

Host choice and host leaving in *Rhopalosiphum padi* (Hemiptera: Aphididae) emigrants and repellency of aphid colonies on the winter host

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Abstract

Host choice and winter-host leaving in emigrants of bird cherry–oat aphid, *Rhopalosiphum padi* (Linnaeus), were investigated in the laboratory. In settling choice tests, emigrants collected from the winter host, *Prunus padus*, preferred this plant over a summer host, oats. Emigrants which had left *P. padus* for up to 24 h did not express a preference as a group, and those which had left for 24–48 h preferred oats. Eighty seven percent of emigrants caged as fourth-instar nymphs on *P. padus* leaves abandoned the host by the second day of adult life, and apparently did not subsequently return to the leaf. In an olfactometer, *P. padus* leaves which had supported spring generations of *R. padi* were repellent to emigrants. Volatiles were entrained from uninfested and *R. padi*-infested *P. padus* using cut twigs in the laboratory as well as intact twigs on a tree in the field. Entrainment extracts from uninfested *P. padus* had no effect on emigrants in the olfactometer, whereas those from twigs infested with nymphal emigrants were repellent. The study indicates that in *R. padi*, host-alternation is driven by behavioural changes which occur in individuals as well as between morphs.

Introduction

The bird cherry–oat aphid *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) is, in northern Europe, a host alternating species that migrates between winter and summer host plants. In the spring, eggs hatch on the winter host, *Prunus padus* (Rosaceae), and the aphids pass through two or three wingless generations before the appearance of the winged emigrant morph (Wiktelius, 1984). The emigrants develop as nymphs on *P. padus* and, after becoming adult, fly to the summer hosts, grasses and cereals (Poaceae), where they give rise to further parthenogenetic generations (Mordwilko, 1907; Wiktelius *et al.*, 1990). The

emigrant therefore lives and feeds on two taxonomically different hosts during its life.

Rhopalosiphum padi populations on *P. padus* increase during May, emigrants begin to leave, and by mid-summer no aphids remain on the plant (Dixon, 1971). The production of winged emigrants is induced both by crowding and poor nutritional quality of the leaves (Dixon & Glen, 1971; Leather & Lehti, 1982; Wiktelius, 1984), and it has been suggested that a change in the aphids intrinsic host preference causes the switch between winter and summer hosts. A considerable amount of work has been devoted to study of the factors contributing to reproductive success and flight capacity of winged morphs, for example fat body content, reproduction and autolysis of wing muscles (Dixon, 1971, 1976; Leather *et al.*, 1983; Walters & Dixon, 1983). However, the influence of different stimuli on the shift in host plant preference in individual emigrants

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has not received much attention. The irreversible change in host preference shown by emigrants, born on the winter host and moving to the summer host, is less common among herbivores. Hypothetically it can be explained either by an intrinsic change in preferences related to physiological age, or to a more complex mechanism where several factors, including probing/feeding experienced, play a priming role.

The behavioural ecology of *R. padi* is influenced by odour stimuli. For emigrants, responses to an aggregation pheromone and a repellent winter host component, methyl salicylate, have been demonstrated (Pettersson, 1994; Pettersson *et al.*, 1994). However, studies of the chemical ecology of *R. padi* have mainly focused on the summer forms and on the remigrants (Pettersson, 1993; Hardie *et al.*, 1994; Quiroz *et al.*, 1997), and the role of semiochemicals for the spring generations on *P. padus* is not well understood.

This study examines in more detail the behavioural and chemical ecology of *R. padi* emigrants. The switch in host preference was investigated in adult emigrants, and the presence of aphid-induced repellent volatiles from the winter host is reported, based on entrainment from *P. padus* in the laboratory and field, and subsequent behavioural bioassay.

Materials and methods

Aphids and plants

Spring morphs of *R. padi* were reared on 4-year-old, *P. padus* saplings, planted in raised beds filled with potting soil (Hasselfors Garden) in an outdoor nethouse under ambient environmental conditions. Non-aphid-infested saplings were maintained under identical conditions. Spring morphs hatched from eggs which were deposited onto the trees by mating females the previous autumn. After hatching, aphids passed through two generations before emigrants were born. Oats, *Avena sativa* cv. Sanna, were grown in a glasshouse at $20 \pm 2^\circ\text{C}$, 16L:8D.

Host leaving of emigrants

Rhopalosiphum padi emigrants were collected from *P. padus* saplings as fourth-instar nymphs (identifiable by the presence of wing buds), and transferred to uninfested saplings in a nethouse. A group of between five and seven leaves were enclosed in a perforated plastic bag (Cryovac, W.R. Grace Ltd, Cambridge, England) and a single aphid introduced. The bag prevented the aphids from leaving the space around the leaf, while allowing them to disperse freely from the leaf after they became winged. Three *P. padus* saplings were used, with ten aphids on each. Aphids were examined several times each day and the number of days after the adult moult on which the aphid flew from the leaf, and later died was recorded.

Host preference of emigrants

Emigrants were collected from *P. padus* saplings as fourth-instar nymphs and placed on uninfested saplings in a nethouse, enclosed on separate leaves using clip cages. Aphids were collected for the experiment at three stages: (i) before leaving the leaf; (ii) having left the leaf for up to 24 h;

and (iii) having left the leaf for 24–48 h. Having abandoned the winter host, *R. padi* emigrants did not return to that plant (R. Glinwood, personal observation). A settling choice bioassay was used to determine host preference. A single excised *P. padus* leaf and a single excised oat leaf were placed side by side on a filter paper disc, and were enclosed under a plastic ring (10 cm diameter with a mesh lid). Emigrants were released individually between the two leaves, and the plant on which they settled was recorded after 2 h. A total of 30 aphids from each experimental group were tested, and the distributions of settling preferences were compared using Chi-square tests.

Entrainment of P. padus volatiles – laboratory experiments

Volatiles were entrained from aphid-infested and uninfested *P. padus* twigs. These were cut, and stood in a beaker of distilled water sealed with Parafilm. The twig was inserted through the Parafilm, and the insertion point was also sealed. Air was dried and purified by passage through activated charcoal and 4 Å molecular sieve, and was drawn at 300 ml min^{-1} through a glass vessel (26 cm high \times 9.5 cm diameter) containing the *P. padus* for 72 h. Volatiles were entrained onto Porapak Q, which had been washed with diethyl ether and heated at 150°C , and subsequently desorbed by elution with diethyl ether. Extracts were concentrated at room temperature under a stream of nitrogen and stored in a refrigerator at 5°C . Entrainments were made from *P. padus* four weeks after leaf emergence, and the plant was either uninfested or infested with a colony of *R. padi* emigrants in the nymphal instars.

Entrainment of P. padus volatile – field experiments

Entrainments were made *in situ* from an uninfested twig, and from a twig infested with mixed instar nymphal emigrants, in the same area of the canopy of a *P. padus* tree (16 years old, 6 m tall) in the field for 48 h between 12 and 14 May 1999. A twig (approximately 20 cm long) was enclosed in a bag made from inert plastic (Cryovac Cellophane). The bag was sealed at one end, and the other held a stopper made from a disc of inert polythene foam plastic (Plastazote PZ940). The stopper had inlets for an air inlet tube containing activated charcoal and 4 Å molecular sieve, the twig, and for the Porapak Q containing tube. The area around the inlets was sealed using Teflon tape. Air was drawn through the bag at 1 l min^{-1} using a vacuum pump connected to an exterior power supply. Volatiles were extracted as above.

Olfactometer bioassay

Extracts of infested and uninfested *P. padus* were tested for their behavioural effects on adult *R. padi* emigrants in a four-way olfactometer (Pettersson, 1970), consisting of an enclosed perspex arena (12 cm diameter) with a central chamber and four side arms. Air was drawn from the centre of the olfactometer using a water pump (3 ml sec^{-1}), establishing discrete air currents in the side arms, and an odour field was created by introducing the air entrainment extract in diethyl ether ($10 \mu\text{l}$ extract applied to a filter paper square) to one of the side arms. To avoid saturating the atmosphere in the olfactometer with solvent, control arms

contained untreated filter paper. However, a separate test showed that diethyl ether alone was neither attractive nor repellent to the aphids (Chi-square test; $n = 16$, $\chi^2 = 0.02$, $P > 0.05$). Tests were also run with living material, which was either a *P. padus* leaf with eight fourth instar emigrants (infested for 48 h), emigrants alone, or a previously infested leaf (10–30 nymphal emigrants for 2 weeks) with the aphids removed and the surface cleaned by gentle washing with damp cotton wool. In these tests, control arms contained clean air.

A single adult migrant (which had left *P. padus* for up to 24 h) was introduced into the olfactometer, and its position recorded every 3 min for 30 min. The distribution of aphid visits to the treatment arm and mean of the control arms was compared using Chi-square tests. Experiments were repeated 14–22 times over at least two days, with the olfactometer turned through 90° between replicates to avoid positional bias.

Results

Host leaving of emigrants

The majority (87%) of emigrants left the leaves of *P. padus* by the second day after becoming adult (table 1). By the third day of adult life, all aphids had left the leaves. Aphid mortality increased rapidly after leaving the leaf (table 1), suggesting that, although no alternative source of food or water was available, the emigrants did not return to *P. padus*.

Table 1. Effect of adult age on host leaving and mortality of *Rhopalosiphum padi* emigrants confined around the winter host plant *Prunus padus* inside perforated polythene bags ($n = 30$).

Adult life (days) ¹	% Aphids on leaf	% Mortality
1	100	0
2	13	0
3	0	57
4	0	87
5	0	97

¹Number of days after the aphid had become adult (i.e. winged).

Table 3. Responses of *Rhopalosiphum padi* emigrants to odours of leaves of the winter host *Prunus padus* and conspecific aphids in a four-way olfactometer.

Test stimulus ¹	Mean no. visits in olfactometer arm	n	χ^2	P	χ^2 value for heterogeneity ²
<i>P. padus</i> leaf infested with eight fourth nymphal instar emigrants for 48 h	1.9	14	1.4	>0.05	6.1
Control	2.6				
<i>P. padus</i> leaf previously infested with 10–30 nymphal emigrants for 2 weeks (aphids removed)	1.6	18	5.7	<0.05	13.0
Control	2.8				
Eight fourth nymphal instar emigrants	2.6	18	0.01	>0.05	3.5
Control	2.5				

¹Control arms contained clean air.

²All values of χ^2 for heterogeneity are non-significant.

Host preference of emigrants

When emigrants were collected before leaving the winter host, *P. padus*, significantly more individuals settled on *P. padus* leaves (Chi-square test; $n = 30$, $\chi^2 = 10.8$, $P < 0.01$) than on oat leaves (table 2). Emigrants that had left *P. padus* for up to 24 h without contacting summer hosts did not settle in significantly greater numbers on either plant (Chi-square test; $n = 30$, $\chi^2 = 1.2$, $P > 0.05$) (table 2). Emigrants which had left *P. padus* for between 24 and 48 h without contacting summer hosts settled in significantly greater numbers on oats (Chi-square test; $n = 30$, $\chi^2 = 19.2$, $P < 0.001$) than on *P. padus* (table 2).

Olfactometry with volatiles

Prunus padus leaves infested with eight fourth-instar emigrants for 48 h had no effect on adult emigrants in the olfactometer (Chi-square test; $n = 14$, $\chi^2 = 1.4$, $P > 0.05$) (table 3). However, leaves which had been infested with 10–30 mixed instar nymphal emigrants for two weeks were significantly repellent (Chi-square test; $n = 18$, $\chi^2 = 5.7$, $P < 0.05$) (table 3). Eight fourth-instar migrants alone had no effect on adult emigrants (Chi-square test; $n = 18$, $\chi^2 = 0.01$, $P > 0.05$) (table 3).

Air entrainment extract containing volatiles of an uninfested, cut *P. padus* twig had no effect on the behaviour of adult emigrants (Chi-square test; $n = 18$, $\chi^2 = 0.3$, $P > 0.05$) (table 4). However, extract containing volatiles of a cut twig infested with fourth instar emigrants was

Table 2. Host preference of *Rhopalosiphum padi* emigrants before and after leaving the winter host plant *Prunus padus*, when offered leaves of *P. padus* and a summer host, oats.

Test aphids	No. aphids settled after 2 h ($n = 30$)		χ^2	P
	<i>P. padus</i>	Oat		
Before leaving <i>P. padus</i>	24	6	10.8	<0.01
Off <i>P. padus</i> < 24 h	18	12	1.2	>0.05
Off <i>P. padus</i> 24–48 h	3	27	19.2	<0.001

Table 4. Responses of *Rhopalosiphum padi* emigrants to volatiles entrained from winter host *Prunus padus* which was either uninfested, or infested with conspecific aphids in a four-way olfactometer.

Air entrainment extract ¹	Mean no. visits in olfactometer arm	n	χ^2	P	χ^2 value for heterogeneity ²
Cut, uninfested <i>P. padus</i> twig	2.8	18	0.3	> 0.05	6.1
Control	2.4				
Cut, <i>P. padus</i> twig infested with fourth instar nymphal emigrants	1.7	20	5.0	< 0.05	12.5
Control	2.8				
Uninfested <i>P. padus</i> twig <i>in situ</i> on a field tree	2.8	19	0.3	> 0.05	11.8
Control	2.4				
<i>P. padus</i> twig infested with nymphal instar emigrants <i>in situ</i> on a field tree	1.8	22	4.0	< 0.05	12.3
Control	2.7				

¹Extract of entrained volatiles in diethyl ether. Control arms contained clean air.

²All values of χ^2 or heterogeneity are non-significant.

significantly repellent to adult emigrants (Chi-square test; $n = 20$, $\chi^2 = 5.0$, $P < 0.05$) (table 4). An extract containing volatiles entrained *in situ* from an uninfested *P. padus* twig on a tree in the field had no behavioural effect on adult emigrants (Chi-square test; $n = 19$, $\chi^2 = 0.3$, $P > 0.05$) (table 4), whereas the extract containing volatiles collected *in situ* from a twig infested with mixed instar nymphal emigrants was significantly repellent (Chi-square test; $n = 22$, $\chi^2 = 4.0$, $P < 0.05$) (table 4).

Discussion

The switching of hosts in host-alternating aphids requires, at some stage of the life cycle, a modification in the host plant preference of the aphid itself. The present study shows that, in the spring, the change in host preference occurs within individuals of *R. padi*. It is not simply explained as a difference between generations born on the winter host. The switch in host preference from winter to summer host did not occur sharply at the adult moult, but formed gradually during the early stages of adult life. Among the group which had left *P. padus* for up to 24 h, some aphids chose the winter host and some the summer host. This suggests that the change in host preference occurs within individuals over a period of hours, rather than days. The change could be mediated by levels of juvenile hormone in the aphid's body (Hardie, 1981; Hardie & Glascodine, 1990). In remigrants of the black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae), Hardie (1981) found that larval instars preferred the summer host, whereas adult remigrants preferred the winter host. However, in this study adults were tested on day 2 after the final moult.

Also linked to time after the adult moult in this study was the behaviour of the aphids caged on *P. padus*. On day 1, all the adult aphids were settled on the leaf, but by day 2, 87% had abandoned the leaf and had settled elsewhere in the cage. After becoming adult, flight muscles may require time to develop fully. In pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae), the flight muscle protein content of winged summer morphs reached a peak two days after the adult moult, and this coincided with take-off by the aphids (Kobayashi & Ishikawa, 1993). In the current study, most of

the aphids had died by day 4, an indication that they did not return to the leaf, even to feed briefly or to ingest fluid. If emigrants do not accept *P. padus* again once the preference for summer hosts has formed, this would ensure that migrating *R. padi* do not accidentally recolonize the winter host after leaving it.

It is interesting that the majority of emigrants left *P. padus* before the preference for the summer host would have been completely formed. Therefore it is possible that an innate switch in host preference is not a causal mechanism in host alternation (Dixon, 1971). However, although the host leaving experiment was performed in a semi-open nethouse, exposed to ambient temperatures, the host leaving pattern can still be regarded as representing what may happen under 'ideal' conditions. Wind and rain, for example, would probably delay the flight of emigrants from the winter host under natural field conditions (Dixon & Mercer, 1983). Moreover, it is possible that a period of flight activity after leaving *P. padus* accelerates the change in host preference.

The olfactometer results indicate that volatiles associated with the infestation of *P. padus* by spring morphs are repellent to emigrants. Aphids alone were not repellent, and a light infestation (8 aphids) for 48 h was insufficient to render a leaf repellent. Heavier infestations (10–30 aphids) over a longer period did cause leaves to become repellent in the olfactometer, even after aphids were removed from them. Volatiles from uninfested *P. padus* were not repellent. Therefore the repellency appears to be induced by aphid infestation. In the context of the *R. padi* life cycle, this may be an additional mechanism ensuring that emigrants do not recolonize the winter host after leaving it. The repellent volatiles might also contribute to the actual leaving of the plant by the emigrants, but this has not been demonstrated. *Rhopalosiphum padi* usually exists at mixed stages of development on *P. padus* in the spring, so adult emigrants are likely to take off from colonies which have feeding nymphal emigrants, or even foundress morphs present. It is possible that the concentration of behaviourally active volatiles in the extracts are higher than would be emitted by an aphid colony/twig in the field, and the olfactometer results should be considered in the light of this possibility. However, it can be noted that there would normally be

many such aphid colonies/twigs on a single tree which may raise the level of volatiles experienced by the aphids.

Rhopalosiphum padi colonization of cereal fields has already been successfully reduced by release of methyl salicylate, a volatile component of *P. padus* which is repellent to this and other cereal aphids (Pettersson *et al.*, 1994). Identification of the substances responsible for the repellent effects reported here may yield further candidates for use in crop protection. This study indicates that factors which influence host preference and winter host leaving by emigrants operate at the level of the individual aphid, as well as the aphid morph.

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