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Nutritional quality of phloem sap in relation to host plant-alternation in the bird cherry-oat aphid

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Summary. Host alternation in aphids has been attributed to complementary growth of host plants, or more specifically to seasonal changes in the nitrogen quality of the phloem sap. In this report, seasonal fluctuation of free amino acids in phloem of the winter and summer host plants (*Prunus padus*, bird cherry and *Hordeum vulgare*, barley) of *Rhopalosiphum padi* (the bird cherry-oat aphid) were investigated in the context of aphid growth and behaviour. Phloem was collected from the cut stylets of aphids taken from plants that were grown outdoors. The total concentration of amino acids in *P. padus* phloem increased between bud break and late flush (spring), decreased in mature leaves (summer), and increased again in early senescent leaves (autumn). In *H. vulgare*, however, amino acid concentration fluctuated less from seedlings to flowering. Spring aphids from *P. padus* grew rapidly on this host from bud break to late flush, but died on mature and early senescent leaves. Summer aphids from *H. vulgare* grew as fast on this host as spring aphids did on flush leaves of *P. padus*. Sexual females grew more slowly than other generations and nearly as well on mature as on early senescent *P. padus* leaves. As judged by aphid growth and phloem nitrogen quality, *P. padus* during spring equals the summer host *H. vulgare*. However, the lower growth rates of *R. padi* on mature and senescent leaves of *P. padus* appear only loosely correlated with phloem amino acid concentrations. Therefore, factors influencing aphid nutrition, or ecology, other than seasonal changes in phloem sap amino acid concentration may explain the existence of host alternation in *R. padi*.

Key words. phloem sap – host alternation – aphid – nutrition – amino acids – *Rhopalosiphum padi* – *Prunus padus* – *Hordeum vulgare* – *Homoptera* – *Aphididae*

Introduction

Aphid life cycles are unusual in many ways, and one fascinating element in some is host plant alternation. Aphid host alternation, or *heteroecy*, is a seasonal shifting between taxonomically unrelated host plant taxa in which different generations specialize on two sets of hosts, often a combination of woody "winter" or "primary" hosts actually used in autumn, winter and spring and herbaceous "summer" or "secondary" hosts. Only about 10% of all aphid species are host alternating (Eastop 1973). However, many non-alternating species within the large family Aphididae are believed to have been derived from host-alternating ancestors (Hille Ris Lambers 1950; Moran 1992).

Hypotheses explaining the evolution of aphid host alternation can be classified into two groups. The first explains host alternation as an optimal use of available host plants, with respect to available nutrients (e.g. Davidson 1927; Mordvilko 1928; Dixon 1971), oviposition sites (Moran 1983), mating rendezvous (Ward 1991), predatory constraints (Way & Banks 1968), or induced host defence (William & Whitham 1986). The second category suggests that evolutionary constraints account for these incomplete host shifts from woody to herbaceous hosts (Mordvilko 1928; Moran 1988).

The first hypothesis from the first category describes host-alternation as a way to exploit the complementary growth patterns of woody and herbaceous plants. According to this view, woody hosts are used in spring and autumn when they are actively growing or senescent, and thus more nutritious due to active nutrient translocation. Herbaceous hosts are used during the summer when they actively grow and when woody hosts are mature and believed to be nutritionally inferior. Nitrogen is one of the basic nutrients for growth and is frequently limiting for strictly phytophagous animals (Mattson 1980). Aphids feed mainly on phloem sap, in which sugars are abundant and free amino acids provide the major source of nitrogen (e.g. Girousse *et al.* 1991; Helden *et al.* 1994). Thus, amino acid content in phloem sap has been the nutritional factor emphasized by most studies. However, the evidence for complementary patterns in phloem nitrogen quality of alternative hosts is rather meager because most studies are based on indirect measurements of phloem composition. A decline in the free amino acid content of whole leaves from woody hosts at mid-summer has been shown to be roughly synchronized with migration to

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herbaceous hosts (Dixon 1971), excretions from aphids and exudates from the cut leaves of one woody host have shown higher amino acid content in early flush than in mature leaves, while another host showed such increase between mature leaves and senescent leaves (Douglas 1993). However, neither whole leaf extracts nor exudates from cut leaves provide reliable estimates of amino acid concentration in phloem sap, especially when different hosts are compared. Exudates give a generally good picture of the relative amino acid composition of phloem but are not so useful for quantification of concentrations (Weibull *et al*. 1990; Girousse *et al*. 1991; Helden *et al*. 1994; Sandström *et al*. 2000), and the correlation between whole leaf extracts and phloem content is less clear. The most reliable method for gathering phloem sap in its natural state is collection through cut aphid stylets, but it is very time-consuming method.

The aims of the present study were to investigate whether seasonal fluctuations of free amino acids in phloem of winter and summer host plants show a complementary pattern and, if so, how this pattern correlates with aphid performance and behaviour. Using aphid stylet exudates from plants grown outdoors, pure phloem was collected in the form naturally used by aphids. Phloem nutritional quality was quantified by measurements of amino acid concentrations, especially amino acids essential for insects in general (Dadd 1985). It is uncertain which amino acids are essential for aphids since intracellular bacterial symbionts (*Buchnera*) provide aphids with several essential amino acids (Douglas 1998). It has been shown that the aphid *Myzus persicae* with the symbiont *Buchnera* requires only three of the ten essential amino acids (Dadd & Krieger 1968).

This study was performed on *Rhopalosiphum padi* (L.) (the bird cherry-oat aphid) (Homoptera: Aphididae) and a pair of host plants on which it alternates, *Prunus padus* (L.) (bird cherry), its woody winter host, and *Hordeum vulgare* (L.) (barley), one of its major summer hosts in the study area. In northern Europe *P. padus* is the sole winter host, but other species of the genus *Prunus* are used in other parts of the world (Rogerson 1948). The summer hosts is a presumed combination of cultivated cereals and later in summer various wild grasses. Wild grasses were probably the sole summer hosts of *R. padi* before human cultivation of cereals.

Material and methods

The study aphid

R. padi, alternates between *P. padus*, its winter host, and various grasses as summer hosts. In Sweden, *R. padi* overwinters as eggs on *P. padus* buds. The eggs hatch in April, and the females start to feed on the breaking buds. The second and part of the third generation continue to feed on the developing *P. padus* shoots. In early summer, the second or third generations develop into alates that migrate to grasses, such as *H. vulgare*, on which *R. padi* reproduce for several generations during the summer. In early September gynoparae (female return migrants) and males are produced, and fly back to *P. padus*, although males migrate later. The gynoparae produce egg-laying, sexual females (oviparae) that mate with males and lay eggs on *P. padus* twigs. Sexual reproduction and oviposition occur once a year, in autumn; all other generations are parthenogenetic.

Aphid collection and rearing

R. padi eggs were collected from *P. padus* trees in March at localities near Uppsala, Sweden. Short pieces of twigs with eggs were placed on lightly moistened filter papers in Petri dishes and stored in darkness at 5°C. When needed, eggs were hatched after about three to five days of exposure to light at 18°C. These first generation nymphs were reared on flush *P. padus* leaves. Second generation aphids reared on flush *P. padus* leaves were used in performance tests on *P. padus* from budbreak to late flush leaves. On mature and senescent leaves, spring generations of later origin were used, along with sexual females. These were all from cultured colonies consisting of mixed clones. To obtain sexual females, the production of gynoparae were induced in a colony reared on *H. vulgare* using short day conditions, artificially (Dixon & Glen 1971), or naturally in autumn. The gynoparae then produced sexual female offspring. Performance tests on *H. vulgare* were made with apterous summer generations of *R. padi* reared from spring migrants. These were kept in culture on *H. vulgare*. For growth stages used, see Table 1 and 2.

Table 1 Concentrations of amino acids found in phloem at different growth stages of *Prunus padus* (mM, means ± S.E.). All samples were obtained from severed aphid stylets of *Rhopalosiphum padi*. Values in the same row, amino acid, with the same letter are not significantly different according to Tukey's test ($P > 0.05$)

Sampling time	early May	late May	mid June	late July	mid September
Plant growth stage	bud break	early flush	late flush	mature	early senescent
n	15	6	9	10	8
Essential amino acids					
arginine	6.8 ± 0.8 b	11.7 ± 1.3 a	10.5 ± 0.8 a	4.9 ± 0.4 b	6.3 ± 0.6 b
histidine	1.7 ± 0.2 b	2.4 ± 0.4 ab	3.2 ± 0.4 a	1.5 ± 0.1 b	3.6 ± 0.3 a
isoleucine	3.0 ± 0.4 a	2.8 ± 0.7 a	2.6 ± 0.3 a	2.9 ± 0.6 a	2.2 ± 0.2 a
leucine	1.9 ± 0.2 a	2.3 ± 0.4 a	1.7 ± 0.2 a	1.7 ± 0.2 a	1.3 ± 0.1 a
lysine	2.2 ± 0.4 a	3.4 ± 0.4 a	3.2 ± 0.4 a	1.7 ± 0.3 a	2.1 ± 0.5 a
methionine	2.2 ± 0.5 a	2.9 ± 0.5 a	2.9 ± 0.3 a	2.2 ± 0.5 a	1.2 ± 0.4 a
phenylalanine	1.7 ± 0.3 a	1.7 ± 0.3 a	1.7 ± 0.2 a	1.4 ± 0.2 a	1.5 ± 0.2 a
threonine	3.3 ± 0.4 b	4.6 ± 0.9 ab	6.3 ± 1.3 a	3.0 ± 0.3 b	3.8 ± 0.9 ab
tryptophan	1.0 ± 0.2 ab	1.6 ± 0.3 a	1.1 ± 0.2 ab	0.9 ± 0.1 ab	0.4 ± 0.1 b
valine	7.6 ± 0.9 abc	10.9 ± 1.2 a	8.2 ± 0.8 ab	7.1 ± 0.6 bc	4.5 ± 0.3 c
Non-essential amino acids	68.5 ± 4.5 b	74.4 ± 12.1 ab	97.3 ± 18.5 ab	65.7 ± 5.4 b	108.5 ± 6.9 a
Sum	99.8 ± 7.2 a	118.7 ± 18.1 a	138.7 ± 21.2 a	92.9 ± 7.4 a	135.3 ± 9.0 a

Table 2 Concentrations of amino acids found in phloem at different growth stages of *Hordeum vulgare* (mM, means ± S.E.). All samples were obtained from severed aphid stylets of *Rhopalosiphum padi*. Values in the same row, amino acid, with the same letter are not significantly different according to Tukey's test ($P > 0.05$)

Sampling time	late May	mid June	late July
Plant growth stage	seedling	tillering	late flowering
n	11	14	11
Essential amino acids			
arginine	2.9 ± 0.5 a	2.9 ± 0.7 a	1.9 ± 0.2 a
histidine	1.9 ± 0.3 a	2.9 ± 0.1 a	2.6 ± 0.5 a
isoleucine	2.3 ± 0.3 a	2.3 ± 0.6 a	1.5 ± 0.3 a
leucine	2.3 ± 0.3 a	2.4 ± 0.7 a	1.5 ± 0.5 a
lysine	2.5 ± 0.4 a	2.0 ± 0.6 a	0.9 ± 0.2 a
methionine	0.5 ± 0.2 a	0.5 ± 0.1 a	0.3 ± 0.1 a
phenylalanine	1.7 ± 0.2 a	2.0 ± 0.7 a	1.1 ± 0.2 a
threonine	5.9 ± 0.9 b	8.2 ± 1.1 ab	11.8 ± 1.9 a
tryptophan	1.0 ± 0.2 a	1.2 ± 0.4 a	0.2 ± 0.1 a
valine	11.0 ± 2.0 a	6.6 ± 1.2 ab	4.8 ± 0.6 b
Non-essential amino acids	103.4 ± 12.2 a	123.0 ± 12.5 a	89.3 ± 8.9 a
Sum	135.4 ± 13.2 a	154.0 ± 17.1 a	115.9 ± 11.4 a

Plants

Test plants of *P. padus* were about 1m in height, three years old, and derived from cuttings rooted in pots. Test plants of *H. vulgare* (cv. Golf) had been planted in pots outdoors in early May, a normal sowing time in the Uppsala region. The pots in which plants from both species were grown were buried in the ground outdoors to prevent pots from heating and accelerating plant growth. Plants were taken indoors only for phloem collection. All plants were planted in potting soil (P-jord, Hasselfors garden, Hasselfors, Sweden) supplemented with fertilizer (Osmocote plus, 200 g/100 dm^3 soil), and watered with tap water under dry weather conditions. Different sets of plants were used for phloem samples and aphid performance tests.

Phloem collection

Phloem samples from each plant species were taken at multiple phenological stages during the seasons 1995–96 (Tables 1 and 2). Each aphid was allowed to freely choose its feeding site on a given plant before its stylet was cut by microcautery according to the method described by Unwin (1978). Phloem sap was then collected under the conditions described by Sandström & Pettersson (1994). Samples from *P. padus* at early May were taken from plants infested 4–5 days earlier with 2–3 adult aphids from the spring generations, later samples in May and June were from developing colonies infested at the same time as the former. Samples from *P. padus* at July and September were taken from plants infested 10–14 days earlier with about 20 nymphs of sexual females. Samples from seedling *H. vulgare* were taken from plants infested with 3 aphids from the summer generations, 2–3 days before the sampling. Later samples from *H. vulgare* were from developing colonies infested at the same time as the former. On *P. padus*, aphids fed on veins on the underside of leaves, causing wrinkling and leaf-folding and ultimately inducing a pseudogall structure that partly enclosed the aphids in late May and mid June. Thus, stylets could only be cut from aphids on moderately galled leaves.

Free amino acids in the phloem were analyzed by high performance liquid chromatography (HPLC) after precolumn derivatisation with o-phtalaldehyde according to Weibull *et al.* (1986). Cysteine and proline are not detectable by this method. They are present in low amounts in the phloem from cultivated grasses and other plants (Fisher & MacNicol 1986; Kuo-Sell 1989; Hayashi & Chino 1990; Girousse *et al.* 1991). All other protein amino acids plus a few non-protein amino acids (i.e. γ-amino butyric acid, o-phosphoserine) were used for calculation of total amino acid concentration.

Amino acids considered essential for insects in general (Dadd 1985) are of special interest and are therefore shown in detail. If they are present in low amounts in phloem, and if the *Buchnera* can not supply the aphid demand of any of these amino acids, aphid growth could be limited. In insects, 10 amino acids are considered essential nutrients: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In addition, the nonessential amino acids tyrosine and cysteine may affect the requirement for phenylalanine and methionine respectively, since they are synthesized only from these essential amino acids (Dadd 1985).

Aphid performance

Two to three adult females were placed on each *P. padus* or *H. vulgare* test plant and allowed to produce five to ten newborn nymphs over a three to five hour period. Then, the adult females were removed, as were one or two nymphs. The remaining nymphs had free access to a shoot of *P. padus* or an entire *H. vulgare* plant, but were prevented from leaving the shoot or plant by enclosure with a micro-perforated plastic bag (Cryovac, W.R. Grace Ltd., Cambridge, England). Test plants were inspected every day. Once the aphids had reached the adult stage, all except one randomly chosen female were removed from the plant. This female was allowed to reproduce until she died. Nymphal survival, weights of the newborn nymphs and adults that were removed, time elapsed from birth to adult stage, and lifetime fecundity were recorded. To estimate the potential reproduction of sexual females, their ovarioles were dissected and the eggs counted. All performance experiments were made outdoors in a screenhouse at ambient conditions at Uppsala, Sweden.

The two weight measurements and the time elapsed between them were used to calculate mean relative growth rate (MRGR) after Fisher (1920). The MRGR were calculated per day-degree to compensate for variable outdoor conditions. The baseline for the calculations of day-degrees was set to 3°C, which is the developmental threshold for growth of *R. padi* (Dean 1974; Hansen 1997). Temperature measurements and day-degrees calculations were obtained from the meteorological station at the Swedish University of Agricultural Sciences, Uppsala, Sweden, situated about 250 m from the experimental site. Among the performance variables measured in this study, MRGR probably best reflects nutritional quality, while other variables such as fecundity might be highly correlated with lifecycle strategies (Wellings *et al.* 1980).

Aphid behaviour in the field

Migration behaviour of *R. padi* in the field was observed in the same years as the experiments were done, 1995–96. Migration from *P. padus* to grasses was monitored by counting the weekly catches of *R. padi* from a permanent suction trap at the Swedish University of Agricultural Sciences, Uppsala, Sweden (data kindly provided by the Department of Applied Plant Protection). Migration back to *P. padus* from grasses was monitored by weekly observations on *P. padus* trees around Uppsala.

Statistical analysis

The results were analyzed using SAS software (SAS institute, Cary, N.C., USA) version 6.12. (SAS Institute Inc 1988). Concentrations of free amino acid in two plants phloem from the five sampling dates, and aphid performance variables were analyzed by one-way analysis of variance (ANOVA). Concentrations of amino acids in phloem sampling dates coincident for the two plant species, i.e. late May, mid June and late July, were analyzed in an additional two-way ANOVA (3 sampling dates * 2 plant species). The SAS procedure GLM was used for the above analyses. For multiple comparison of means, Tukey's test was used (Zar 1996).

Results

Seasonal changes in phloem sap

Phloem samples from the winter host, *P. padus*, showed significant changes in total free amino acid concentration during the season ($F_{4,47} = 2.87$, $P = 0.034$). Concentration showed an increase between bud break (early May) and flush leaves (mid June), then a decline in mature leaves (late July), and another increase in early senescent leaves (mid September) (Fig. 1A). Multiple comparisons using Tukey's test, which compensates for experimentwise errors, revealed non-significant differences in these cases ($P = 0.06–0.07$). In the summer host, *H. vulgare*, no significant changes were observed during the season ($F_{2,35} = 1.71$, $P = 0.20$) (Fig. 1B). The concentration of amino acids in the phloem of *H. vulgare* was slightly higher than in the phloem of *P. padus* of the coincident samplings at late May, mid June and late July (Fig. 1). However, the differences was not significant when tested in a two-way ANOVA, i.e. no effect of plant species ($F_{1,60} = 2.70$, $P = 0.106$).

The concentrations of some of the individual essential amino acids changed significantly in the phloem sap of *P. padus* during the season, arginine ($F_{4,47} = 10.68$, $P = 0.0001$), histidine ($F_{4,47} = 12.02$, $P = 0.0001$), threonine ($F_{4,47} = 2.99$, $P = 0.029$), tryptophan ($F_{4,47} = 4.00$, $P = 0.008$) and valine ($F_{4,47} = 5.63$, $P = 0.001$) (Table 1). The concentrations of these amino acids were higher in early flush leaves in late May and/or late flush leaves in mid June. Significantly higher concentrations of arginine and histidine were also found in early senescent leaves in mid September. In phloem sap from *H. vulgare* only threonine ($F_{2,35} = 5.07$, $P = 0.012$) and valine ($F_{2,35} = 4.36$, $P = 0.021$) showed significant changes during the season (Table 2); the threonine concentration was highest in late flowering plants in late July and valine in seedlings in late May.

The phloem concentrations of some of the essential amino acids differed between the two plant species at the coincident samplings dates. The effect of plant in the two-way analysis of variance was significant for arginine ($F_{1,60} = 119.33$, $P = 0.0001$), lysine ($F_{1,60} = 4.53$, $P = 0.039$) and methionine ($F_{1,60} = 95.06$, $P = 0.0001$) which all were present in higher concentrations in *P. padus*, and for threonine ($F_{1,60} = 15.11$, $P = 0.0003$) which was present in higher concentration in *H. vulgare*.

Besides differences between the plants phloem sap concentrations of essential amino acids, large differences were observed in the remaining non-essential amino acids. Phloem sap from *P. padus* was dominated by the non-essential amino acids asparagine and glutamine from bud break to late flush leaves. In early senescent leaves there was a reduction in asparagine and an increase in serine and glutamine. By contrast, *H. vulgare* was dominated by the non-essential amino acids glutamic acid, aspartic acid and serine in seedlings and tillering plants. In early flowering plants there was a shift to lower concentrations of glutamic acid and higher concentrations of glutamine.

Aphid performance

Mean relative growth rate (MRGR) of second spring generation *R. padi* on *P. padus* was similar on breaking buds and later on flush leaves (Fig. 1A) ($F_{1,19} = 0.01$, $P = 0.92$) and performance was good as long as flush, light green leaves were available. Mature leaves on non-growing shoots were rejected by the second spring generation as were early senescent leaves. All nymphs died on plants from these stages (Table 3).

Sexual females survived well on both mature and early senescent leaves (Table 3), but the MRGR were much lower than that of the second spring generation on young leaves (Fig. 1A). MRGR of sexual females was slightly higher on early senescent leaves than on mature leaves but not significantly so ($F_{1,19} = 3.52$, $P = 0.077$) (Fig. 1A). Performance of sexual females was not tested on leaves at bud break or on flush leaves.

MRGR of summer generations on *H. vulgare* were high and comparable to spring generations on young leaves of *P. padus*. MRGR of summer generations differed significantly among plant phenological stages ($F_{1,29} = 22.00$, $P = 0.0001$) (Fig. 1B), with highest values on tillering plants.

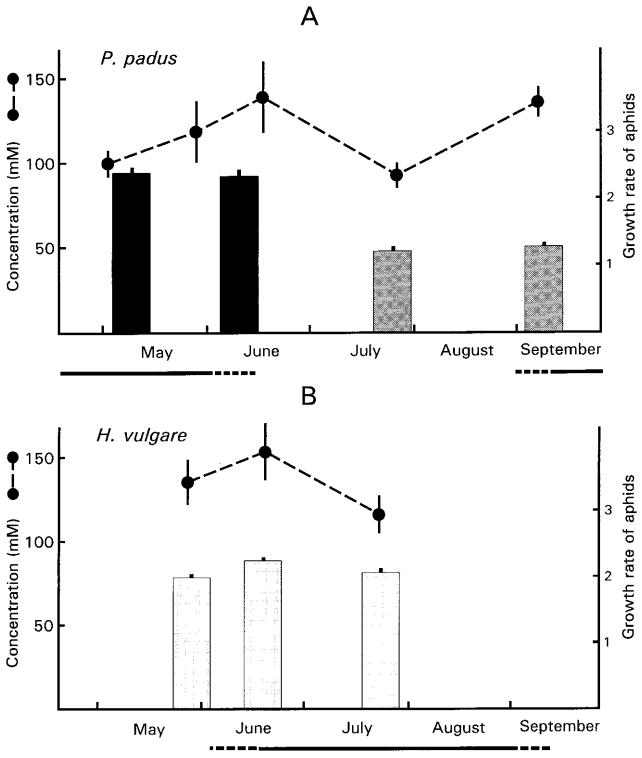

Fig. 1 Seasonal fluctuation in total amino acid concentration in phloem from *Prunus padus* (**A**) and *Hordeum vulgare* (**B**) collected from stylets of *Rhopalosiphum padi* (line and filled circles, left scale). Mean relative growth rates (mg/mg * 100 day °C) of *R. padi* on *P. padus* (**A**) and *H. vulgare* (**B**) at different growth stages (bars, right scale). On *P. padus* (**A**) black bars are growth rates of second spring generation aphids, and stippled bars are rates of sexual females. Standard error of means shown as vertical bars. Lines under months indicate normal presence of *R. padi* on the two plants in Uppsala, Sweden. For phloem samples n = 6–15, for aphid growth rate n = 10

Table 3 Performance of second spring generation, and sexual females of *Rhopalosiphum padi* on *Prunus padus* of different seasonal growth stages (means ± S.E.). Values in the same column with the same letter are not significantly different according to Tukey's test ($P > 0.05$). n.t. = not tested

Plant growth stage at start of experiment	Second spring generation				Sexual females			
	n	Adult weight (mg)	Nymphal mortality (%)	Lifetime reproduction	n	Adult weight (mg)	Nymphal mortality (%)	# Eggs in ovarioles
bud break	10	1.92 ± 0.05 a	2 ± 2 a	151 ± 10 a		n.t.		
flush leaves	10	1.78 ± 0.03 b	4 ± 3 a	139 ± 9 a		n.t.		
mature leaves	10	–	100 ± 0 b	–	10	0.250 ± 0.007 a	12 ± 4 a	5.1 ± 0.2 a
early senescent leaves	10	–	100 ± 0 b	–	10	0.272 ± 0.007 b	8 ± 3 a	5.4 ± 0.2 a

Other measured performance variables paralleled MRGR within each aphid generation, i.e. adult weight, reproduction and mortality (Tables 3 and 4). The different lifehistory strategies of different aphid generations make it difficult to interpret intergenerational comparisons of variables other than growth rate. The growth rates and other performance values reported here correspond closely to those measured in other studies of *R. padi* on *P. padus* and cereals (e.g. Dixon 1971; Leather & Dixon 1981).

Aphid behaviour in the field

In 1995, spring migration of *R. padi* from *P. padus* to grasses peaked at 8–11 of June and in 1996 at 17–23 of June. Migration back to *P. padus* at autumn started in 1995 and 1996 about 5–10 September.

Discussion

The growth rates of *R. padi* on the host plants, *P. padus* in spring, and on *H. vulgare* in summer, reveal these two plants to be of almost equal nutritional quality for the aphid: the change in host plant at mid June did not alter the growth rate much. Similar growth rates may reflect the nutritionally similar amino acid content in phloem of these two plants, i.e. *P. padus* and *H. vulgare* have similar total amino acid concentrations and there are only a few major differences between the two plants' concentrations of individual essential amino acids. Woody winter host plants of host-alternating aphids are generally believed to be of nutritionally inferior quality than herbaceous summer hosts (Llewellyn 1982). This is because aphids typically show lower production (P) to energy consumption (C) ratios on the woody winter host, i.e., they do not use energy as efficiently. Low P/C ratios could simply be an effect of high sugar, and thus energy, concentrations in the phloem of woody plants and therefore offer misleading estimates of plant quality. Nitrogen P/C may offer more biologically relevant estimates of quality, since nitrogen, and not sugar, is usually in low concentrations in phloem. This study shows that the woody winter host of *R. padi*, *P. padus*, is a high quality host in spring as judged by both aphid growth and phloem amino acid concentrations. Wild grasses were probably the original summer hosts of *R. padi* before human cultivation of cereals. Performance and growth rate of *R. padi* on wild grasses are generally lower than on cultivated cereals (Leather & Dixon 1981, 1982; Sandström *unpub*.). Thus, *P. padus* in springtime might be of superior quality in comparison with wild grasses.

The data show that staying on *P. padus* after mid-June is unfavourable. The natural switch to grass is thus well synchronized with a reduced aphid growth rate and lowered phloem nitrogen quality in mature *P. padus* leaves. The phloem quality of mature leaves seem to be of lower quality than flush *P. padus* leaves and *H. vulgare* leaves; i.e., lower total amino acid concentration together with lower concentrations of some essential amino acids. However, these changes are probably not the sole explanation for the high mortality of the spring generations and the lower growth rate in the sexual generation on mature *P. padus* leaves. Aphid growth rates are neither lowered on breaking buds of *P. padus*, where the phloem is low in total amino acid concentration nor on *H. vulgare*, which has lower concentrations of the essential amino acids, arginine, lysine and methionine compared to flush *P. padus* leaves. Many aphid species that use woody hosts have developed mechanisms to escape their woody host during mid-summer, whether by alternating to another host, or by aestivation, or by life cycle abbreviation (Moran 1992). Mature leaves of woody plants are apparently poor hosts for most aphids during mid-summer cessation of plant growth, but, as this study suggests, not necessarily because of changing amino acid concentrations in phloem itself. Cessation of growth and maturity of leaf tissue might be correlated with changes in phloem characteristics that might restrict aphid phloem ingestion rates, e.g. phloem sealing mechanisms might be more developed. This factor that has been proposed to affect aphid performance on grasses (Girousse & Bournoville 1994; Caillaud & Niemeyer 1996). In this study, phloem sap flowed for a longer time from stylets cut on young *P. padus* leaves and *H. vulgare* (mean 93 and 112 min, many still flowing after collection was finished) than on mature and early senescent *P. padus* leaves (mean 4 and 12 min). This could be an indication of more efficient wound healing mechanism in mature leaves. But it might also be an artifact of the sampling technique, since much smaller aphids were used for sampling from mature and early senescent leaves. Not all aphids perform poorly on mature mid-summer leaves of woody hosts compared to flush or senescent

leaves, e.g. *Pterocallis alni* on *Alnus* (Gange & Pryse 1990), and three species of pecan aphids on *Carya illioensis* (Kaakeh & Dutcher 1992). Likewise, this study shows that sexual females of *R. padi* can utilize mature leaves of *P. padus*, an adaptation that maybe has been lost in other generations. Even the *R. padi* generation that migrates to *P. padus* in autumn (gynoparae) seems to have lost this ability, as they are short-lived and do not feed after arriving to *P. padus* (Leather 1982; Walters *et al.* 1984). In contrast, the colonizers of grass in mid-June are able to feed on both hosts (Dixon 1971).

The changes in *P. padus* phloem between mature and early senescent leaves, i.e. higher total concentration and shifts in the dominant non-essential amino acids, are probably a result of protein degradation and increased translocation. Similar changes in EDTA-exudates from senescing leaves of woody plants were observed by Douglas (1993) who also observed a continued increase in total concentration of amino acids in later senescent stages. These changes seem to present sexual females with a slight advantage, as also observed by Dixon (1971). However, mature leaves can support a nearly normal growth rate of the sexual generation. This suggests that the return to *P. padus* at autumn is not solely correlated to changes in amino acid concentrations of phloem sap. Other factors contributing to host alternation in autumn might be important, e.g. enabling presence on *P. padus* early in spring when it is of high quality, providing mating rendezvous for sexuals in autumn, allowing better overwintering conditions for the eggs and close access to food in spring, and permitting an early spring microclimate on *P. padus* that might be favourable.

With regard to phloem amino acid profiles, the two hosts differed markedly. *P. padus*, with high levels of asparagine, had phloem similar to that collected from cut stylets on leguminous plants (Girousse *et al.* 1991; Sandström & Pettersson 1994). *H. vulgare* phloem, dominated by glutamic acid and aspartic acid and low levels of asparagine, resembled phloem typically collected on other cultivated grasses, e.g. oat (Kuo-Sell 1989), wheat (Fisher & MacNicol 1986), and rice (Hayashi & Chino 1990). Large differences in the concentrations of these amino acids are apparently encountered by *R. padi* during alternation between *P. padus* and *H. vulgare*. Whether such differences between spring and summer hosts pose any major physiological problems for the aphid is doubtful since glutamine, glutamic acid, aspartic acid and asparagine are all non-essential and are interchangeable by transamination in aphids (Febvay *et al.* 1995). The difference between *P. padus* and *H. vulgare* in concentrations of essential amino acids is potentially of greater biological importance. The switch from *P. padus* to *H. vulgare* probably brings about lower ingested amounts of arginine, lysine and methionine. Aphids possess symbiotic intracellular bacteria that provide their host with certain essential amino acids, i.e. methionine and tryptophan (Douglas 1988; Douglas & Prosser 1992). Further, for the synthesis of leucine and tryptophan it has been shown that the symbiont genes are amplified in symbionts of certain aphid species (Lai *et al.* 1994; Thao *et al.* 1998). Thus, the symbionts can probably compensate aphids for variable supply of some essential amino acids from the plants phloem, but it is unclear to what extent the symbionts of *R. padi* are capable of compensating for the differences found between *P. padus* and *H. vulgare*.

The concentration of amino acids in the phloem of *P. padus* increased between bud break and late flush leaves. This could be an effect of plant phenology, but it could also be an effect of the developing pseudogall induced by *R. padi* feeding. Gall formation might be accompanied by a change in phloem content, as aphids appear to gain nutritional advantages from galled tissue (Forrest 1971). Direct evidence that some aphids are able to alter the phloem amino acid concentrations is documented from aphids causing chlorotic lesions on grasses, which ingest phloem with an enhanced nitrogen quality (Sandström *et al.* 2000). Leather & Dixon (1981) showed that previously galled *P. padus* leaves promote better aphid growth; they suggested that galling was a way to prolong the suitability of the *P. padus* leaves for *R. padi*, possibly by delaying maturation. Natural changes in climate might also affect phloem nitrogen composition. For example, colder conditions in spring could yield changes in the plants' metabolic rate, thus affecting phloem content.

Host alternation in aphids has often been explained as an optimal use of host plants with complementary growth patterns. The present study does not rule out the importance of complementary growth. However, seasonal changes in phloem amino acid concentration are not alone sufficient to explain advantages of host alternation in *R. padi*, other factors that influence aphid nutrition probably play an important part, e.g. phloem access or seasonal patterns of nutritional components other than amino acids (e.g. vitamins, trace metals). It must also be emphasized that nutrition is not the only factor influencing patterns of host plant use.

Table 4 Performance of *Rhopalosiphum padi* on *Hordeum vulgare* of different seasonal growth stages (means $\pm$ S.E.). All test aphids were unwinged offspring from summer generations cultivated on *H. vulgare*. Values with the same letter are not significantly different according to Tukey's test ($P > 0.05$)

Growth stage at start of experiment	n	Adult weight (mg)	Nymphal mortality (%)	Lifetime reproduction
seedling	10	0.708 ± 0.016 a	2 ± 2 a	75 ± 4 a
tillering	10	0.703 ± 0.019 a	0 ± 0 a	63 ± 5 a
flowering	10	0.859 ± 0.018 b	4 ± 2 a	66 ± 5 a

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