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ORIGINAL PAPER

Probing behavior of bird cherry-oat aphid *Rhopalosiphum padi* (L.) on native bird cherry *Prunus padus* L. and alien invasive black cherry *Prunus serotina* Erhr. in Europe and the role of cyanogenic glycosides

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Abstract The probing behavior of bird cherry-oat aphid *Rhopalosiphum padi* was studied on its natural winter host in Europe, the bird cherry *Prunus padus,* and on the invasive black cherry *Prunus serotina,* on which spring generations of *R. padi* do not survive. The EPG-recorded behavior of *R. padi* on bird cherry and black cherry showed differences in crucial aspects of probing and feeding. The period of the pre-phloem penetration was twice as long and rarely interrupted in aphids on bird cherry as opposed to aphids on black cherry. On black cherry, there was a considerable delay between finding and accepting the phloem. Aphids that had sampled phloem sap either refused to ingest it or the ingestion periods were very short. Amygdalin and prunasin (cyanogenic glycosides present in leaves of *Prunus*) seriously impeded ingestion activities when applied in pure sucrose diet. The role of amygdalin and prunasin in winter host plant selection and host alternation in *R. padi* is discussed.

Keywords Bird cherry · Black cherry · *Prunus padus* · *Prunus serotina* · Probing behavior · EPG · Prunasin · Amygdalin · Host selection

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Introduction

The bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) (Hemiptera, Sternorrhyncha: Aphididae) is one of the most important pests of temperate cereal crops, causing damage as a virus vector and by direct feeding (Vickerman and Wratten 1979). *R. padi* is a holocyclic and host-alternating aphid species, which means that it alternates parthenogenetic with bisexual reproduction, thus starting another cycle by laying winter eggs on winter host. The switch requires a change of the host plant. Holocyclic development, basically in areas where winters are generally cold starts with the hatching of virginoparous females (*fundatrices*) from the eggs that overwintered in bark crevices of the aphid's primary (i.e., "winter") host plant. *Fundatrices* give birth to wingless virginoparous *fundatrigeniae*. In spring, after two or three virginoparous generations on primary host, winged aphids called spring migrants appear and fly to the secondary hosts: cereals and other Poaceae. During summer, a number of virginoparous generations develop on these plants. In autumn, the shortening days trigger the production of the winged parthenogenetic females (*gynoparae*) that fly to the primary host and give birth to wingless mating females (*oviparae*). Later, winged males are also produced on secondary host plants and they migrate to winter host as well, to mate with the *oviparae*. The latter lay the overwintering eggs (Dixon 1971; Powell and Hardie 2001). Anholocyclic populations of *R. padi* have been described, too. These populations overwinter in the form of virginoparous females on winter cereal crops and other Poaceae, where conditions permit and where the basic primary host, bird cherry (*Prunus padus* L. subsp. *padus* = syn. *Padus avium* Mill.), or alternative primary hosts are not available (Simon et al. 1991; Blackman and Eastop 2006).

R. padi is currently characterized by Holarctic distribution but it is difficult to determine its origins unequivocally (Blackman and Eastop 2007). Within its present range, *R. padi* overwinters on plants that are native to particular regions: choke cherry (*Prunus virginiana* (L.) M. Roem) in North America (Stoetzel and Miller 2001), blackthorn (*Prunus spinosa* L.) in Asia (Aslan and Uygun 2005), or bird cherry (*Prunus padus*) in Europe (Blackman and Eastop 1984). As in other host-alternating aphid species, the specificity to the primary host in *R. padi* is very high. However, non-indigenous plants that are co-occurring in a given area are likely to provide vacant ecological niches and thus be used as reserve hosts (Blackman and Eastop 1984). Indeed, *R. padi* can develop on dwarf Russian almond (*Amygdalus nana* L. syn. *Prunus tenella* Batsch) and spring cherry (*Prunus subhirtella* Miq.) (Hille Ris Lambers 1971). Cichocka and Lubiarz (2003) reported the bird cherry-oat aphid infesting cherry plum (*Prunus cerasifera* Ehrh.) grown as trimmed hedges in southeastern Poland.

Black cherry (*Prunus serotina* Erhr.) and bird cherry are the most common *Prunus* species in Europe. *P. serotina* is native to North America and it has successfully been introduced into Europe in the seventeenth century. At present, the species is regarded as an intensively spreading invasive alien that has the ability to form monospecific stands in nature (Starfinger et al. 2003). The recent study by Klueken et al. (2012) demonstrated that the colonization of summer hosts by *R. padi* depends on the density of winter hosts which are a source of early season migrants. It is likely that the increasing abundance of *P. serotina* may contribute to the bird cherry-oat aphid population build-up in the adjacent cereal crops. In Poland, it occurs commonly, especially in lowlands on dry soils and in many forest cover types (Olszewska 2005). *P. serotina* shows close phylogenetic affinity to *P. padus* (Bortiri et al. 2001). Therefore, theoretically, it might present a suitable primary host for *R. padi* within its ancestral as well as present range. Actually, Hille Ris Lambers (1971) mentioned black cherry as a plant on which *R. padi* might develop in autumn. However, he did not consider *P. serotina* precisely as a host plant, but treated it merely as a temporary food plant. The *fundatrices* hatching from the eggs laid on black cherry, perish in spring without giving birth to wingless spring morphs (*fundatrigeniae*) (Hille Ris Lambers 1971). Conversely, *R. padi* populations overwintering on *P. padus* do survive, despite the considerable winter egg mortality reaching as much as 80 % (Leather 1981). On *P. padus*, each individual that hatches from an egg in spring starts a new colony on new leaves. Their offspring (*fundatrigeniae*) then remain on the primary host, and altogether, two or three generations develop on bird cherry (Leather and Dixon 1981). Considering these facts, this study was carried out to explore one of many possible explanations of this situation. The absence of *R. padi* on *P. serotina* in spring despite successful reproduction on this plant in autumn may be a sign of some kind of antibiotic or antixenotic interaction between these two organisms. Both phenomena, antibiosis (adverse effects on insect survival) and antixenosis (adverse effects on insect behavior), involve plant resistance mechanisms based on toxic or deterrent character of the food consumed by insects (Smith 2005). It is possible that factors related to food uptake and quality are responsible for the successful development of *R. padi* population on bird cherry and the collapse on black cherry.

The leaves of *P. padus* and *P. serotina* are well equipped with constitutive chemical defense against herbivores. The compounds of special importance are cyanogenic glycosides (Santamour 1998) and phenolic compounds, mainly flavonoids (Olszewska 2005). *R. padi* is able to detoxify phenolic compounds using salivary enzymes polyphenol oxidase and peroxidase (Urbanska and Leszczynski 1992). It is also well adapted to plant cyanogenic compounds, since it uses beta-cyanoalanine synthetase and rhodanese to detoxify the toxic cyanide (Urbańska et al. 2002). The leaves of both plants, bird cherry and black cherry, contain cyanogenic glycosides. However, whereas *P. padus* contains only prunasin, *P. serotina* contains two: prunasin and amygdalin. The ratio of prunasin to amygdalin in *P. serotina* changes from 16:1 in spring and summer to 34:1, in autumn (Santamour 1998). Cyanogenic glycosides are translocated in the phloem sap (Calatayud et al. 1994). It is possible that high concentration of amygdalin in *P. serotina* in spring, which is the time of emergence of *fundatrices*, impedes the development of this aphid morph. In autumn, when amygdalin content in relation to prunasin is two times lower than in spring, the presence of this cyanogenic glycoside may appear inconsequential for *oviparae* and males. Interestingly, amygdalin was also detected in *P. virginiana*, the primary host of *R. padi* in North America (Santamour 1998). However, the total content of cyanogenic glycosides is quite low (three and two times lower than in *P. padus* and *P. serotina*, respectively) (Patton et al. 1997). The prunasin-to-amygdalin ratio in *P. virginiana* is 29:1 and it does not change throughout the season (Santamour 1998).

The aim of the present study was to compare the probing and feeding behavior of *R. padi* apterous spring morphs *fundatrigeniae* on *P. padus* and *P. serotina*. Moreover, the effect of prunasin and amygdalin on aphid probing behavior was studied on 15 % sucrose diets containing these cyanogenic glycosides. In order to trace aphid activities in plant tissues and diets, EPG (Electrical Penetration Graph) technique was used.

Materials and methods

Cultures of aphids and plants

Fundatrices of bird cherry-oat aphid (*Rhopalosiphum padi*) were collected in the second decade of April from the bird cherry (*Prunus padus*) tree at Krzeczyn, 40 km east from Wrocław, Poland. Despite the observations being made at the same time on black cherry (*Prunus serotina*), no *R. padi* had been found on that plant. The aphids from *P. padus* were reared on a branch of this same host plant in controlled environment at 75 % R.H., 20 °C, and long day photoperiod (L16:8D). The 30-cm-long twigs of *P. padus* and *P. serotina* were used for the experiments. The twigs had their first leaves completely unfolded, and the stem axis was clearly developed. The twigs were cut of the maternal plant and put in polystyrene tubes filled with water for the entire time of the EPG recording. One- to two-day-old adult *fundatrigeniae*, born in laboratory, were selected and starved approximately 1 h before the beginning of EPG recording.

Sucrose diets

The basic diet used in all experiments was 15 % sucrose solution. Pure 15 % sucrose diet was chosen because it is highly phagostimulatory for *R. padi* (Hewer et al. 2010), and any effect of nutrients on aphid probing behavior on cyanogenic glycoside diets could be avoided. The cyanogenic glycosides, prunasin (Santa Cruz Biotechnology Inc. USA), or amygdalin (Sigma-Aldrich) were incorporated into sucrose diets. The natural content of cyanogenic glycosides in plants varies depending on ecological and physiological conditions (Calatayud et al. 1994; Santamour 1998). Considering this, prunasin and amygdalin were applied at final concentration of 0.1 %, which is typical for studies on aphid response to xenobiotics (Polonsky et al. 1989; Urbańska et al. 2002; Dancewicz et al. 2008). Control treatment included only sucrose. The diet was sandwiched between two layers of stretched Parafilm M®, following the idea described by Sadeghi et al. (2009). Fresh diet was prepared just before the start of EPG recording.

Aphid probing and feeding behavior

Aphids detect the chemical components of plant tissues with the epipharyngeal gustatory organ (Douglas 2003; Pettersson et al. 2007). The uptake of small samples of mesophyll cell contents and the ingestion of phloem sap are necessary for the recognition and acceptance of the host plant (Wensler and Filshie 1969; Harrewijn 1990). It is not possible to evaluate aphid probing visually; therefore, the bird cherry-oat aphid stylet penetration in plant tissues (= probing) and especially the phloem sap uptake (=feeding) by *R. padi* were monitored using the EPG technique that is frequently employed in insect–plant relationship studies (Tjallingii 1995; Pettersson et al. 2007). The parameters describing aphid behavior during probing and feeding, such as total time of probing, number of probes, duration of phloem sap ingestion, and duration of sap ingestion from one sieve element are good indicators of plant suitability or interference of probing by chemical or physical factors in particular plant tissues (Mayoral et al. 1996). In this experimental setup, 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. Aphids were attached to a golden wire electrode with conductive silver paint and starved for 1 h prior to the experiment. Probing behavior of *fundatrigeniae* was monitored for 8 h continuously with the eight-channel DC EPG recording equipment (EPG-Systems; Dillenburg 12, 6703 CJ Wageningen, the Netherlands). Each aphid was given access to a freshly prepared twig or diet. The recordings of all replicates of a same treatment were performed during two consecutive days due to equipment limitations. All experiments were started at 10–11 a.m. Signals were saved on the computer and analyzed using the PROBE 3.1 software provided by dr. W. F. Tjallingii (EPG-Systems; Dillenburg 12, 6703 CJ Wageningen, the Netherlands). On plants, the following aphid behaviors were distinguished: non-penetration (baseline "np" in EPG recording—aphid stylets outside the plant), pathway phase—penetration of non-phloem tissues: epidermis and mesophyll ("C" = waveforms 'ABC'), watery salivation into sieve elements (waveform "E1"), ingestion of phloem sap (waveform "E2"), and ingestion of xylem sap (waveform "G") (Tjallingii 1995). On diets, we followed the interpretation of signals by Sauvion et al. (2004) and we distinguished two main waveform patterns: "C" and "G" by analogy to those described for plants and representing stylet pathway activity in the diet, including sheath salivation, and an active ingestion of the diet, respectively. Additionally, the non-penetration ("np") phases were distinguished. The parameters derived from EPGs were analyzed according to their frequency, duration, and sequence. The numerical data were analyzed statistically using Mann–Whitney *U* test (aphids on plants) and Kruskal–Wallis test (aphids on artificial diets).

Results

Bird cherry-oat aphid probing behavior on bird cherry and black cherry

The bird cherry-oat aphid showed all kinds of activities on *Prunus padus* as well as on *P. serotina*, that were non-

Table 1 Probing activities of bird cherry-oat aphid on bird cherry and black cherry

EPG parameters		*Prunus padus*	*Prunus serotina*	*p*
General aspects of aphid probing behavior[a]				
Total duration of probing	Min	380.2 ± 75.0 ($n = 12$)	359.8 ± 99.3 ($n = 12$)	0.7075
Total duration of pathway	Min	170.8 ± 90.3 ($n = 12$)	295.3 ± 85.6 ($n = 12$)	0.0051
Total duration of phloem phase	Min	209.4 ± 138.9 ($n = 12$)	64.5 ± 70.6 ($n = 12$)	0.0036
Total duration of phloem sap ingestion phase	Min	196.2 ± 134.9 ($n = 12$)	48,3 ± 59.5 ($n = 12$)	0.0017
Proportion of phloem phase in total probing	%	52.0 ± 27.3 ($n = 12$)	15.9 ± 15.9 ($n = 12$)	0.0020
Number of probes	#	11.7 ± 4.4 ($n = 12$)	29.1 ± 16.4 ($n = 12$)	0.0010
Mean duration of a probe	Min	36.9 ± 15.7 ($n = 12$)	15.4 ± 8.0 ($n = 12$)	0.0004
Activities in peripheral tissues before phloem phase				
Number of probes before first phloem phase[b]	#	2.9 ± 1.9 ($n = 11$)	7.8 ± 4.6 ($n = 10$)	0.0083
Number of probes <3 min. before first phloem phase[b]	#	2.6 ± 1.5 ($n = 7$)	4.2 ± 3.5 ($n = 10$)	0.3539
Time from first probe to first phloem phase[c]	Min	72.3 ± 99.6 ($n = 12$)	97.7 ± 93.8 ($n = 10$)	0.1062
Activities in sieve elements[b]				
Duration of first phloem phase	Min	92.2 ± 150.9 ($n = 12$)	15.2 ± 11.2 ($n = 10$)	0.2225
Number of phloem sap ingestion phases	#	4.1 ± 3.3 ($n = 12$)	4.8 ± 2.4 ($n = 9$)	0.4773
Duration of first phloem sap ingestion phase	Min	91.5 ± 150.8 ($n = 12$)	9.5 ± 8.2 ($n = 9$)	0.1179
Mean duration of phloem sap ingestion phase	Min	104.4 ± 143.8 ($n = 12$)	11.9 ± 8.7 ($n = 9$)	0.0006
Proportion of salivation in phloem phase	%	7.7 ± 7.2 ($n = 12$)	38.6 ± 28.7 ($n = 10$)	0.0027

Values represent mean ± SD, statistically significant difference at $p < 0.05$ (Mann–Whitney *U* test)

[a] All aphids were included in analysis and if an aphid did not show a phloem phase, the value of a given parameter for such individual was zero

[b] Only aphids that showed a phloem phase (E1) or phloem sap ingestion phase (E2) were included in statistical analysis

[c] Time to reach phloem phase was 8 h–first np period for aphids that did not show this activity until the end of experiment

probing, pathway activities in mesophyll ("ABC"), phloem salivation ("E1"), phloem sap ingestion ("E2"), and xylem sap ingestion ("G"). Xylem sap ingestion occurred sporadically; therefore, it was included in pathway activities in all calculations.

On bird cherry, aphid probing activities occupied 80 % of the 8-h recording period. The total number of probes was approximately 12 per aphid and they were ca. 40 min long. The period of the pre-phloem penetration (i.e., the time to the first phloem phase from the onset of probing) was 1.2 h on average and consisted of less than three probes, on average. These probes were mainly of epidermal character (i.e., shorter than 3 min). The feeding on phloem sap occupied more than half of the probing time. The number of phloem sap ingestion phases was approximately four per aphid and their mean duration reached almost 2 h. The first phloem sap ingestion phase was 1.5 h long, on average. In total, the sap ingestion occupied 92 % of aphid activities in sieve elements (Table 1). On *P. padus*, all aphids reached sieve elements (i.e., showed at least E1 waveform that represents watery salivation into sieve elements) and almost 80 % of them showed E2 waveform longer than 10 min, which is interpreted as sustained sap ingestion, during the first hour of the experiment. By the end of the 8-h recording, all aphids were ingesting phloem sap in a sustained manner (Fig. 1). The time devoted to phloem sap

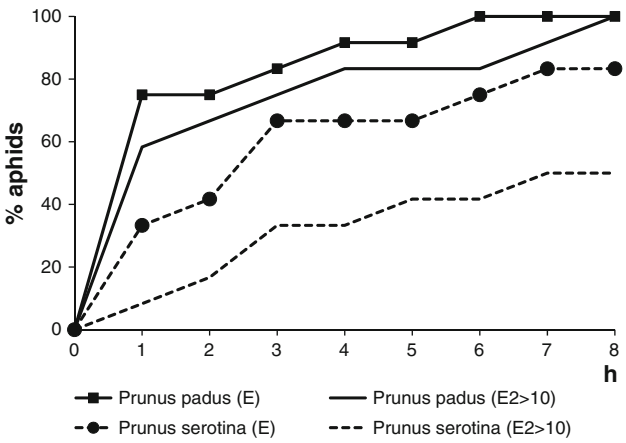

Fig. 1 Cumulative percent of bird cherry-oat aphids *Rhopalosiphum padi* that reached phloem vessels (E) and showed sustained phloem sap ingestion (E2 > 10 min.) on bird cherry *Prunus padus* and black cherry *Prunus serotina* during 8-h EPG experiment

ingestion increased up to 70 % at the end of the 8-h experiment, the duration of pathway activities decreased, and non-probing became marginal at the end of experiment (Fig. 2). On black cherry, total probing time occupied 75 % of the 8 h of the aphid EPG-recorded probing. The total number of probes was approximately 30 per aphid and they were usually 15 min long. The period of the pre-

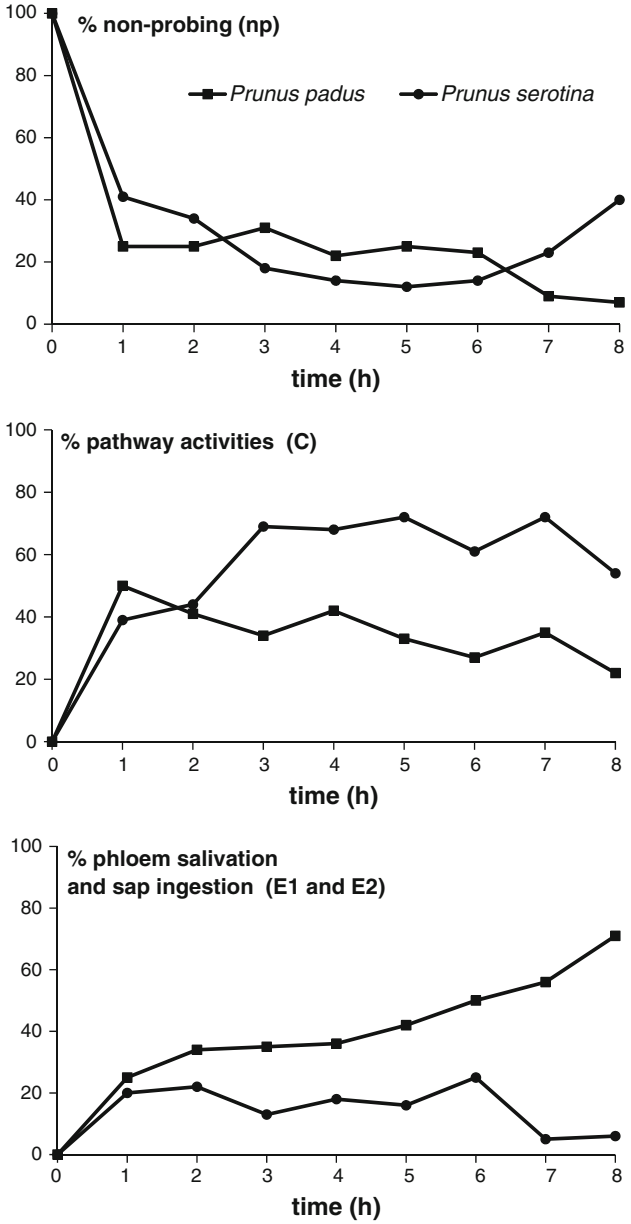

Fig. 2 Sequential changes in the bird cherry-oat aphid *Rhopalosiphum padi* probing behavior on bird cherry *Prunus padus* and black cherry *P. serotina* during the continuous 8-h EPG recording

phloem penetration was 1.6 h on average and consisted of eight probes, on average. 50 % of these probes were epidermal probes. Sap ingestion occupied 18 % of probing time. The number of phloem sap ingestion phases was approximately five per aphid and their mean duration was 12 min. The first phloem sap ingestion phase was 15 min long, on average. In total, the sap ingestion occupied 61 % of aphid activities in sieve elements. Additionally, there occurred frequent E1/E2 switches (data not shown), which resulted in the substantial proportion (39 %) of E1 salivation during phloem phase (Table 1). On *P. serotina*, 20 % of aphids did not reach phloem elements within 8 h after access to the plants and 50 % of aphids did not start sustained ingestion. 30 % of aphids reached phloem vessels during the first hour of the experiment (Fig. 1). The time devoted to phloem sap ingestion made up to 20 % of all aphid activities during 1–6th hour of the experiment, and during 7th and 8th hour, it decreased to 5 and 6 %, respectively. The proportion of time that aphids spent on non-probing decreased during 1–6th hour, then increased up to 23 and 40 % in 7th and 8th hour, respectively (Fig. 2).

The comparative analysis of data presented above exposed clear differences in crucial aspects of probing behavior of *R. padi* on bird cherry and black cherry. Although the total duration of stylet penetration in plant tissues during the 8-h experiment was similar on both plant species, the proportion of phloem phase in total probing was more than three times higher on bird cherry than on black cherry. The period of the pre-phloem penetration was twice as long in aphids on black cherry as on bird cherry, and that activity on bird cherry was rarely interrupted as opposed to aphids on black cherry. Moreover, the total number of probes was three times lower, and the probes were two times longer on bird cherry than on black cherry. On the other hand, the average number of epidermal probes (i.e., shorter than 3 min) before the first phloem phase was similar on both plant species. On black cherry, there was a considerable delay between finding and accepting the phloem, aphids that had sampled phloem sap either refused to ingest it or the ingestion periods were very short, and the proportion of phloem phase in aphid activities decreased in the course of time. In contrast, on *P. padus* the time devoted to phloem sap ingestion increased up to 70 % at the end of the 8-h experiment, the duration of pathway activities decreased and non-probing became marginal, on *P. serotina*, the duration of non-probing and pathway activities increased and phloem phase turned out to be marginal. On *P. padus*, all aphids were ingesting phloem sap in a long-term manner while on *P. serotina*, 20 % of aphids failed to reach phloem vessels and 40 % of aphids that did show phloem phase did not start committed ingestion. The number of ingestion phases was similar on two plant species but the mean duration of a single sap intake period was approximately nine times longer on *P. padus* than on *P. serotina*.

Bird cherry-oat aphid probing behavior on sucrose and cyanogenic glycoside–sucrose diets

Aphid probing was not restrained on either control or cyanogenic glycoside diets: stylet activities occupied from 30 % up to 50 % of experimental time on prunasin–sucrose, amygdalin–sucrose, and control (=sucrose) diets, respectively. All aphids made similar number of probes

Table 2 Comparison of probing and feeding behavior of bird cherry-oat aphid *fundatrigeniae* on control, amygdalin-, and prunasin-containing diets

	Control $n = 10$	Amygdalin $n = 10$	Prunasin $n = 8$	p
Number of probes	8.2 ± 7.7	15.8 ± 10.6	22.4 ± 15.4	0.0547
Number of C phases	13.6 ± 9.6	31.2 ± 21.4	28.1 ± 16.1	0.0529
Number of G phases	6.0 ± 4.7	16.3 ± 14.1	6.4 ± 5.3	0.0679
Proportion of np in total probing (%)	47.6 ± 20.0	47.4 ± 18.0	68.2 ± 25.5	0.1198
Proportion of C in total probing (%)	21.2 ± 8.7b	40.3 ± 16.2a	26.5 ± 21.8 ab	0.0306
Proportion of G in total probing (%)	31.2 ± 20.5a	12.3 ± 10.6ab	5.3 ± 5.7b	0.0020
Mean duration of np (min)	62.2 ± 58.0	31.2 ± 38.7	32.3 ± 50.9	0.3803
Mean duration of C (min)	9.6 ± 4.4	10.1 ± 9.9	5.4 ± 4.1	0.1438
Mean duration of G (min)	32.0 ± 21.8a	4.4 ± 3.4b	3.5 ± 1.9b	0.0040
Time to first G from the start of recording (min)	135.4 ± 111.4	73.9 ± 77.0	214.7 ± 164.1	0.1887
Duration of first C (min)	4.3 ± 5.3	15.1 ± 33.0	2.2 ± 1.6	0.4024
Duration of first G (min)	50.7 ± 63.4a	7.2 ± 14.7ab	1.6 ± 2.1b	0.0024
Duration of first np after first ingestion (min)	35.0 ± 82.8	36.2 ± 59.5	18.2 ± 22.3	0.7892
Time from the end of first ingestion to next ingestion (min)	6.2 ± 6.9	61.4 ± 63.4	52.8 ± 71.6	0.1022
Proportion of probes with ingestion (%)	45.9 ± 32.5a	46.6 ± 29.6a	10.8 ± 12.4b	0.0115

Data show mean ± SD. Different letters in rows indicate significant differences between groups determined in Kruskal–Wallis test ($p < 0.05$)

that contained similar number of pathway ("C") and ingestion ("G") phases and the non-probing intervals were of similar duration. However, the proportion of the two activities during probing differed among the treatments. Generally, the ingestion was the main activity of aphids on control diet but pathway activities predominated in aphids on cyanogenic glycoside–sucrose diets. All aphids, irrespective of treatment, started the ingestion after similar time from the onset of the experiment but the duration of the first ingestion period was seven and thirty times shorter on amygdalin and prunasin diet than on control, respectively. Likewise, the mean duration of an ingestion phase was approximately eight times shorter on cyanogenic glycoside than on pure sucrose diets. Additionally, the proportion of probes with an ingestion phase was considerably reduced in aphids on prunasin–sucrose diets: almost 90 % of probes did not contain the ingestion activity in aphids on that diet in contrast to aphids on sucrose and amygdalin–sucrose diets, which had 50 % feeding success (Table 2). The sequential analysis of aphid behavior showed that the proportion of non-probing activities was generally stable during the 8-h experiment on sucrose and amygdalin–sucrose diets and ranged from 65 % at the end of the first hour, down to 35 % at the end of the experiment. Likewise, the proportion of ingestion increased gradually to reach approximately 30 % of all activities 8 h after aphids had access to the diets. In contrast, on prunasin–sucrose diet, non-probing prevailed over all other aphid activities from the start until the end of experiment and the ingestion made up a small portion of probing activities, reaching the maximum of 15 % in the 8 h (Fig. 3).

Discussion

Generally, aphid probing activity on plants can be divided into three distinct phases: pathway, xylem, and phloem phases. During pathway phase, aphid stylets penetrate epidermis and parenchymatous tissues, xylem phase represents the uptake of water to compensate for water stress, and phloem phase comprises the main feeding (Pettersson et al. 2007). According to the data presented above, it may be assumed that the probing of the bird cherry-oat aphid *fundatrices* in peripheral tissues was not impeded in a significant way on either of the plant species. However, the high number of short probes on *P. serotina* (ca. 15 min. duration) in contrast to few long probes on *P. padus* (ca. 40 min.) during the whole of the probing period might indicate the existence of deterrent factors in epidermis and mesophyll of the black cherry. On the other hand, the average number of epidermal probes (i.e., shorter than 3 min) before the first phloem phase was similar on both plant species. It is likely that the long duration of an average probe on *P. padus* resulted from the fact that those probes usually contained a prolonged period of phloem sap ingestion, which is shown in the high value of the E/C ratio. The effect of frequently repeated insertion and withdrawal of the stylets on *P. serotina* may partly be the consequence 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 have walked away from the unsuitable host after a short probe had they been free to move (Powell et al., 1993; Gabryś et al., 1997). In contrast to the probing phase in peripheral tissues, aphid

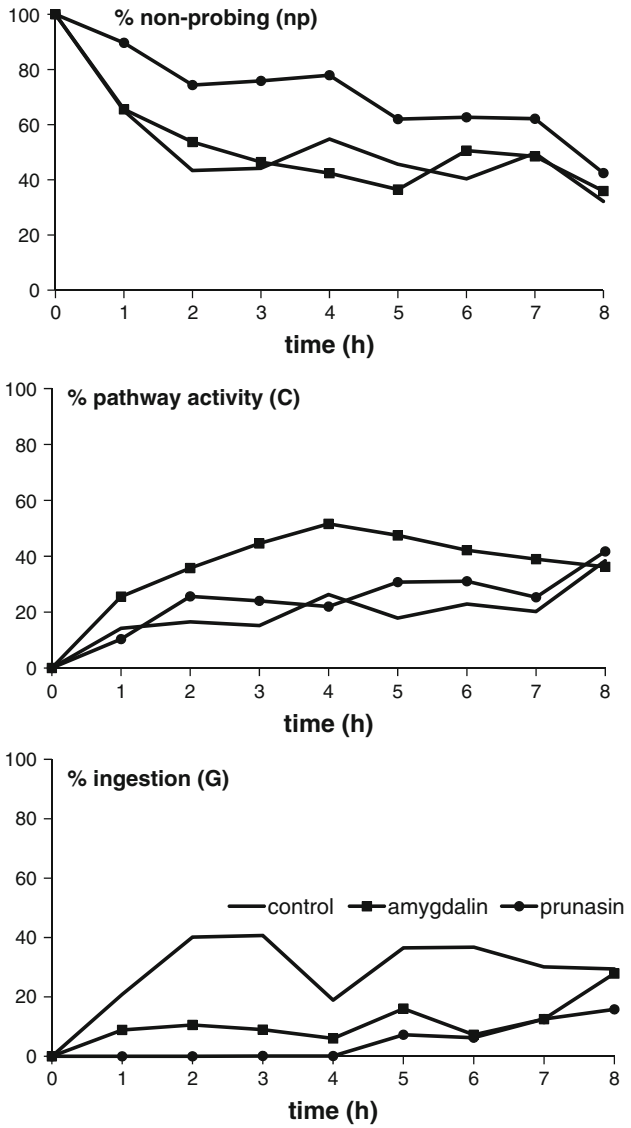

Fig. 3 Sequential changes in the bird cherry-oat aphid *Rhopalosiphum padi* probing behavior on 15 % sucrose (control), 15 % sucrose +0.01 % amygdalin, and 15 % sucrose +0.01 % prunasin diets during the continuous 8-h EPG recording

behavior during the phloem phase on black cherry was seriously altered as compared to aphids on bird cherry. The data obtained in this study lead to the conclusion that the presence of deterrent factor(s) in the phloem sap of *P. serotina* is very likely. *R. padi* consumes very little sap when on black cherry and the high proportion of salivation during the phloem phase may indicate the necessity of detoxication of xenobiotics. The lack of deterrent factors in the sieve elements, which allows long and uninterrupted periods of sap ingestion, is probably the explanation of the high suitability of *P. padus* for *R. padi*. The prolonged watery salivation, which was found in aphids on *P. serotina*, has often been reported the characteristic of aphid behavior on resistant plant cultivars or non-host plants (van Helden and Tjallingii, 1993; Wilkinson and Douglas, 1998; Gabryś and Pawluk, 1999). Aphid saliva contains various enzymes, such as UDP glucose transferases and polyphenol oxidases, which may detoxify plant allelochemicals (Miles 1990; Douglas 2003). At the beginning of this study, we made the hypothesis that prunasin, or more likely amygdalin that is present in black cherry and absent in bird cherry, could act as deterrent for the bird cherry-oat aphid feeding on *P. serotina*. Although both compounds and especially prunasin impeded *R. padi* ingestion activities when incorporated in pure sucrose diet, the results of our study do not allow concluding that either one or the other is responsible for the non-acceptance of *P. serotina* by *fundatrices*.

Given the role of cyanogenic glycosides as factors that determine the rejection of *P. serotina* by *fundatrigeniae* of *R. padi* can be excluded, further study will be needed to explore alternative possibilities. Indeed, the study by Sandström and Pettersson (2000) showed that behavioral morph-dependent aspects can be responsible for the winter host plant specificity of *R. padi.* These authors found that this specificity is controlled mainly by the preference of the females remigrating (*gynoparae*) to the winter host, *P. padus*, in autumn, while the other generations living on the winter host, that is, sexual females and males accept a broader range of winter hosts (Sandström and Pettersson 2000). On the other hand, bird cherry-oat *gynoparae* and males use benzaldehyde, the volatile breakdown product of cyanogenic glycosides, as a cue to find winter host (Park et al. 2000; Pope et al. 2007). It is likely that impaired survival of *R. padi fundatrigeniae* on *P. serotina* is a consequence of an inadequate host plant selection by the autumn migrants, the *gynoparae* and (or) males, that use a universal cue for winter host finding and it is estimated that less than 1 % of the aerial population of *R. padi gynoparae* successfully locates and colonizes a bird cherry tree (Powell and Hardie 2001). It is possible that deterrent, other than cyanogenic glycosides, component(s) of black cherry phloem sap is detectable only in spring when *fundatrices* hatch from the eggs or that autumn migrants do not respond to them. In fact, the *gynoparae* do not feed after the arrival on winter host (Walters et al. 1984), so they have no opportunity to assess plant suitability by gustation.

The results of this study may be interpreted also in the context of the bird cherry-oat aphid host-alternation behavior, especially aphid emigration from primary hosts in spring. Leszczyński et al. (2003) found that the appearance of *fundatrices* coincides with the highest content of the cyanogenic glycosides and the highest cyanogenesis potential in the youngest leaves of *P. padus.* Moreover, the aphid population build up occurs when the content of the cyanogenic glycosides and the cyanogenesis potential decreases and the winged migrants began to fly off from the primary host onto secondary hosts when the

level of cyanogenic glycosides is the lowest. Leszczyński et al. (2003) suggested that it is likely that the changing content of plant allelochemicals, especially the decrease in cyanogenic glycosides, may, in part, be responsible for host alternation of *R. padi.* The present study of aphid behavior on artificial diets showed that these compounds, especially prunasin, inhibit sap ingestion by *R. padi fundatrigeniae,* although these latter are able to detoxify cyanogenes. It may be speculated then that cyanogenic glycoside-rich sap in primary hosts may contribute to the set of signals for this generation of bird cherry-oat aphids to prepare the population for leaving the primary host. This signal may act as early as at the time of aphid emergence in spring. As it was shown by Sandström (2000), factors influencing aphid nutrition, or ecology, other than seasonal changes in the nutritional value of the phloem sap may play a role in the phenomenon of host alternation in *R. padi*. Nevertheless, a detailed study on the seasonal and phenological changes in plant secondary chemistry is planned to clarify the role of cyanogenic glycosides in host selection by *R. padi* more precisely.

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