

Behavioural differences during host selection between alate virginoparae of generalist and tobacco-specialist *Myzus persicae*

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Abstract

Host plant selection and acceptance by aphids involves four consecutive steps: (1) prealighting behaviour, (2) leaf surface exploration and probing of subepidermal tissues, (3) deep probing of plant tissues, and (4) evaluation of the phloem sap. Host specialisation in aphids may involve not only different performances on potential hosts, but also different strategies for host selection and acceptance. *Myzus persicae* s.s. (Sulzer) (Homoptera: Aphididae) is one of the most polyphagous aphid species, although a tobacco-adapted subspecies, *M. persicae nicotianae*, has been described. These two taxa constitute a good system for studying the effect of host range on host selection strategies. We studied the first two steps in the host selection process by alate virginoparae of *M. persicae* s.s. and *M. persicae nicotianae* on host and non-host plants, using three types of behavioural assays: wind tunnel, olfactometry, and video-recording. Alate virginoparae of *M. persicae nicotianae* recognised and chose their host plant more efficiently than *M. persicae* s.s., on the basis of olfactory and visual cues, and factors residing at cuticular and subcuticular levels. Host recognition was evident before phloem tissues were contacted. Olfactory cues were apparently not involved in host selection by *M. persicae* s.s.

Introduction

The majority of herbivorous insect species are very selective feeders that choose their host plants based on visual, mechanical, and chemical stimuli (Prokopy, 1986; Bernays & Chapman, 1994; Price, 1997; Bernays, 1998). In aphids, host plant selection may be divided into four consecutive steps: (1) prealighting behaviour, (2) leaf surface exploration and probing of subepidermal tissues, (3) deep probing of plant tissues, and (4) evaluation of the phloem sap (Niemeyer, 1990; Caillaud, 1999; Caillaud & Via, 2000). The host or non-host nature of a plant can be recognised before alighting on the plant (Chapman et al., 1981; Nottingham & Hardie, 1993), on the leaf surface (Powell et al., 1999), at subepidermal levels (Bernays & Funk, 2000; Caillaud & Via, 2000; Powell & Hardie, 2000; Funk & Bernays, 2001), and in the phloem itself (van Helden & Tjallingii, 1993; Saugé et al., 1998). The relative importance of each of these

four stages may be affected by the degree of host specialisation (Bernays & Funk, 1999; Funk & Bernays, 2001), and by the species studied (Tosh et al., 2003). The efficient recognition of a host will depend on its capacity for processing information about environmental cues, as was proposed in the neural constraints hypothesis (Bernays, 1998, 2001).

An aphid species that may be particularly suited for studying the effect of host range on host selection behaviour is the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Myzus persicae* is a generalist aphid that uses peach (*Prunus persica* L.) as its normal primary host on which sexual reproduction occurs, and more than 400 plant species from more than 40 families as secondary hosts on which parthenogenetic reproduction occurs (van Emden et al., 1969; Weber, 1986; Bernays & Chapman, 1994; Blackman & Eastop, 2000). A form of *M. persicae* which is specialised on tobacco has been known for over 60 years (Brain, 1940). Recent evidence shows that there is a sufficient degree of isolation of the tobacco-adapted genome for the tobacco specialist to be treated as a subspecies (Margaritopoulos et al., 2003; Blackman & Eastop,

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in press). Following Blackman & Eastop (in press), we refer to the generalist and specialist aphids as *M. persicae* s.s. and *M. persicae nicotianae*, respectively.

A study of the host preference of both aphid taxa did not reveal differences between them, and host selection was described as a random process (Clements et al., 2000). In contrast, Nikolakakis et al. (2003) detected differences in host preference between *M. persicae* from tobacco and non-tobacco growing regions. However, both these studies were carried out with apterous morphs. Given that alate virginoparae are mostly responsible for finding and colonising new secondary hosts (Walters & Dixon, 1982; Klingauf, 1987; Blackman, 1990), in the present study we test the prediction that generalists and specialists will differ in their ability to discriminate between hosts and non-hosts, based on their respective neural capacities (Bernays, 1998, 2001), by comparing host selection behaviours between alate individuals of *M. persicae* s.s. and *M. persicae nicotianae*. We hypothesise that these taxa will show behavioural differences in response to plant volatiles, leaf surface and subepidermal tissue characteristics, which will be related to their respective host range.

Materials and methods

Aphids and plants

Different patterns of performance have been found for *M. persicae* s.s. and *M. persicae nicotianae*, depending on the host plant (Clements et al., 2000). The specialist on tobacco shows a better performance on tobacco, although it may be reared on several species of five plant families under laboratory conditions (Semtner et al., 1998; Nikolakakis et al., 2003). On the other hand, the generalist thrives on a large variety of hosts (Blackman & Eastop, 2000), but shows a poor performance on tobacco (Boiteau & Lowery, 1989; Nikolakakis et al., 2003). Sugar beet has been described as a host for *M. persicae* s.s. in the UK (Heathcote et al., 1965; Thornhill & Heathcote, 1987); in Chile, *M. persicae* s.s. has been described as a major pest of sugar beet, presumably due to an overlap of successive growing seasons of the crop (Stevens & Dewar, 1996). In the laboratory, high density colonies of *M. persicae* s.s. on sugar beet produced a large proportion of alatae. Hence, sugar beet was considered to be a host for *M. persicae* s.s. and a non-host for *M. persicae nicotianae*, while tobacco was considered to be a host for *M. persicae nicotianae* and a non-host for *M. persicae* s.s.

Aphids were collected from parthenogenetic populations; *M. persicae* s.s. was collected from sugar beet (*Beta vulgaris* L.; Chenopodiaceae), a common crop in the tobacco growing regions of Chile, and *M. persicae nicotianae* was collected from tobacco (*Nicotiana tabacum* L. cv. BY 64;

Solanaceae). Aphid genotype characterisation with micro-satellite markers and morphometric multivariate analysis were done to separate the populations (Fuentes-Contreras et al., 2004). The colony of *M. persicae* s.s. was multiclinal. The colony of *M. persicae nicotianae* was monoclonal, as was shown by Fuentes-Contreras et al. (2004) for all the Chilean populations of this aphid they studied. Both aphid taxa were reared in laboratory cabinets on the same host plants from which they were collected, at 21 ± 2 °C and under a long day photoperiod (L16:D8), for more than three generations before the beginning of the experiments. All experiments were performed with 3-day-old alate virginoparae obtained as follows: alataids were withdrawn from the colony and transferred to their respective hosts; when the alates emerged, they were again transferred to their respective hosts, to be used when they were 3 days old. Experiments were performed in a room at 21 ± 2 °C, and between approximately 09.00 and 15.00 hours.

Morphometric features related to aphid movement on the plant surface (length of hind tibia and femur), were also evaluated. Alate virginoparae of both aphid taxa were mounted as described by Blackman & Eastop (2000). The slides with mounted aphids and a scale of ± 0.01 mm were placed under a microscope with a close-up video system (Sony SSC-DC34); digital photographs (Sony MVC-FD95) of the monitor screen were taken, and measurements were performed using Sigma Scan Pro 5.0 Software ($n = 30$ for each aphid taxon). Morphometric measurements from both aphid taxa were compared using a one-way ANOVA.

Wind tunnel

Tests were performed using a horizontal wind tunnel (215 × 82 × 82 cm), the floor and walls of which consisted of agglomerated wood coated with white melamine. Lights were placed above the Plexiglas roof, and filters were placed at the air entrance in order to retain impurities and to guarantee a laminar flow which was free of turbulence; air passed through at a linear speed of 0.01 m s^{-1} . Uninfested potted plants of tobacco and sugar beet with similar foliar surface areas were placed near the point entrance of the air, and equidistant from the aphid platform (5 × 5 cm, 40 cm above ground) at the downwind end. In order to ensure that aphids reached the plants only by flight, each pot was surrounded by double-sided adhesive tape at its base. In each experiment, 20–25 alate virginoparae of one of the studied taxa were placed on the aphid platform inside the wind tunnel and exposed to tobacco and sugar beet plants for 4 h. The proportions of aphids that were present on the host and non-host plants were quantified at hourly intervals, as well as at the end of the experiment (4 h). Eight replicates were done with *M. persicae* s.s. and 14 with

M. persicae nicotianae. These numbers of replicates were sufficient to quantitatively account for the clear trends exhibited by the two taxa. Host selection within each aphid taxon was assessed by comparing proportions of aphids on host and non-host plants with a Wilcoxon signed-ranks Z-test for paired data because the proportions on each plant were co-dependent within the sample (Siegel & Castellan, 1988). For between-taxa comparisons, the proportion of aphids landing on either host or non-host plants were compared using a Mann–Whitney U-test since they constituted independent samples (Zar, 1996). Data from intermediate time intervals did not comply with the requirements of a repeated measures parametric ANOVA; therefore they were not analysed statistically.

Olfactometry

The four-way olfactometer used in the experiments was originally designed by Pettersson (1970). Two opposing arms of the olfactometer were connected by Teflon tubing to a glass bell-jar which contained one stimulus source, while the other two arms were connected to a glass bell-jar containing the other stimulus source. The stimuli used were: a host plant, a non-host plant, and a control consisting of a pot with soil but without a plant. Charcoal filters at the inlet of the bell-jars were used to eliminate external odours during the experiments. The airflow (150 ml min^{-1}) was obtained with a vacuum compressor pumping air out from the olfactometer through a hole in its centre. A white paper cylinder (10 cm in height) surrounded the olfactometer in order to avoid visual stimuli from the plants inside the bell-jars. Pseudoreplication was avoided by using a single aphid in each replicate, testing each aphid only once, and by using a clean olfactometer for each replicate, as was suggested by Ramírez et al. (2000). To avoid bias, the olfactometer was periodically rotated, and lighting was provided from above. Four treatments were carried out: *M. persicae* s.s. exposed to volatiles from sugar beet vs. control, and tobacco vs. sugar beet, and *M. persicae nicotianae* confronted with volatiles from tobacco vs. control, and tobacco vs. sugar beet. Treatments with host and control stimuli were included in order to assess attraction to host volatiles, while treatments with host and non-host were included in order to assess the host discriminatory capacity of each aphid taxon (Prokopy, 1986; Visser, 1986; Pickett et al., 1992). Fifteen replicates were done for each treatment. The aphids (alate virginoparae) were given a 2 min adaptation time after being introduced into the arena. Continuous observations lasted 30 min and the time spent in each stimulus zone was recorded and analysed with The Observer program (Noldus Information Technology, 1995). All individuals remaining immobile for longer than 15 min were discarded (16.8% of all cases). The comparison of the

total time spent by the aphid in arms with different stimuli was performed with a Wilcoxon signed-ranks Z-test for paired data (Siegel & Castellan, 1988).

Video-recording

Alate virginoparous aphids were studied on host and non-host plant leaf surfaces. A stereomicroscope with a close-up video system (Sony SSC-DC34) was used to accurately monitoring the behaviour of freely moving aphids on the leaf surface (Hardie et al., 1992; Caillaud & Via, 2000; Powell & Hardie, 2000), as well as for recording videotapes for later analysis using The Observer program (Noldus Information Technology, 1995). A freshly excised leaf (tobacco or sugar beet), with wet cotton wool around its petiole to avoid wilting, was attached to a stand with a system of pincers which allowed its rotation in such a way that the position of the aphid rostrum in relation to the leaf surface could always be assessed. Previous studies with apterous aphids have shown that stylet penetration can be determined accurately by observing aphid antennal and body movements (Hardie et al., 1992; Powell et al., 1993); similar criteria were used in the present study. Thus, the start of a probe was assumed when the rostrum contacted the leaf surface perpendicular to the aphid's body, and the antennae stopped waving and pointed backwards forming an angle smaller than 90° with the aphid's body. Stylet withdrawal was assumed to start when the antennae waved forwards again prior to resuming walking or moving. Experiments began when an insect was placed on the centre of the adaxial surface of a leaf using a fine camel hair paintbrush. When the aphids occasionally walked off the leaf onto the pincers, they were returned immediately to the leaf surface using a paintbrush (Powell & Hardie, 2000). Recordings stopped when the aphid flew away from the plant or when it performed a probe for 5 min (defined herein as a long-duration-probe), or when video recording had proceeded for 60 min. Individuals remaining immobile for longer than 30 min without a long duration probe were discarded and substituted with new replicates (only six cases).

The following behavioural variables were analysed: time to first probe, duration of first probe, proportion of total time spent probing, total time spent probing before the long-duration probe, number of probes before the long-duration probe, time to the long-duration probe, proportion of individuals making a long-duration probe, time before take-off, and proportion of individuals that flew away (see Caillaud & Via, 2000; Powell & Hardie, 2000). Thirty replicates for each of the four aphid/plant combinations were carried out. Behavioural variables were compared across aphid/plant combinations using the Scheirer–Ray–Hare extension of the Kruskal–Wallis H-test

Table 1 Means (\pm SE) and proportions of behavioural parameters on host and non-host plants of generalist *Myzus persicae* s.s. and tobacco specialist *Myzus persicae nicotianae*. Parameters were compared across aphid/plant combinations. Within a row, different letters denote significant differences across aphid/plant combinations (see text for details of the statistical tests performed)

| | Tobacco | | Sugar beet | |
|---|--------------------------------------|----------------------------------|----------------------------|----------------------------------|
| | <i>Myzus persicae</i> s.s. | <i>Myzus persicae nicotianae</i> | <i>Myzus persicae</i> s.s. | <i>Myzus persicae nicotianae</i> |
| Time to first probe (s) | 191.8 \pm 50.6 a (21) ^a | 162.3 \pm 73.1 a (28) | 35.77 \pm 9.6 b (29) | 32.73 \pm 5.2 b (27) |
| Duration of the first probe (s) | 33.5 \pm 9.1 b (19) | 58.4 \pm 15.7 a (23) | 55.8 \pm 15.7 a (23) | 39.3 \pm 9.8 b (18) |
| Proportion of total time spent probing | 0.15 \pm 0.03 b (19) | 0.30 \pm 0.05 a (23) | 0.31 \pm 0.04 a (23) | 0.17 \pm 0.04 b (18) |
| Number of probes before long-duration probe | 2.5 \pm 0.4 a (17) | 1.7 \pm 0.3 b (27) | 1.3 \pm 0.3 c (17) | 0.5 \pm 0.1 d (19) |
| Time to long-duration probe (s) | 680.1 \pm 147 a (17) | 428.1 \pm 94 b (27) | 198.3 \pm 28.7 c (17) | 66.7 \pm 10.5 d (19) |
| Total time spent probing before long-duration probe (s) | 159.1 \pm 41.4 a (17) | 116.4 \pm 23 b (27) | 64.6 \pm 18.8 c (17) | 10.4 \pm 3.1 d (19) |
| Proportion of individuals performing a long-duration probe | 0.57 b (30) | 0.90 a (30) | 0.57 b (30) | 0.63 b (30) |
| Proportion of individuals that did not perform a long-duration probe, and remained on the plant | 0.36 a (30) | 0.03 b (30) | 0 b (30) | 0 b (30) |
| Proportion of individuals that did not perform a long-duration probe, and took-off | 0.07 b (30) | 0.07 b (30) | 0.43 a (30) | 0.37 a (30) |
| Time to take-off (s) | 1917–224 ^b (2) | 359–189 ^b (2) | 183 \pm 23.3 a (13) | 246 \pm 79.9 a (11) |
| Proportion of individuals that performed at least one probe before take-off | 0.50 ^b (2) | 0.50 ^b (2) | 0.92 a (13) | 0.72 a (11) |
| Frequency of foot-lifting (min ⁻¹) | 32.6 \pm 9.7 b (10) | 41.6 \pm 14.8 b (10) | 77.9 \pm 16.0 a (10) | 68.3 \pm 12.1 a (10) |
| Percentage of times a leg rested on a trichome | 87.7 \pm 16.9 a (10) | 17.5 \pm 8.8 b (10) | | |

^a(n), number of replicates for each treatment.

^bValues correspond to the two individuals that flew away from the plant.

(two-way non-parametric ANOVA; Sokal & Rohlf, 1998; Dytham, 1999) because the data could not be normalised after transformation, and post hoc comparisons were performed with Dunnett's tests. Only those replicates in which the behaviour examined occurred within the duration of the experiment were used in the analysis; these numbers are given in brackets in Table 1. Since take-off behaviour mainly occurred on sugar beet, time to take-off was compared using the Mann–Whitney U-test. Comparisons between proportions were performed with the Z-test for proportions and Yates' correction for continuity (Zar, 1996).

To evaluate differences between taxa on their movement during leaf surface exploration, the video recordings were re-analysed to evaluate the following motion variables: the number of times a foot was lifted, which was counted for each of the four aphid/plant combinations, and the position of the tarsus once that foot was lowered to a resting position, which was determined only on tobacco. The analysis was performed on randomly chosen 1-min recording periods where the aphid was performing a continuous walking behaviour, and repeated 10 times for each treatment. Two-way and one-way ANOVA were performed on the two sets of data, respectively.

Results

Host finding

In the wind tunnel, most *M. persicae* s.s. individuals took off from the aphid platform in all tests, but they were observed in minimal proportions on plants, and no significant differences were found between the proportions observed on either plant species (Figure 1). On the other hand, a significantly higher proportion of individuals of *M. persicae nicotianae* were observed on tobacco than on sugar beet (Wilcoxon signed-ranks test: $Z = 3.06$, $P < 0.01$; $n = 14$) (Figure 1). The proportion of individuals observed on their host plant was significantly different between *M. persicae* s.s. and *M. persicae nicotianae* (Mann–Whitney U-test: $U = 8$, $P < 0.01$; $n_1 = 8$, $n_2 = 14$). The proportions of individuals observed on non-host plants were not different between taxa (Figure 1). It should be noted that a relatively high proportion of aphids of both taxa ended up on the floor, walls, and roof of the wind tunnel (*M. persicae* s.s. 39 ± 22 , 11 ± 12 , and $3 \pm 4\%$; *M. persicae nicotianae*: 35 ± 13 , 12 ± 8 , and $4 \pm 5\%$, respectively).

Olfactometry tests revealed a significant attraction of *M. persicae nicotianae* to volatiles from tobacco plants relative to the control (Wilcoxon signed-ranks test: $n = 15$, $Z = 2.33$,

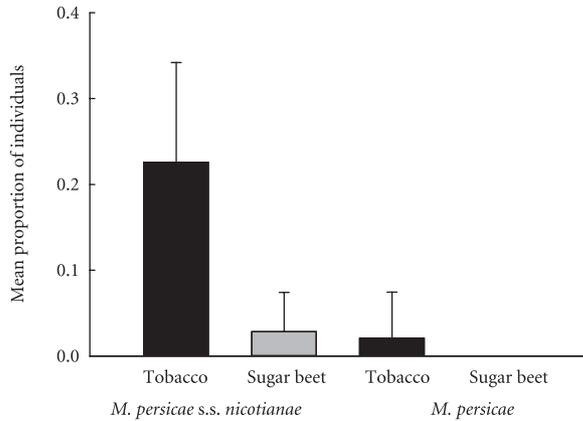


Figure 1 Host finding in the wind tunnel, expressed as the mean proportion of individuals of *Myzus persicae* s.s. and *Myzus persicae nicotianae*, observed on tobacco and sugar beet plants after 4 h of exposure.

$P < 0.05$) (Figure 2). On the other hand, no significant attraction was found for *M. persicae* s.s. to volatiles from sugar beet relative to control (Wilcoxon signed-ranks test: $Z = 0.57$, ns; $n = 15$). Neither the generalist nor the specialist on tobacco preferred host over non-host odour stimuli.

Exploration of leaf surface and subepidermal tissues

The time to first probe on tobacco ranged from 3.8 to 1860 s, while on sugar beet it was never longer than 222 s; this variable was significantly shorter for sugar beet than for tobacco (two-way non-parametric ANOVA: $H_{1,105} = 10.123$, $P < 0.005$), but there were no significant differences between *M. persicae* s.s. and *M. persicae nicotianae* (Table 1). The

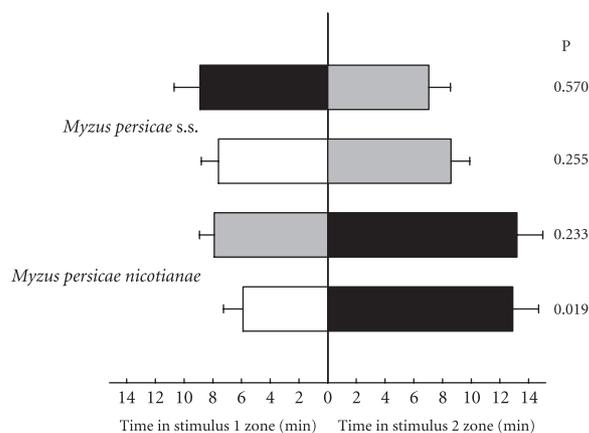


Figure 2 Mean time spent by *Myzus persicae* s.s. and *Myzus persicae nicotianae*, on the olfactometer zones permeated by stimuli 1 and 2. Stimuli used were tobacco plants (black bars), sugar beet plants (grey bars), or no plants (white bars). P-values for each treatment are shown on the right of the bars.

duration of the first probe lasted longer on the host than on the non-host, and the proportion of total time spent probing was about twice as great on the host than on the non-host, for both aphid taxa (Table 1); these variables showed significant aphid–plant interactions (two-way non-parametric ANOVA: $H_{1,83} = 4.159$, $P < 0.05$ and $H_{1,80} = 8.947$, $P < 0.005$, respectively).

For both aphid taxa, the number of probes before a long-duration probe was smaller, the time to achieve a long-duration probe was shorter, and the total time spent probing before a long-duration probe was also shorter on sugar beet than on tobacco (two-way non-parametric ANOVA: $H_{1,80} = 13.468$, $P < 0.001$; $H_{1,80} = 25.335$, $P < 0.001$; $H_{1,79} = 16.398$, $P < 0.001$; for the three variables, respectively) (Table 1). In addition, *M. persicae nicotianae* made fewer probes (two-way non-parametric ANOVA: $H_{1,80} = 5.610$, $P < 0.025$), performed a long-duration probe sooner (two-way non-parametric ANOVA: $H_{1,80} = 8.254$, $P < 0.005$), and spent less time probing (two-way non-parametric ANOVA: $H_{1,79} = 4.038$, $P < 0.05$) than *M. persicae* s.s., both on host and non-host plants (Table 1).

Myzus persicae nicotianae, compared with *M. persicae* s.s., showed a significantly greater proportion of individuals performing a long-duration probe when they were on tobacco (Z-test for proportions: $Z = 2.162$, $P < 0.05$; $n = 120$) but not when they were on sugar beet (Z-test for proportions: $Z = 0.211$, ns; $n = 120$) (Table 1). Among the individuals remaining on tobacco (i.e., not taking off), a greater proportion of *M. persicae* s.s. did not perform a long-duration probe as compared with *M. persicae nicotianae* (Z-test for proportions: $Z = 2.900$, $P < 0.01$; $n = 11$) (Table 1), while on sugar beet all individuals which did not take off performed a long-duration probe (Table 1).

The proportion of individuals that left the plant before performing a long-duration probe differed between plants (Z-test for proportions: $Z = 4.048$, $P < 0.001$; $n = 28$), but not between aphid taxa (Z-test for proportions: $Z = 0.172$, ns; $n = 28$) (Table 1). Only two individuals of each aphid taxon left tobacco. In sugar beet, about 40% of individuals of both aphid taxa flew away after they had remained on the plant for 3–4 min, but no significant differences were found between *M. persicae* s.s. and *M. persicae nicotianae* (Table 1). Among individuals that rejected the plants, a greater proportion of both aphid taxa flew away from sugar beet making at least one probe before take off (Z-test for proportions: $Z = 2.045$, $P < 0.05$; $n = 13$ for *M. persicae* s.s., $Z = 4.067$, $P < 0.001$; $n = 11$ for *M. persicae nicotianae*), while on tobacco, one of the two individuals of each taxa that rejected the plant made at least one probe before take off (Table 1).

Foot-lifting was significantly less frequent in tobacco than in sugar beet (two-way ANOVA: $F_{1,40} = 71.073$,

$P < 0.001$), but there were no significant differences between *M. persicae* s.s. and *M. persicae nicotianae* (Table 1). Footsteps ended more frequently on a trichome in *M. persicae* s.s. than in *M. persicae nicotianae* ($87.8 \pm 16.9\%$ and $17.5 \pm 8.8\%$, respectively, one-way ANOVA: $F_{1,20} = 59.612$, $P < 0.001$).

Differences were observed in the length of hind tibia and femur between the two aphid taxa (hind tibia: 1.11 ± 0.07 and 1.41 ± 0.09 mm for *M. persicae* s.s. and *M. persicae nicotianae*, respectively; one-way ANOVA: $F_{1,60} = 165.971$, $P < 0.001$; hind femur: 0.70 ± 0.04 and 0.81 ± 0.06 mm for *M. persicae* s.s. and *M. persicae nicotianae*, respectively; one-way ANOVA: $F_{1,60} = 55.216$, $P < 0.001$).

Discussion

Host finding

Most studies on the response of alate aphids to distant host plants have been based on olfactometer tests, few have employed wind tunnel experiments, and none to our knowledge have combined both techniques. Wind tunnel experiments combine visual and olfactory stimuli in host finding by alate individuals, and represent biological reality better than olfactometers when evaluating the process of host selection. On the other hand, olfactometers evaluate the importance of volatile semiochemicals in the host finding process, independently of visual stimuli.

Our results in the wind tunnel show clear differences in host finding behaviour between *M. persicae* s.s. and *M. persicae nicotianae*: while *M. persicae* s.s. was not able to find its host within the duration of the experiment, *M. persicae nicotianae* readily did so. Classical studies on flying aphids (see Kennedy, 1976, for review) suggest that aphids depend on visual orientation for landing, and that host selection relies primarily on arresting stimuli received after landing. Wind tunnel observations at 1 h intervals showed that the number of aphids on plants increased over time, at least in the case of the tobacco specialist alighting on tobacco (Figure 3). Furthermore, during the periods when aphids were counted (ca. 15 min every hour), aphids were never observed to be taking off from plants. These observations suggest that attraction took place, rather than random colonisation and selective take off. Interestingly, Del Campo et al. (2003) have suggested that the feeding behaviour of the pea aphid, *Acyrtosiphon pisum*, is determined by the recognition of host-specific chemicals rather than the avoidance of deterrents.

The olfactometric results show that *M. persicae* s.s. was unable to make a choice between volatiles emitted by sugar beet and control, while *M. persicae nicotianae* chose volatiles emitted by tobacco over the control, indicating the importance of olfactory stimuli in host finding (Figure 2). This preference of the specialist on tobacco for tobacco volatiles

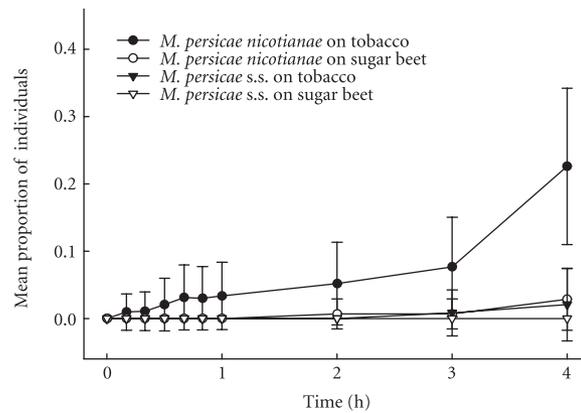


Figure 3 Mean proportion of individuals found on plants at hourly intervals in the wind tunnel experiment.

did not show up in the experiments offering a choice between volatiles emitted by both plants. This fact may be attributed to a masking effect of host volatiles by non-host volatiles (Prokopy, 1986; Nottingham et al., 1991; Hardie et al., 1994; Hori & Komatsu, 1997). Furthermore, although we made several attempts using different techniques to capture the headspace volatiles of both plant species, the GC-MS analyses did not provide clear results on the presence of volatiles; it was most likely that volatiles were collected at extremely low concentrations under the conditions employed.

The evidence presented from wind tunnel and olfactometers support the notion that alate virginoparae of *M. persicae* s.s. exhibit a diminished capability for host discrimination at the prealightment stage compared with *M. persicae nicotianae*, whereas in alate virginoparae of *M. persicae nicotianae*, host finding is an olfactory process complemented by a visual process, as shown by wind tunnel results where, despite a potential masking effect of the mixture of tobacco and sugar beet volatiles, *M. persicae nicotianae* successfully located its host plant.

It is interesting to assess how this conclusion fits into the general patterns reported for olfactometric and wind tunnel studies of aphid morphs with different host-plant ranges. Table 2 summarises the data available from the literature according to the host range of aphid species or morphs assigned by Blackman & Eastop (2000), and shows that while monophagous and oligophagous morphs consistently prefer volatiles from host plants or host plant parts, not all polyphagous morphs were sensitive to such olfactory stimuli in the olfactometer.

These results suggest an integration of visual and olfactory stimuli in host finding by the tobacco specialist, and point to an effect of host range on efficient host selection prior to landing.

Table 2 Response of different aphid morphs in the olfactometer (O) and in the wind tunnel (WT). Morphs used were alate virginoparae unless otherwise noted (^a = alate fundatrigeniae; ^b = gynoparae; ^c = males). Stimuli were whole plants unless otherwise noted (^d = leaf volatiles; ^e = leaves)

| Aphid species/morph | Method | Preference for host features | References |
|--|--------|------------------------------|---|
| Monophagous | | | |
| <i>Phorodon humuli</i> ^{a,d} | O | yes | Campbell et al. (1993) |
| <i>Neuquenaphis sensoriata</i> ^d | O | yes | Quiroz et al. (1999) |
| <i>Rhopalosiphum padi</i> ^{b,e} | O | yes | Glinwood & Pettersson (2000a) |
| <i>Rhopalosiphum padi</i> ^b | O | yes | Sandström & Pettersson (2000) |
| <i>Sitobion fragariae</i> ^b | O | yes | Lilley & Hardie (1996) |
| <i>Rhopalosiphum padi</i> ^c | O | yes | Sandström & Pettersson (2000) |
| <i>Uroleucon ambrosiae</i> (specialist race) | WT | yes | Bernays & Funk (1999) |
| Oligophagous | | | |
| <i>Rhopalosiphum padi</i> ^a | O | yes | Pettersson et al. (1994); Glinwood & Pettersson (2000b) |
| <i>Rhopalosiphum padi</i> | O | yes | Quiroz & Niemeyer (1998) |
| <i>Uroleucon gobonis</i> | O | yes | Hori (1999) |
| <i>Uroleucon adenophorae</i> | O | yes | Hori (1999) |
| <i>Uroleucon kikioense</i> | O | yes | Hori (1999) |
| <i>Rhopalosiphum maidis</i> | O | yes | Bernasconi et al. (1998) |
| <i>Brevicoryne brassicae</i> ^e | O | yes | Nottingham et al. (1991) |
| <i>Brevicoryne brassicae</i> | O | yes | Pettersson (1973); Pettersson & Stephansson (1991) |
| <i>Lipaphis erysimi</i> | O | yes | Pettersson & Stephansson (1991) |
| <i>Capitophorus formosartemisiae</i> | O | yes | Hori (1999) |
| <i>Uroleucon ambrosiae</i> (generalist race) | WT | yes | Bernays & Funk (1999) |
| <i>Myzus persicae nicotianae</i> | O,WT | yes | this work |
| Polyphagous | | | |
| <i>Macrosiphum euphorbiae</i> | O | no | Hori (1999) |
| <i>Aphis craccivora</i> | O | yes | Pettersson et al. (1998) |
| <i>Aphis craccivora</i> | O | no | Hori (1999) |
| <i>Aphis fabae</i> ^e | O | yes | Nottingham et al. (1991); Hardie et al. (1994) |
| <i>Aphis gossypii</i> | O | no | Storer & van Emden (1995); Hori (1999) |
| <i>Myzus persicae</i> s.s. | O, WT | no | this work |

Assessment of the leaf surface and subepidermal tissues

After alighting on a plant, aphids evaluate the physical and chemical traits of the leaf surface and subepidermal tissues by means of chemoreceptors at the antennae and tarsi (Anderson & Bromley, 1987) and mechanoreceptors in the labium (Tjallingii, 1978), as well as through gustatory receptors in the foregut (Miles, 1987) after stylet insertion. Both aphid taxa studied responded in a similar way with regard to the time they took to perform the first probe, which was longer on tobacco than on sugar beet. In addition, the completion of a long-duration probe was delayed on tobacco relative to sugar beet, and the number of probes and total time spent probing before a long-duration probe were increased on tobacco relative to sugar beet, for both taxa. However, *M. persicae nicotianae* on tobacco was clearly faster than *M. persicae* s.s. These observations indicate that both taxa may be affected during probing to a similar extent by the physical and

chemical surface characteristics of the plants they attempt to feed on. The leaves of sugar beet are glabrous and smooth, and there is no evidence of the epicuticular compounds which are deterrent to aphids (Williams et al., 1999); on the other hand, tobacco leaves possess trichomes which, through their sticky secretions, constitute a physical barrier to aphid movement and settlement – as shown by Tingey & Laubengayer (1981), Lapointe & Tingey (1984, 1986), and Neal et al. (1990) for the effect of trichomes of wild potato leaves on *M. persicae* – as well as a chemical barrier due to the toxicity of chemicals in them (Hagimori et al., 1993; Lin & Wagner, 1994).

The duration of the first probe and the proportion of total time spent probing showed that both the aphid taxa recognised and spent more time probing subepidermal tissues on the host than on the non-host, suggesting that both taxa recognise their host plants soon after their stylets have penetrated the plant leaf surface, probably during the first

probes and clearly before finding the phloem. Similar behavioural patterns were shown to occur in the pea aphid, *Acyrtosiphon pisum* Harris (Caillaud & Via, 2000), and in genetically identical individuals of the black bean aphid, *Aphis fabae* Scop., with different plant preferences (Powell & Hardie, 2000). Accordingly, the significantly different proportion of *M. persicae nicotianae* individuals compared with *M. persicae* s.s. that achieved a long-duration probe on tobacco is further evidence of the greater efficiency of the specialist in recognising its host plant. Although leaf excision for bioassays may affect physical or chemical traits and may in turn affect aphid behaviour, both aphids were exposed to the same conditions and there was no evidence of leaf damage, as most of the aphids performed a long-duration probe.

Host rejection

Host rejection by *M. persicae* s.s. and *M. persicae nicotianae* generally occurred after a brief probe of subepidermal tissues. In sugar beet, nearly 40% of individuals of both aphid taxa flew away after a similar searching period on the plant, and all remaining individuals achieved a long-duration probe. In contrast, a high proportion of individuals of both aphid taxa remained on tobacco (93% in both taxa), but among these, a highly significant proportion (36%) of *M. persicae* s.s. did not carry out a long-duration probe while all remaining individuals of *M. persicae nicotianae* achieved a long-duration probe. Therefore, host rejection was not only displayed through taking off but also through the absence of long-duration probes. The rejection of tobacco plants while staying in contact seems to be related to entrapment by exudates of trichomes on the leaf surface (Hagimori et al., 1993; Lin & Wagner, 1994). This entrapment on tobacco leads to slower aphid movements (lower frequency of foot-lifting) on tobacco than on sugar beet (which lacks physical barriers on its surface). Additionally, on tobacco, while no differences were observed between the two aphid taxa in relation to frequency of foot-lifting, the position of the tarsus after a footstep was more often on a trichome in the case of *M. persicae* s.s. than in the case of *M. persicae nicotianae* (Table 1). This capability of the studied *M. persicae nicotianae* individuals to avoid the sticky surface of trichomes is likely to be related to its longer feet (tibia and femur), as compared to *M. persicae* s.s. (Table 1). These differences may arise from the fact that the two taxa studied were reared on different hosts. It should be noted that Blackman (1987) and Margaritopoulos et al. (2000) did not find that the length of the hind femur was a discriminatory character between both taxa, although these authors only examined apterae. This trichome avoidance mechanism may be compared with that described for the aphid *Macrosiphum mentzeliae* Wilson on *Mentzelia pumila*

Torr. and Gray (Loasaceae), a plant which is densely covered with hooked trichomes: while these trichomes prove lethal to a wide variety of insects, the specialised aphid is able to tiptoe through the trichomes (Eisner et al., 1998); furthermore, in the aphid genus *Uroleucon*, Moran (1986) has described correlations between features of the host plant surface and aphid morphological traits.

The relatively high proportion of individuals of *M. persicae* s.s. that achieved a long-duration probe on tobacco (57%), may be a consequence of an opportunistic behaviour: since they cannot take off from the plant, then they will continue probing it, in spite of it not being a host. A similar behaviour was observed by Caillaud & Via (2000) when different genotypes of the pea aphid were confined to an alternate host for their entire life. This was particularly notable in the less specialised genotypes studied.

Behavioural differences between generalist and tobacco-specialist *M. persicae* were found at different stages of the host selection process, from prealighting behaviour, to host volatile recognition, and leaf surface and subepidermal exploration. It is apparent from the above discussion that the generalist shows a less efficient host recognition behaviour when compared with the specialist on tobacco, which performs a more efficient searching and acceptance behaviour. The host specialisation on tobacco points to behavioural and morphological adaptations to the physical and chemical defence mechanisms of tobacco (Blackman, 1987; Margaritopoulos et al., 2000).

This paper has discussed behavioural traits that may contribute to differential host selection process between *M. persicae* s.s. and *M. persicae nicotianae*, and which have made possible the exploitation by *M. persicae* s.s. of a new resource, the tobacco plant, resulting in a new specialised subspecies. The evolutionary mechanisms underlying the trade-off between the investment in these adaptations and the benefits of acquiring a new host, remain an open question. The data presented provide additional evidence for the neural constraint hypothesis (Bernays, 2001). Neurophysiological studies addressing possible changes in the perception of volatiles and of semiochemicals within plant tissues of the two aphid taxa studied would be desirable to define the mechanisms underlying the behavioural changes described.

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