stored in a freezer until the analysis.

Analysis of polyols in plants and aphids

Soluble carbohydrates in plants and aphids were extracted and assayed as described previously (Lahuta 2006), with minor modifications. In plant samples, sugars were extracted from 50 mg lyophilized tissue using 1 ml 50% ethanol containing 100 µg xylitol as the internal standard. An internal standard (10 µl of xylitol at 10 mg/ml concentration) was also added to tubes containing aphids in 80% ethanol. Both types of samples: plants tissues and aphids were shaken for 2 min (1,300 rpm) and heated at 80°C for 20 min. After centrifugation, supernatant was deionized, centrifuged again and clear supernatant was dried in a speed-vacuum rotary evaporator. The derivatization of carbohydrates extracts and conditions of gas chromatographic separation were identical to those described previously (Lahuta 2006). Standards of sugars: glucose, fructose, sucrose, and trehalose were obtained from Sigma (USA). *Myo*-inositol, D-*chiro*-inositol, and D-pinitol were obtained from Fine Chemicals (Industrial Research Ltd, Lower Hutt, New Zealand). Carbohydrate content was calculated from standard curves of the appropriate component.

Effect of polyols on aphid feeding (EPG)

Aphid probing behaviour was monitored using the Electrical Penetration Graph (EPG) technique that is frequently employed in insect–plant relationship studies (Tjallingii 1995). The parameters describing aphid behaviour during probing and feeding, such as total time of probing, proportion of phloem patterns E1 and E2, number of probes, *etc*., are good indicators of plant suitability or interference of probing by chemical or physical factors in certain plant tissues (Mayoral *et al.* 1996). 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 females per studied cyclitol/aphid combination was monitored continuously, for 8 h with a four-channel DC EPG recording equipment. Each aphid was given access to

freshly prepared pea explant kept in a given cyclitol solution or in water (control experiment). Aphids were placed on the leaf blades. Signals were saved on the computer and analysed using the PROBE 2.1 software provided by W. F. Tjallingii (EPG-Systems, Dillenburg 126703 CJ, Wageningen, The Netherlands). 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).

RESULTS

Accumulation of polyols in plants and aphids

The representative results of GC analysis of *Pisum sativum* explants treated with cyclitols, and aphids that fed on these explants are shown in figure 1. The application of cyclitols had a significant effect on the total content of carbohydrates in plant tissues. The total content of soluble carbohydrates ranged from approximately 33 mg/g d.w. in the control *Pisum sativum* to 87 (D-pinitol), 91 (D-*chiro*-inositol), and 95 mg/g d.w. (cyclitol mixture-treated plants) (Table 1). The highest concentration of sugars occurred in leaf petioles as compared to leaf blades and stems. However, there were no qualitative differences in the content of soluble carbohydrates among leaf blades, leaf petioles, and stems in any treatment. The tissue of control plants incubated in water contained glucose, fructose, sucrose, *myo*-inositol, and galactose (at the level < 1 mg/g d.m., data not shown). In explants fed with D-pinitol or D-*chiro*-inositol, the appropriate cyclitols were found in the tissues of leaf blades, petioles, and stems. Both cyclitols constituted ca 40–50% of total soluble carbohydrates. In explants fed with D-pinitol, traces of D-*chiro*-inositol were found.

In the bodies of the control pea aphid *Acyrthosiphon pisum*, trehalose predominated among the sugars (Table 1). The remaining identified sugars were glucose, mannitol, sucrose, and *myo*-inositol. Aphids that fed on D-pinitol – treated plants also contained D-pinitol (4% of all sugars). Likewise, aphids on D-*chiro*-inositol plants contained D-*chiro*-inositol (4%), and aphids on cyclitol mixture plants contained both, D-pinitol and D-*chiro*-inositol (1 and 4%, respectively). The total content of soluble carbohydrates in the bodies of the pea aphid ranged from 28 μg (aphids on D-pinitol treated plants) to 54 μg per individual (aphids on D-pinitol+D-*chiro*-inositol treated plants) (Table 1).

Effect of polyols on aphid feeding (EPG)

Electronic monitoring of aphid probing behaviour revealed that the pea aphids showed all kinds of activities on the control as well as on cyclitol-treated peas. These activities were: non-probing, pathway activities in mesophyll ('C'), phloem salivation ('E1'), phloem sap ingestion ('E2'), xylem sap ingestion ('G'), and 'probing difficulties' ('F') (Table 2). Stylet penetration in plant tissues made up approximately 90% of the experimental time. Zero up to 26% of aphids showed activities associated with the uptake of xylem sap (pattern 'G'). However, the duration of this activity was relatively short: 0–3% of probing time. The percentage of aphids that showed short periods of pattern 'F': unidentified probing difficulties during stylet penetration in mesophyll, was 13–33%. The relative duration of this activity was 0.5–7% of the probing time. Nearly all aphids showed phloem sap ingestion activity that took 55–67% of the probing time irrespective of the

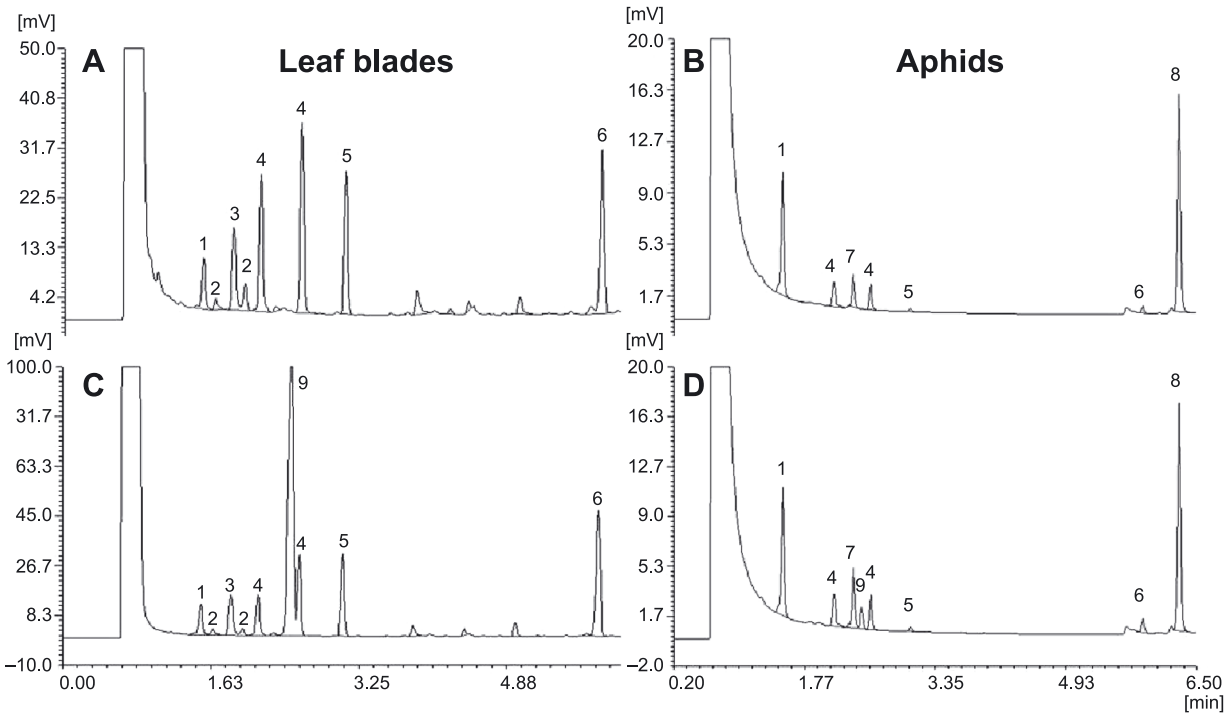

Fig. 1. Gas chromatograms of TMS-derivatives of soluble carbohydrates extracted from pea leaves (A, C) of control explants (A) or explants fed with D-*chiro*-inositol (C), and aphids that fed on respective leaves (B – control, D – fed with D-*chiro*-inositol). Abbreviations: 1 –internal standard (xylitol); 2 – galactose; 3 – fructose; 4 – glucose; 5 – *myo*-inositol; 6 – sucrose; 7 – mannitol; 8 – trehalose; 9 – D-*chiro*-inositol

Table 1. Soluble carbohydrates in *P. sativum*[1,2] and *A. pisum*[1,3] after application of cyclitols

Carbohydrates supplied solution	Water (control)	D-*chiro*-inositol	D-pinitol	*chiro*-inositol + D-pinitol
Leaf blades				
D-*chiro*-inositol	–*	32.74±1.07	1.38±0.23	19.38±2.15
D-pinitol	–	–	49.88±7.25	27.71±3.38
myo-inositol	3.55 ±0.59	5.24±0.17	6.94±1.08	4.42±0.37
Glucose	11.8±1.35	15.89±1.99	24.45±6.87	13.09±2.29
Fructose	3.27±0.54	5.74±2.06	7.65±2.06	7.43±1.10
Sucrose	4.74±2.30	7.58±0.90	5.07±3.40	16.10±1.74
Total	23.35±3.05 a	67.20±3.60 b	95.37±14.21 b	88.15±9.05 b
Leaf petioles				
D-*chiro*-inositol	–	38.57±8.09	1.74±0.49	37.97±4.08
D-pinitol	–	–	46.13±9.92	47.12±7.79
myo-inositol	6.44±1.22	7.64±1.50	6.13±0.87	6.11±0.63
Glucose	33.27±1.38	56.47±7.41	39.91±7.82	24.51±1.01
Fructose	3.45±1.51	4.35±0.73	4.89±1.14	3.93±1.11
Sucrose	1.24±0.28	0.0±0.0	0.19±0.19	3.82±3.82
Total	44.40±2.26 a	107.03±12.79 b	98.99±6.20 b	123.46±10.82 b
Stems				
D-*chiro*-inositol	–	43.94±5.93	1.02±0.09	13.42±1.69
D-pinitol	–	–	27.49±3.1	17.33±2.39
myo-inositol	3.45±0.07	6.82±1.05	4.00±0.16	3.35±0.01
Glucose	21.37±1.56	39.09±7.1	26.96±2.10	24.05±0.67
Fructose	3.94±0.30	7.1±1.42	3.99±0.86	3.69±1.01
Sucrose	3.92±0.95	0.85±0.51	4.65±1.63	13.65±2.23
Total	32.69±2.83 a	97.80±14.18 b	68.11±3.96 a	75.48±5.92 b
Aphids				
D-*chiro*-inositol	–	2.22±0.99	–	2.15±0.17
D-pinitol	–	–	1.16±0.32	0.59±0.11
myo-inositol	0.27±0.0	0.71±0.13	0.24±0.08	0.26±0.01
Glucose	2.98±2.02	4.06±3.98	2.25±0.73	2.68±0.70
Sucrose	2.37±1.04	1.66±0.43	0.77±0.28	7.22±4.23
Trehalose	42.62±8.10	36.29±2.88	22.56±5.74	38.01±2.38
Mannitol	2.55±0.53	5.06±2.65	1.57±0.52	2.70±1.04
Total	50.53±6.59	48.67±10.80	28.30±7.67	54.14±6.65

*not detected; [1] values represent means ±SD; [2] mg/g dry matter; [3] µg/aphid

Plants: statistically significant ($p < 0.05$) differences in total content of carbohydrates among explants treated with cyclitols are marked with different letters (a–b) after Tukey's correction for multiple comparisons

Aphids: no statistically significant differences were found at $p = 0.05$ (Kruskal-Wallis test)

Table 2. Behavioural effects of cyclitols on *A. pisum*

		Control	D-*chiro* –inositol	D-pinitol	D-*chiro* -inositol D-pinitol	P
Total duration of non-probing 'np'	min	43.4±9.2	39.7±6.1	48.2±10.5	56.0±14.7	0.9553
Total duration of pathway 'C'	min	133.5±16.7	136.2±28.1	149.8±15.6	148.5±22.1	0.5107
Total duration of xylem sap ingestion 'G'	min	0.0±0.0	4.7±3.1	13.6±6.2	5.6±5.6	0.1180
Total duration of probing difficulties 'F'	min	32.0±15.9	2.6±1.8	29.0±12.8	12.5±7.0	0.2830
Total duration of phloem sap ingestion 'E'	min	271.1±27.6	296.8±33.7	239.3±21.2	257.4±31.5	0.1997
Proportion of phloem phase in all probing E/C+F+G+E		0.62	0.67	0.55	0.60	0.1715
Proportion of salivation in phloem activities E1/E1+E2		0.03	0.02	0.04	0.05	0.0940
Number of probes	#	16.2±3.2	25.33±4.1	24.38±3.5	20.2±2.9	0.2317
Number of phloem phases	#	2.5±0.7	1.7±0.2	3.4±0.7	2.9±0.7	0.4078
Number of phloem phases longer than 10 min	#	1.9±0.3	1.5±0.2	2.4±0.4	1.5±0.3	0.1886

Values represent means ±SE. Kruskal-Wallis test at $p = 0.05$

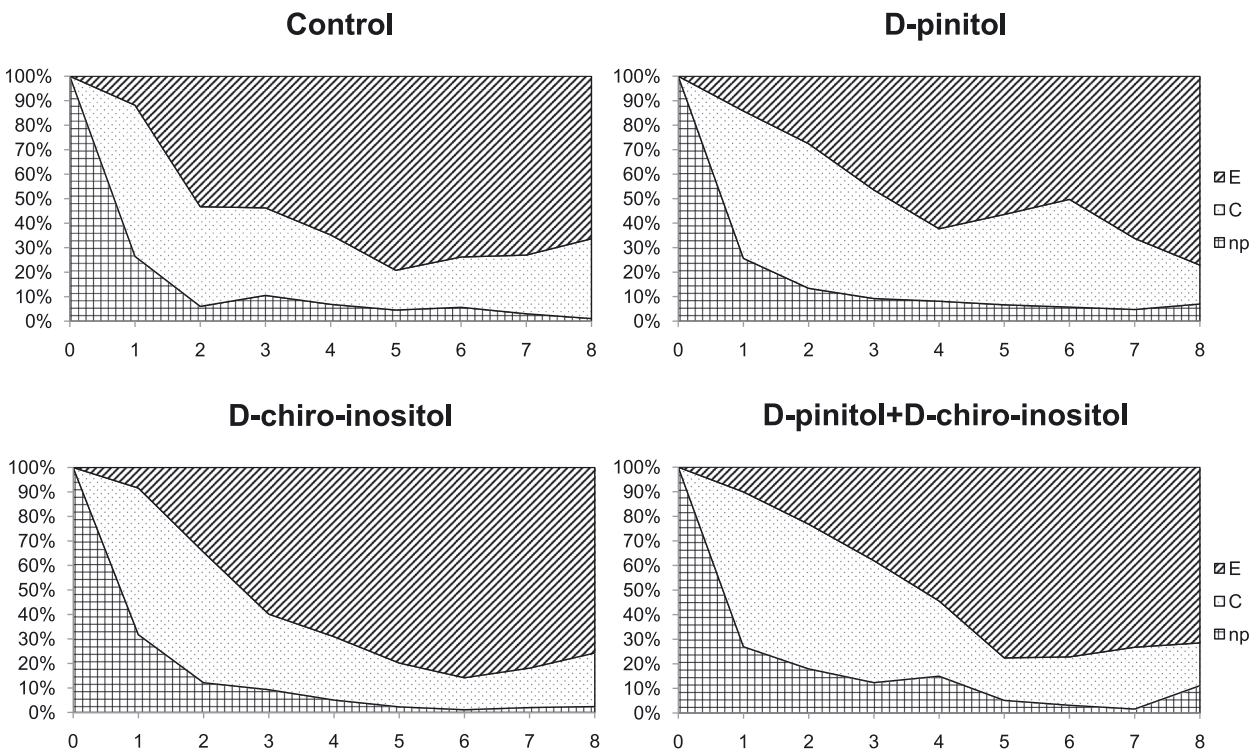

Fig. 2. Proportion of different activities in relation to the complete probing of *A. pisum* on *P. sativum* treated with cyclitols during 8-hour EPG experiment. np – no probing, C – pathway, E – phloem activities (salivation and sap ingestion)

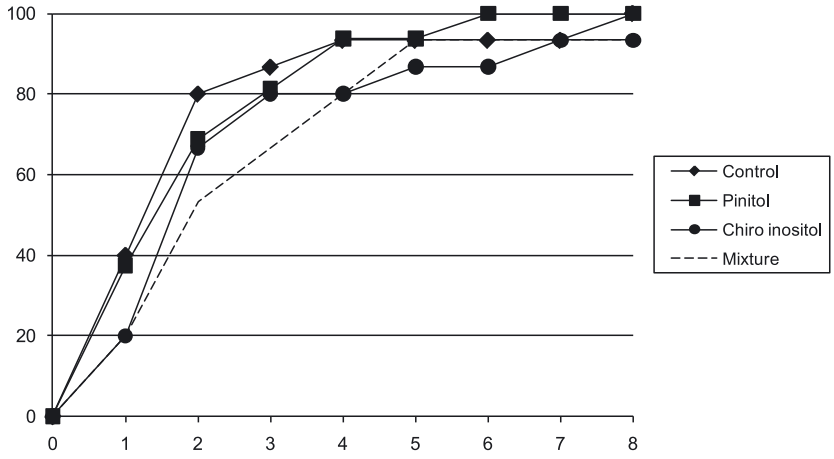

Fig. 3. Cumulative percent of *A. pisum* reaching sieve elements on *P. sativum* treated with cyclitols during 8-hour EPG experiment

treatment. The periods of sustained sap ingestion ('E2' > 10 min.) were rarely interrupted: there were a minimum of 1.5 (D-*chiro*-inositol plants) and up to a maximum of 2.4 (D-pinitol plants) phloem phases per aphid on average, and the duration of an individual sustained ingestion phase was relatively long, i.e., maximum 3.3 (D-*chiro*-inositol plants) – minimum 1.7 hours (D-pinitol plants) (Table 2). In the course of the experiment, the proportion of phloem activities increased in relation to all probing events in all aphids, irrespective of plant treatment (Fig. 2). However, aphids on D-pinitol+D-*chiro*-inositol-treated plants reached phloem vessels relatively later than aphids on the control and D-*chiro*-inositol plants (Fig. 3). The proportion of salivation into sieve elements ranged from 2 to 5% of all stylet activities in the phloem. There were frequent switches between E1 and E2 (data not shown) and consequently a slight increase in proportion of salivation (E1) up to 4 and 5% in relation to all probing activities in sieve elements. The switches and salivation increase occurred during the phloem phase on D-pinitol and D-pinitol+D-*chiro*-inositol - treated plants, respectively (Table 2).

DISCUSSION

Among the three cyclitols identified in the analyzed plant parts of the cyclitol-fed experimental peas *P. sativum,* the only naturally occurring cyclitol was *myo*-inositol. The control plants contained only this compound. The quantity of *myo*-inositol was similar in all plant parts, irrespective of the treatment. In our experiments, all explants of *P. sativum* accumulated D-pinitol and D-*chiro*-inositol in their tissues after they had been fed with a water solution of these polyols. This showed the ability of pea plants to take up cyclitols from the media in which they were grown. Moreover, the uptake of cyclitols might have changed the metabolism and accumulation of other soluble carbohydrates in the plants. Plants fed with D-*chiro*-inositol accumulated the new cyclitol in the stem, leaf blades, and leaf petioles. In the leaf blades and in stems, the content of D-*chiro*-inositol was higher than the content of glucose, the predominating sugar among soluble carbohydrates in the control plants. However, the presence of D-*chiro*-inositol was associated with an increase of glucose content in plants, compared with the control. In D-*chiro*-inositol – treated plants, the content of fructose and *myo*-inositol also increased, while the content of sucrose decreased. In D-pinitol – treated plants, an increase in glucose, fructose, and *myo*-inositol content was found. Additionally, the content of sucrose also increased, which was in contrast with D-*chiro*-inositol – treated plants. Plants accumulating D-pinitol also contained D-*chiro*-inositol, although this cyclitol was not included in the solution applied to plants. This indicates that active enzymes capable of demethylation of D-pinitol are present in pea tissues. The application of a mixture of cyclitols caused their accumulation in all plant organs. However, the content of each cyclitol was lower than when they were applied separately. In each plant part, the content of D-pinitol was higher than the content of D-*chiro*-inositol. This suggests that there is competition in cyclitol translocation among plants organs. In the experimental peas, there was no increase in the amount of monosaccharides but the quantity of sucrose was considerably higher (*ca.* four times) than in the control plants, especially in leaf blades and stems. This is the first report indicating the transport of D-pinitol and D-*chiro*-inositol in pea plants.

The composition of essential carbohydrates in all aphids was typical for these insects: small amounts of glucose and sucrose, and trehalose as the main sugar in the hemolymph. Such a composition illustrates the adaptation of aphids to deal with the high osmotic pressure of the consumed phloem sap that contains high concentrations of sucrose (Wilkinson *et al.* 1997). The content of mannitol in all aphids irrespective of the treatment in this study, reflects the adaptation of aphids to deal with the temperature of the environment. These findings correspond with the findings of Hendrix and Salvucci (1998), who found mannitol in aphids at temperatures comparable to those in our experimental chamber (21±2°C). The three cyclitols detected in the tissues of cyclitol-treated plants, *i.e.*, *myo*-inositol, D-pinitol, and D-*chiro*-inositol were also found in the pea aphids that fed on these plants, which confirms the ability of aphids to absorb them from the consumed sap (Campbell and Binder 1984). The ingestion of phloem sap is the only possibility for aphids to take up high quantities of plant allelochemicals. In the present experiment, aphids spent 55–67% of their probing time ingesting phloem sap. Short cell punctures during pathway (*i.e.*, 'potential drops' during the 'C' phase in mesophyll) serve only for the ingestion of small sap samples for gustatory purposes (Gabryś and Tjallingii 2002). Generally, there were no significant differences in aphid behaviour on plants treated either with individual cyclitols or with their mixture. The total duration and frequency of various probing activities and especially the ingestion of phloem sap were similar in all aphids. Such results indicate that the studied cyclitols might not have had a clear effect on aphid probing. However, a slight delay in reaching the phloem took place, and there occurred frequent switches between E1 and E2 during the phloem phase on D-pinitol- and D-pinitol+chiro-inositol – treated plants. These phenomena might reflect a difficulty in uptaking the phloem sap. Such a difficulty is probably of a chemical nature. Similar trends were observed in the probing of aphids on resistant plants or on unsuitable hosts (Klinger *et al.* 1998; Gabryś and Pawluk 1999; Pettersson *et al.* 2007). The waveform E1 is associated with the egestion of watery saliva into the sieve elements. Short periods of salivation always precede the sap ingestion. In this context, E1 is perceived as a kind of 'preparation' of sieve elements for prolonged ingestion of sap by aphids (Pettersson *et al.* 2007). On suitable host plants, the sap ingestion periods (E2) may last for many hours with no interruption. The watery saliva contains various enzymes associated with detoxification of plant allelochemicals, such as UPD-glucose transferases, polyphenol oxidases, and peroxidases (Leszczyński *et al.* 1992; Pettersson *et al.* 2007). Therefore, the long duration or frequent periods of phloem salivation that interrupt sustained sap ingestion during the phloem phase may be associated with plant defense mechanisms based on xenobiotics. Frequent and relatively long periods of phloem salivation occurred mainly on D-pinitol- and D-pinitol+chiro-inositol – treated plants. Natural populations of the pea clones of *A. pisum* do not contact either cyclitol in the phloem sap of their host plants because they do not occur in *P. sativum* in nature. Therefore, these compounds may be at least in part, responsible for the low suitability of narrow-leaved lupin as a host plant to the pea clone of *A. pisum*.

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POLISH SUMMARY

WPŁYW CYKLITOLI ŁUBINU NA ZACHOWANIE MSZYCY GROCHOWEJ PODCZAS ŻEROWANIA

Badano wpływ cyklitoli: D-pinitolu i D-*chiro*-inozytolu oraz ich mieszaniny na zachowanie mszycy grochowej (*Acyrthosiphon pisum*) podczas różnych faz żerowania, głównie penetracji kłujki w tkankach miękiszowych i przewodzących. D-pinitol i D-*chiro*-inozytol zostały podane w roztworze, w którym zanurzono końce odciętych pędów grochu *Pisum sativum*. W naturze tkanki grochu są pozbawione tych cyklitoli. Natomiast są one obecne

w tkankach łubinu wąskolistnego *Lupinus angustifolius*, który nie jest akceptowany przez mszycę grochową. Zachowanie mszyc podczas żerowania badano za pomocą techniki Elektronicznej Rejestracji Żerowania (Electrical Penetration Graph – EPG). Egzogenna aplikacja cyklitoli w stężeniu 10 mM spowodowała akumulację tych substancji w pędach, ogonkach i blaszkach liściowych grochu. Obecność cyklitoli stwierdzono również w organizmie mszyc żerujących na badanych pędach grochu. Cyklitole nie wpływały istotnie na całkowity czas trwania i częstość poszczególnych etapów penetracji kłujki tak w tkankach peryferyjnych, jak i przewodzących. Jednak stwierdzono, że po podaniu mieszaniny cyklitoli (D-pinitolu i D-*chiro*-inozytolu), wśród aktywności związanych z żerowaniem przeważał brak penetracji i penetracja tkanek peryferyjnych. Ustalono również opóźnienie w rozpoczęciu żerowania, jak też częste zmiany modeli E1 (wydzielanie śliny do floemu) i E2 (pobieranie soku floemowego). Zjawiska te mogą wskazywać na trudności w żerowaniu spowodowane przez cyklitole. Zawartość D-pinitolu i D-*chiro*-inozytolu w tkankach łubinu wąskolistnego może być jedną z przyczyn braku akceptacji tej rośliny przez mszycę grochową.