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Changes in aphid probing behaviour as a function of insect age and plant resistance level

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Abstract

Aphids perform a series of behaviours to assess feeding suitability and, hence, to select a plant. Little information, however, is available on such behaviour after aphids have settled on a plant. Observation of probing behaviour over an extended period of time can improve our understanding of insect-plant interactions and is instrumental in the study of crop resistance. Here, we assessed the influence of aphid age and plant resistance level on aphid behaviour. An electrical penetration graph (EPG) technique was implemented to monitor the behaviour of potato aphid, *Macrosiphum euphorbiae*, alates on potato, *Solanum tuberosum*, and on both a susceptible and a resistant genotype of a wild *Solanum* species, *S. chomatophilum*. The behaviour was measured at daily intervals for the first seven days following adult emergence. The results indicated independent and interacting effects of aphid age and plant genotype on probing behaviour. Some behavioural discrepancies between susceptible and resistant genotypes were only observed after the first day, thus highlighting the limits of punctual one-day behavioural studies to assess plant resistance mechanisms. Our work supports the hypothesis that aphids continuously adapt their behaviour to the plant characteristics.

Keywords: probing behaviour, EPG, multivariate analysis, *Solanum chomatophilum*, crop resistance mechanism, adaptation

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Introduction

Probing behaviour depends on the combined effect of informational and physiological variations (Mangel, 1993). For aphids, probing behaviour is influenced by the host plant (Powell *et al.*, 2006) but also by previous plant feeding experiences and starvation (Ramírez & Niemeyer, 2000).

Aphids exhibit several probing behaviours which have been studied in the context of host-plant selection (Powell *et al.*, 2006). These behaviours generally occur in the following order: (i) pre-alighting behaviour, which appears to have little effect on host-plant selection since aphids have little control over the direction of their flight (Dixon, 1998); (ii) initial plant contact and assessment of surface cues before probing (insertion of stylets); (iii) probing the epidermis; (iv) stylet pathway activity in the mesophyll; (v) sieve element puncture and phloem salivation; and, lastly, (vi) phloem acceptance and sustained ingestion. Xylem sap consumption is also occasionally observed (Spiller *et al.*, 1990; Ramírez & Niemeyer, 2000) and is related to osmoregulation (Pompon *et al.*, 2010b, 2011b). The selection of the plant is considered to be completed when a phloem sap feeding period longer than ten minutes has been observed (Prado & Tjallingii, 1997).

Plant resistance mechanism can be inferred from aphid behaviour (Alvarez *et al.*, 2006; Le Roux *et al.*, 2008; Pompon

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et al., 2010a). For instance, a reduction in the duration of phloem sap ingestion is caused by toxic phloem sap (Givovich & Niemeyer, 1995). Studies assessing aphid behaviour on resistant crop, however, usually evaluate the behaviour of young individuals, most of the time naive to the tested plant, and for a punctual short period of time.

Electrical penetration graph (EPG) is commonly used to study the probing behaviour of aphids (Tjallingii, 1995). Numerous variables related to particular behavioural activities are calculated from EPG and can most efficiently be analysed through multivariate analysis approaches (Pompon *et al*., 2010a). The EPG experimental set up can interfere with behaviour (Prado & Tjallingii, 1999; Pelletier & Giguere, 2009), is a destructive method (Tjallingii, 1995) and affects life history parameters (Tjallingii, 1986). For these reasons, it cannot be conducted for long periods without the risk of significantly affecting the results obtained (Tjallingii, 1986).

The aim of the present study was to document the probing behaviour of aphids over an extended period of time with the insects in continuous contact with hosts of different suitability. We assessed the influence of plant genotype (with different resistance levels) and aphid age on probing behaviour of a major aphid potato pest, the potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (Radcliffe, 1982). Behaviour was monitored every day for seven days on potato, *Solanum tuberosum* L., and on one susceptible and one resistant genotype of a wild *Solanum* species, *S. chomatophilum* (Pompon *et al*., 2011a).

Material and methods

Plants and insects

Two genotypes of *S. chomatophilum* (Bitter) accession PI243340, previously identified as resistant (chmR, previously named RES6) and susceptible (chmS, previously named RES10) to *M. euphorbiae*, were propagated by cuttings (Pompon *et al*., 2011a). Potato plants (*Solanum tuberosum* L.) var. Shepody (tbr) were grown from Elite II seed tubers (McCain Produce Inc., Florenceville, NB, Canada). Both plant species were used when they reached 5–7 weeks-old. The *M. euphorbiae* colony was initiated from one asexual individual collected in a potato field in Fredericton, NB, Canada (45°55′ 32.92″N; 66°36′22.23″W) during the summer of 2000. Ever since, aphids have been reared on potted tbr plants in wood frame cages (1 m high, 50 cm deep and wide, all sides and ceiling screened), which allow alate individuals to take off. Alate aphid production was induced by crowding (Muller *et al*., 2001). Alates were age-standardized by collecting them from the ceiling of the rearing cage (described above) 14 h after removing all alate aphids that were flying or walking on the walls and ceiling of the same cage. As alate aphids engage in flight less than 24 h after adult moulting and once settled on a suitable plant do not take off (Robert, 1988), collected aphids were assumed to have moulted less than 24 h before collecting them. The collection day is referred to as day 0. Collected aphids were either used the same day (day 0) for experiments, or caged (cage: 15 cm diameter, 30 cm long piece of Plexiglas™ tubing closed at one end with fine white screening) on the same plant genotype as used for later experiments (day 1–6) in groups of 30 to 50 individuals until they were assessed. Plant production and aphid rearing were performed in growth chambers set to 16:8 h (light:dark), 24:20°C (day:night) and 50% relative humidity. Manipulations of aphids were realized with a soft-bristled paint brush. All behavioural experiments started around 9:00 am.

Fecundity assessment

Aphid fecundity was assessed in order to confirm the resistance status of the plant genotypes. Four plants of each plant genotype (chmR, chmS and tbr) were used. On each plant, five clip-cages (MacGillivray & Anderson, 1957), each containing one newly moulted (day 0) adult alate aphid, were attached to five different young leaves (2nd or 3rd fully expanded leaf from the apex) (20 replicates per aphid age × plant genotype combination). Fecundity was recorded every day from the same aphids until day 6. All plants were studied at the same time, and their positions were randomized within the same growth chamber where aphids were reared.

Probing behaviour

EPG analysis was used to monitor probing behaviour of alate adult aphids every day from day 0 to day 6 on the three plant genotypes. Prior to the experiment, young aphids were reared on the same plant genotype on which they were assessed. Plants and aphids were used once. Using water-based silver conductive paint, a fine gold wire (2–3 cm long and 12 µm in diameter) was glued on the dorsum of each aphid while immobilized with a vacuum device. Inside a Faraday cage, one tethered aphid was rapidly (< 30 min after collecting it from the rearing plant) placed on a young leaf of one potted plant, which had a copper electrode inserted in the soil. Wires and electrodes were connected to a 10^9 Ω input resistance Giga 8™ amplifier (EPG-systems, Wageningen, The Netherlands). Probing behaviour was recorded for 5 h using a USB data acquisition board (DT9806, Data Translation, Marlboro, MA, USA) and Scope software, version 2.2.0.30 (Data Translation). A previous study showed that aphids do not take off from the same plant species studied here within 5 h after placing them on plants (Pompon *et al*., 2010a). Twelve to 22 continuous records per aphid age × plant genotype combination were conducted in laboratory conditions under constant light and at 20°C. EPG records were interpreted with respect to the waveforms identified by Tjallingii (1995) and were used to calculate 50 different variables (table 1).

Statistical analysis

One-way repeated measures ANOVA was carried out to determine the effect of plant genotype on aphid fecundity. Normal distribution of data was verified using the Kolmogorov-Smirnov's test.

For the few EPG variables that could not be calculated because the behaviour associated to it did not occur, we took the following conservative approach to modify their values: for variables describing time to a behaviour (t_1Pr, t_1G, t_1E1rec, t_1E1, t_1E2rec, t_1E2, t_1Esrec, and t_1Es; see table 1), we ascribed the maximum possible value (5 h); for variables describing the average duration of a behaviour (a_Pr, a_C, a_G, G prop, a_E1, a_E1-E2, a_E2, a_Es, and a_F; see table 1), we ascribed the value of zero; for variables counting the number of bouts of a behaviour before the first occurrence of another behaviour (n_Pr > E1, n_Pr > E2, n_Pr > Es, n_E1 > E2 and n_E2 > Es; see table 1) that was not observed, we ascribed the total number of bouts of the behaviour that was counted; and for variables measuring the duration of a behaviour before

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Table 1. Description and abbreviation of EPG behavioural variables, categorized with respect to five probing activities.

Activity	Behavioural variable	Abbreviation
Pathway activity in epidermis / mesophyll	Time from release on the leaf to first probe	t>1Pr
	Number of penetrations	n_Pr
	Total duration of probes[1]	s_Pr
	Average duration of a probe	a_Pr
	Number of probes before the first phloem salivation	n_Pr>E1
	Number of penetrations before the first phloem consumption	n_Pr>E2
	Number of penetrations before the first sustained phloem consumption[1]	n_Pr>Es
	Number of pathway activity bouts[1]	n_C
	Total duration of pathway activity[1]	s_C
	Average duration of a pathway activity bouts	a_C
	Number of brief (<3 min) probes[1]	n_bPr
	Total duration of brief probes[1]	s_bPr
	Number of cell punctures	n_pd
	Average duration between two cell punctures[1]	a_pd
Xylem sap consumption	Number of xylem consumption bouts[1]	n_G
	Total duration of xylem consumption[1]	s_G
	Average duration of a xylem consumption bout[1]	a_G
	Time from aphid release on the leaf to the first xylem consumption[1]	t>1Grec
	Time first probe to the first xylem consumption[1]	t>1G
	Xylem consumption proportion over total sap intake [dG/(dG+dE2)] [1]	G prop
Phloem salivation	Number of phloem salivation bouts[1]	n_E1
	Total duration of phloem salivation	s_E1
	Average duration of phloem salivation bouts	a_E1
	Number of phloem salivation bouts before the first phloem consumption[1]	n_E1>E2
	Total duration of phloem salivation before the first phloem consumption	s_E1>E2
	Average duration of phloem salivation before the first phloem consumption[1]	a_E1>E2
	Number of phloem salivation bouts without subsequent phloem consumption[1]	n_E1–E2
	Total duration of phloem salivation without subsequent phloem consumption	s_E1–E2
	Average duration of phloem salivation bouts without subsequent phloem consumption	a_E1–E2
	Number of phloem salivation bouts before the first xylem consumption[1]	n_E1>G
	Total duration of phloem salivation before the first xylem consumption	s_E1>G
	Duration of phloem salivation before the first xylem consumption[1]	a_E1>G
	Time from aphid release on the leaf to the first phloem salivation	t_1E1rec
	Time from penetration to the first phloem salivation[1]	t_1E1
Phloem sap consumption	Number of phloem consumption bouts	n_E2
	Total duration of phloem consumption	s_E2
	Average duration of a phloem consumption bout	a_E2
	Number of sustained (>10 min) phloem consumption bouts[1]	n_Es
	Total duration of sustained phloem consumption	s_Es
	Average duration of a sustained phloem consumption bout	a_Es
	Number of phloem consumption bouts before first sustained phloem consumption[1]	n_E2>Es
	Duration of phloem consumption before first sustained phloem consumption	a_E2>Es
	Time from aphid release on the leaf to the first phloem consumption	t_1E2rec
	Time from first probe to the first phloem consumption	t_1E2
	Time from aphid release on the leaf to the first sustained phloem consumption[1]	t_1Esrec
	Time from first probe to the first sustained phloem consumption	t_1Es
	Phloem consumption index [100×dE2/(total EPG duration–d-0:E2)]	Ei
Stylet mechanical derailements	Number of stylet derailments	n_F
	Total duration of stylet derailments[1]	s_F
	Average duration of a stylet derailment	a_F

[1] variable selected by the stepwise discriminant analysis.

the occurrence of another behaviour (a_E1>E2, and a_E2>Es; see table 1) that was not observed, we ascribed the total duration of the behaviour that was timed. Percentage of aphids that did not exhibit one of the behaviours is detailed in table 2, but cannot be used in the analysis as there is no variance associated with each value. Such a modification of variables was applied to limit any bias caused by the elimination of samples that did not exhibit one of the behaviours, as multivariate analysis deals with missing values by eliminating the entire sample.

The influence of plant genotype and aphid age on the EPG variables was determined by applying multivariate analyses (Pompon *et al.*, 2010a). EPG variables were then submitted to backward stepwise discriminant analysis (classic) at a tolerance of 0.001, and F-to-enter and F-to-remove equal to 0.02, to select a subset of variables relevant to group each

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Table 2. Percentage of aphids that did not exhibit one of the behaviour recorded on one of the plant genotypes.

Behaviour	EPG waveform	Plant genotype		
		S. tuberosum	*S. chomatophilum* susceptible	*S. chomatophilum* resistant
Epidermis/mesophyll activity	C	0	0	0
Cell puncture	pd	0	0	0
Xylem ingestion	G	68	39	25
Phloem salivation	E1	5	10	11
Phloem ingestion	E2	10	27	83
Sustained phloem ingestion	Es	15	44	95
Stylet mechanical derailments	f	94	68	75

combination of plant genotype × aphid age (Sokal & Rohlf, 1995). Factorial analyses based on a correlation matrix with varimax rotation option (orthogonal rotation minimizing the number of variables with high loadings), and a minimum eigenvalue of 1 (factors with eigenvalues lower than 1 were not retained) were applied to the EPG variables selected through the discriminant analysis. The biological interpretation of each factor was derived using the variables contributing the most to the factors. Factors are orthogonal to each other, allowing the use of factor scores as explanatory variables in general linear models and eliminating the problem of co-linearity (Quinn & Keough, 2002) inherent to behavioural variables. To assess the effect of plant genotype and aphid age on the behavioural factors, two-way ANOVA was applied to the factor scores. Statistical analyses were performed with Systat 12.0 (Systat Software, San José, CA, USA).

Results

Plant resistance level measured by fecundity

Fecundity was influenced by plant genotype (between subjects: df = 2, 57; F = 12.20; $P < 0.001$), aphid age (within subjects: df = 6, 342; F = 40.95; $P < 0.001$), and the interaction between plant genotype and aphid age (within subjects: df = 12, 342; F = 4.48; $P < 0.001$). Fecundity was similar on tbr and chmS, and was the lowest on chmR, although these patterns were most apparent on days 3–6 (fig. 1).

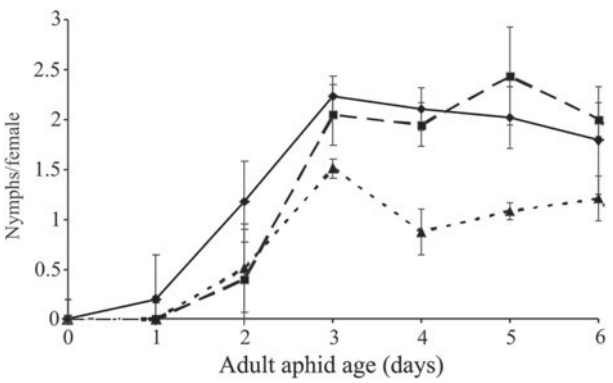

Fig. 1. Mean (±SEM) fecundity of adult alate *Macrosiphum euphorbiae* maintained on *S. tuberosum* (tbr) and two *S. chomatophilum* genotypes (chmS and chmR). Fecundity was measured from day of adult emergence (day 0) until aphids were six days old (day 6) (—◆—, tbr; – -■–, chmS; - -▲ -, chmR).

Probing behaviour

Of the 50 EPG variables calculated (table 1), 24 were retained by discriminant analysis (table 3). Factorial analysis using the 24 variables produced six factors explaining 77% of the total variance (table 3). According to factor loadings, factor 1 was related to 'xylem sap ingestion', factor 2 to 'brief probe', factor 3 to 'phloem salivation bout', factor 4 to 'pathway activity', factor 5 to 'probing activity' and factor 6 was related to 'phloem salivation duration preceding sap ingestion'.

'Xylem sap ingestion' factor (F1) scores were affected by aphid age, plant genotype, and their interaction (table 4). It was lower for tbr than for the other plants, decreased with aphid age for tbr and chmS, and increased for chmR after four days (fig. 2). 'Brief probe' factor (F2) was affected by aphid age, plant genotype and their interaction. It was generally the lowest for tbr and the highest for chmR, was constant on tbr and increased for one and two days for chmS and chmR, respectively, before decreasing. 'Phloem salivation bout' factor (F3) was independent of the effect tested. 'Pathway activity' factor (F4) was influenced by aphid age and the interaction between aphid age and plant genotype. It was very variable for tbr and followed a similar variable pattern for both chmS and chmR. 'Probing activity' factor (F5) was influenced by aphid age and the interaction between aphid age and plant genotype. It was generally the lowest for chmR, on which it particularly varied as a function of age. 'Phloem salivation duration preceding sap ingestion' factor (F6) was only influenced by aphid age. Overall, scores of every factor were similar for chmS and chmR on day 0.

Discussion

Our study demonstrates the combined and independent effects of aphid age and plant resistance level on aphid behaviour. The fecundity results confirmed the resistant and susceptible status of chmR and chmS, respectively (Pompon *et al.*, 2011a). Using EPG and a multivariate statistical approach, we identified six behavioural factors on three distinct plant genotypes with different resistance levels.

During brief probes (<3 min), aphid stylets can only reach the epidermal layers (Powell *et al.*, 2006), from which they gain sufficient information to reject and leave the plant (Caillaud, 1999; Powell & Hardie, 2000). This decision can be based on primary and secondary metabolites acting as token signs (Bernays, 2001), nutritional cues and/or chemical signals

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Table 3. Factors defined through factorial analysis of behavioural variables measured daily for alate *Macrosiphum euphorbiae* on different plant genotypes for the first six days following the adult moult. The biological meaning of each factor was estimated by the loading value of the variables contributing the most to the factors. Loading values contributing the most to each factor are indicated in bold.

Behavioural variables	Factors[1]					
	1	2	3	4	5	6
s_Pr	0.041	−0.055	0.009	0.077	**0.350**	0.046
n_Pr>Es	−0.012	**0.220**	−0.049	0.057	0.045	−0.001
n_C	−0.007	**0.231**	0.021	0.022	0.089	−0.065
s_C	−0.043	0.085	0.131	**0.458**	0.053	−0.088
n_C<3	−0.008	**0.229**	−0.044	−0.070	0.094	−0.013
s_C<3	−0.013	**0.213**	−0.014	−0.001	0.064	−0.026
s_pd	0.069	0.073	−0.001	**−0.417**	0.079	0.030
n_G	**0.179**	−0.030	0.060	−0.045	0.056	−0.068
s_G	**0.188**	−0.047	−0.005	−0.066	0.117	−0.007
s_G	**0.185**	−0.038	−0.004	−0.093	0.112	0.020
t>1Grec	0.108	0.028	−0.023	−0.066	0.062	0.045
t>1G	**−0.160**	−0.006	−0.074	0.060	0.106	0.082
G prop	**0.185**	0.001	0.046	−0.027	0.047	−0.011
n_E1	0.002	−0.023	**0.292**	−0.024	0.018	−0.115
n_E1>E2	0.032	−0.031	**0.319**	0.005	−0.155	0.015
a_E1>E2	0.043	−0.056	−0.034	−0.122	0.026	**0.529**
n_E1-E2	0.038	−0.001	**0.297**	0.050	−0.055	−0.004
n_E1>G	−0.096	0.017	0.113	−0.005	0.058	−0.025
a_E1>G	−0.054	−0.009	−0.102	−0.080	0.073	**0.534**
t_1E1	−0.017	−0.003	−0.097	**0.349**	0.108	−0.128
n_Es	−0.042	−0.020	−0.007	−0.138	0.222	−0.102
n_E2>Es	−0.033	−0.068	0.187	−0.093	−0.078	−0.157
t_1Esrec	0.047	0.046	0.063	0.193	−0.219	0.031
s_F	−0.052	−0.117	0.115	−0.021	**−0.536**	−0.072
Total variance explained by each factor (%)	23	18	13	8	8	7

[1] Factor 1 was related to 'xylem sap ingestion', factor 2 to 'brief probe', factor 3 to 'phloem salivation bout', factor 4 to 'pathway activity', factor 5 to 'probing activity' and factor 6 to 'phloem salivation duration preceding sap ingestion'.

Table 4. Effect of alate aphid age and plant genotype on probing behaviour factor scores. Factors are described in table 3.

Sources	df	F1: Xylem sap consumption		F2: Brief probe		F3: Phloem salivation bout		F4: Pathway activity		F5: Probing activity		F6: Phloem salivation preceding sap consumption	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Aphid age	6	53.27	<0.001	43.50	<0.001	1.01	0.366	5.33	0.005	13.02	0.001	9.37	<0.001
Plant genotype	2	17.89	<0.001	7.58	<0.001	0.71	0.643	1.98	0.068	1.64	0.136	0.85	0.531
Aphid age x plant genotype	12	4.31	<0.001	2.38	0.006	1.24	0.255	3.00	0.001	2.65	0.002	1.30	0.220
Error	297												

involved in plant defence (Li *et al.*, 2002), although these cues are different in epidermis from the ones in phloem (Tosh *et al.*, 2003). Phloem sap nitrogen quality has no impact at this stage (Nowak & Komor, 2010), as aphids have not reached phloem bundles. Brief probe factor (F2) scores were low and constant for *S. tuberosum* during the six days following the adult moult, likely because the brief probe stage quickly leads to the next behavioural stage of host-plant selection (pathway activity) on suitable plants (Tosh *et al.*, 2003). Conversely, the increase of the brief probe factor scores for chmR and chmS after day 0 suggests that previous experience on these plants influenced the probing of superficial cues. Aphids may have associated superficial cues with the toxicity of deeper tissues, such as phloem sap, which is ingested during day 0 on *S. chomatophilum* (Pompon *et al.*, 2010a). Probing behaviour can be modified through associative learning in insects (Mangel, 1993) and was suspected for the blackberry-grain aphid, *Sitobion fragariae* (Walker), which modifies its probing behaviour to avoid hydroxamic acids after previous exposure to plants containing high levels of hydroxamic acid (Ramírez *et al.*, 1999).

Pathway activity towards phloem bundles consists of inserting stylets into intercellular spaces in epidermal and mesophyll tissues. This stage usually includes the puncture of cells, which provides gustatory information (Powell *et al.*, 2006). Cell puncture frequency can be influenced by symplastic compounds (Chen *et al.*, 1997) and is suspected to trigger parturition (Powell *et al.*, 2004). Early reproductive decisions, based on peripheral cues, provide a fitness advantage (Powell *et al.*, 2006). The variation of the pathway activity with cell puncture factor score (F4) is difficult to interpret on *S. tuberosum*, whereas the factor score increase on chmS and chmR may be interpreted as changes in the sensitivity to cues

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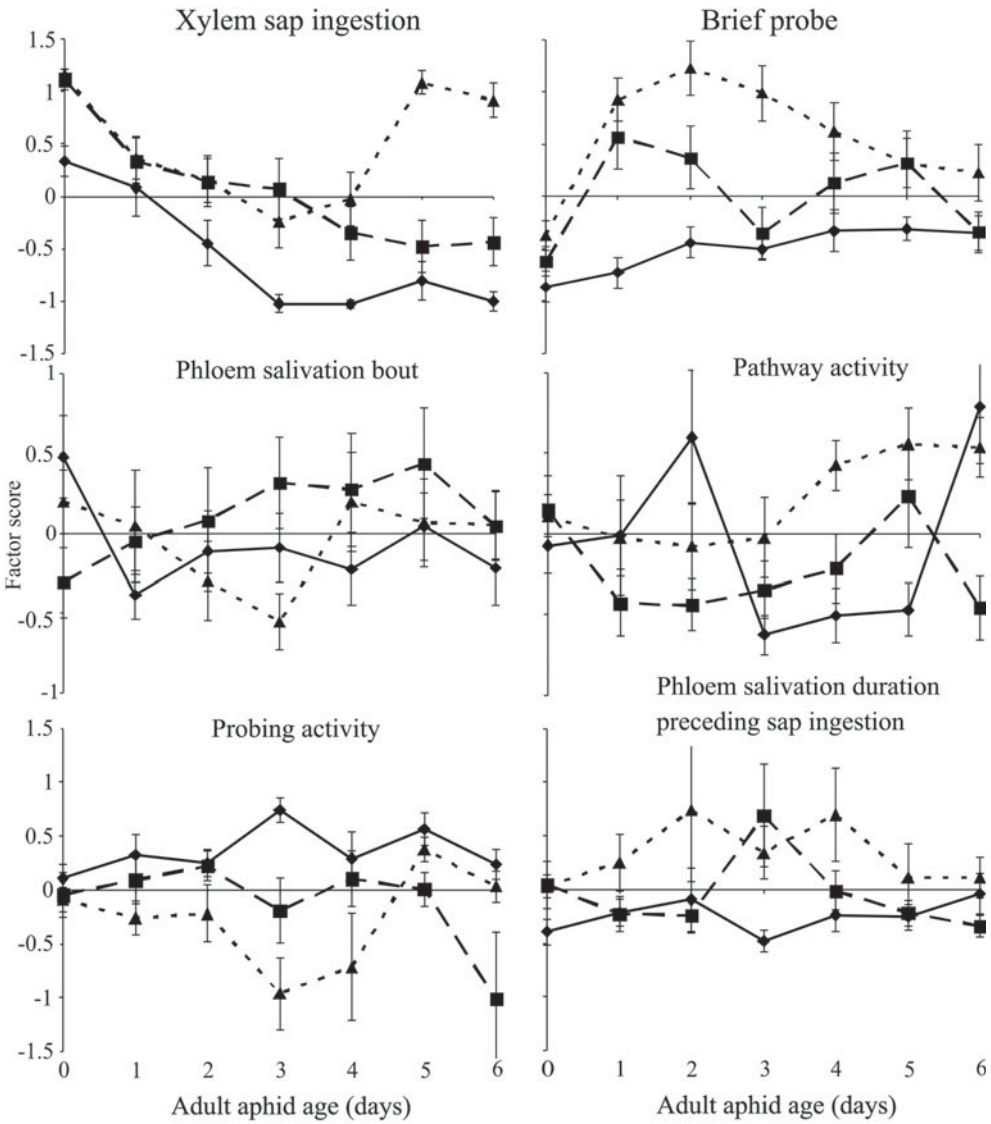

Fig. 2. Mean (±SEM) scores at different ages after adult moult of six factors (detailed in table 3) describing the probing behaviour of alate *Macrosiphum euphorbiae* maintained on *S. tuberosum* (tbr) and two *Solanum chomatophilum* genotypes (chmS and chmR) (—, tbr; -■-, chmS; - - ▲- -, chmR).

of deeper plant tissues, similar to those observed for brief probes. Alternatively, the factor could be related to the ability to penetrate the mesophyll tissue (Mutti *et al*., 2008).

Derailed stylet mechanics is visualized as the waveform F (Tjallingii, 1988). Little is known about the reasons for such activities, except that it is related to the gelling saliva (Tjallingii, 1988), depends on plant age and virus infection (Alvarez *et al*., 2007) and can last from several minutes to hours (data not shown; Tjallingii, 1987). The gelling saliva is continuously excreted during the intercellular stylet penetration of the mesophyll (pathway activity) and forms a salivary sheath enveloping the stylets (Tjallingii, 2006), potentially protecting stylets from plant contacts. Stylet derailment is suspected to reflect adverse conditions (Caillaud *et al*., 1995), as exemplified by its increase on *S. stoloniferum* resistant plants for the peach-potato aphid, *Myzus persicae* (Sulzer) (Alvarez *et al*., 2006). We found that probing activity factor (F5), mainly made of the stylet derailment variable, was more prominent on the resistant genotype but varied with aphid age.

Phloem salivation is the first behaviour performed after reaching phloem bundles and always occurs before phloem ingestion. It may provide information about phloem sap quality (Tjallingii, 2006), but especially it is supposed to prevent phloem protein clogging, which is part of the plant wound response (Will *et al*., 2007). Phloem salivation duration increases when aphids face nutritionally unbalanced (Ponder *et al*., 2000) or toxic phloem sap (Ramírez & Niemeyer, 1999), while the saliva composition may adjust to (Tjallingii, 2006) and modify phloem sap quality (Girousse *et al*., 2005; Nowak & Komor, 2010). The number of phloem salivation bouts (F3) is related to the difficulty to detect suitable phloem vessels,

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whereas phloem salivation duration (F6) indicates either difficulties in preventing plant sap clogging or a reaction to phloem sap quality (Tjallingii, 2006). Our results showed that aphids did not experience difficulty in finding phloem bundles on the genotype tested, but that their general reaction to phloem sap quality (illustrated by F6) varied with adult age and did not follow a regular pattern.

Xylem sap ingestion is a general response to osmotic stress (Pompon *et al*., 2011b). The xylem sap ingestion factor (F1) was the most clearly divergent between the plant genotypes, and strikingly increased in old aphids on the resistant genotype (chmR), while it steadily decreased with age on chmS as on *S. tuberosum*. Xylem sap ingestion might have succeeded in limiting the negative impact of the resistance mechanism on chmS. We speculate that toxins in phloem sap, which have been suggested to trigger xylem sap ingestion (Givovich & Niemeyer, 1995), could have been diluted below their deleterious concentration in chmS by ingesting xylem sap. For chmR, higher toxin concentrations may have restricted the beneficial impact of xylem sap ingestion, resulting in shorter survival (Pompon *et al*., 2011a) and lower fecundity. Further analyses of phloem sap constituents are required to test that hypothesis.

Our study showed that behaviour differences between resistant and susceptible genotypes can develop days after host-plant selection. This questions conclusions on resistance mechanisms drawn from punctual behavioural studies. Previous studies that assessed behaviour during the first hours an aphid encountered a plant revealed that xylem sap ingestion increased on resistant *S. chomatophilum* accessions (Le Roux *et al*., 2008; Pelletier *et al*., 2010; Pompon *et al*., 2010a). In our study, we only observed a difference in the duration of xylem sap ingestion between a susceptible and a resistance genotype of *S. chomatophilum* after six days, confirming that *S. chomatophilum* resistance influences xylem sap ingestion. Performance evaluation is conducted over several days and is better accounted for by behaviour observation over the same time-period. Our results support the hypothesis that aphids modify their behaviour to react and/or adapt to plant characteristics after host selection, pointing to complex dynamic interactions between insects and plants.

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References

Alvarez, A.E., Tjallingii, W.F., Garzo, E., Vleeshouwers, V., Dicke, M. & Vosman, B. (2006) Location of resistance factors in the leaves of potato and wild tuber-bearing *Solanum* species to the aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata* **121**, 145–157.

Alvarez, A.E., Garzo, E., Verbeek, M., Vosman, B., Dicke, M. & Tjallingii, W.F. (2007) Infection of potato plants with potato leafroll virus changes attraction and feeding behaviour of *Myzus persicae*. *Entomologia Experimentalis et Applicata* **125**, 135–144.

Bernays, E.A. (2001) Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology* **46**, 703–727.

Caillaud, M. (1999) Behavioural correlates of genetic divergence due to host specialization in the pea aphid, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* **91**, 227–232.

Caillaud, C.M., Pierre, J.S., Chaubet, B. & Di Pietro, J.P. (1995) Analysis of wheat resistance to the cereal aphid *Sitobion avenae* using electrical penetration graphs and flow charts combined with correspondence analysis. *Entomologia Experimentalis et Applicata* **75**, 9–18.

Chen, J.Q., Rahbé, Y., Delobel, B., Sauvion, N., Guillaud, J. & Febvay, G. (1997) Melon resistance to the aphid *Aphis gossypii*: behavioural analysis and chemical correlations with nitrogenous compounds. *Entomologia Experimentalis et Applicata* **85**, 33–44.

Dixon, A.F.G. (1998) *Aphid Ecology*. London, UK, Chapman & Hall.

Girousse, C., Moulia, B., Silk, W. & Bonnemain, J.L. (2005) Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. *Plant Physiology* **137**, 1474–1484.

Givovich, A. & Niemeyer, H.M. (1995) Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomologia Experimentalis et Applicata* **74**, 115–119.

Le Roux, V., Dugravot, S., Campan, E., Dubois, F., Vincent, C. & Giordanengo, P. (2008) Wild *Solanum* resistance to aphids: Antixenosis or antibiosis? *Journal of Economic Entomology* **101**, 584–591.

Li, X., Schuler, M.A. & Berenbaum, M.R. (2002) Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature* **419**, 712–715.

MacGillivray, M.E. & Anderson, G.B. (1957) Three useful insect cages. *Canadian Entomologist* **89**, 43–46.

Mangel, M. (1993) Motivation, learning and motivated learning. pp. 158–173 *in* Papaj, D.R. & Lewis, A.C. (*Eds*) *Insect Learning: Ecological and Evolutionary Perspectives*. New York, USA, Chapman and Hall.

Muller, C.B., Williams, I.S. & Hardie, J. (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology* **26**, 330–340.

Mutti, N.S., Louis, J., Pappan, L.K., Pappan, K., Begum, K., Chen, M.S., Park, Y., Dittmer, N., Marshall, J., Reese, J.C. & Reeck, G.R. (2008) A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 9965–9969.

Nowak, H. & Komor, E. (2010) How aphids decide what is good for them: experiments to test aphid feeding behaviour on *Tanacetum vulgare* (L.) using different nitrogen regimes. *Oecologia* **163**, 973–984.

Pelletier, Y. & Giguere, M.A. (2009) Effect of manipulations on the host selection behavior of *Sitobion avenae* (Homoptera: Aphididae). *Journal of Insect Behavior* **22**, 165–171.

Pelletier, Y., Pompon, J., Dexter, P. & Quiring, D. (2010) Biological performance of *Myzus persicae* and *Macrosiphum euphorbiae* (Homoptera: Aphididae) on seven wild *Solanum* species. *Annals of Applied Biology* **156**, 329–336.

Pompon, J., Quiring, D., Giordanengo, P. & Pelletier, Y. (2010a) Role of host plant selection in resistance of wild *Solanum* species to *Macrosiphum euphorbiae* (Thomas) and *Myzus*

persicae (Sulzer) (Hemiptera: Aphididae). *Entomologia Experimentalis et Applicata* **137**, 73–85.

Pompon, J., Quiring, D., Giordanengo, P. & Pelletier, Y. (2010b) Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). *Journal of Insect Physiology* **56**, 610–615.

Pompon, J., Li, X.Q. & Pelletier, Y. (2011a) Resistance level to an aphid potato pest varies between genotypes from the same *Solanum* accession. *Journal of Economic Entomology* **104**, 1075–1079.

Pompon, J., Quiring, D., Goyer, C., Giordanengo, P. & Pelletier, Y. (2011b) A phloem-sap feeder mixes phloem and xylem sap to regulate osmotic potential. *Journal of Insect Physiology* **57**, 1317–1322

Ponder, K.L., Pritchard, J., Harrington, R. & Bale, J.S. (2000) Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid *Rhopalosiphum padi* on nitrogen deficient barley (*Hordeum vulgare*) seedlings. *Entomologia Experimentalis et Applicata* **97**, 203–210.

Powell, G. & Hardie, J. (2000) Host-selection behaviour by genetically identical aphids with different plant preferences. *Physiological Entomology* **25**, 54–62.

Powell, G., Tosh, C. & Hardie, J. (2004) Parturition by colonizing aphids: no correlation with phloem ingestion. pp. 485–489 *in* Simon, J.C., Dedryer, C.A., Rispe, C. & Hull, M. (*Eds*) *Aphid in a New Millenium*. Paris, France, INRA.

Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology* **51**, 309–330.

Prado, E. & Tjallingii, W.F. (1997) Effects of previous plant infestation on sieve element acceptance by two aphids. *Entomologia Experimentalis et Applicata* **82**, 189–200.

Prado, E. & Tjallingii, W.F. (1999) Effects of experimental stress factors on probing behaviour by aphids. *Entomologia Experimentalis et Applicata* **90**, 289–300.

Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge, UK, Cambridge University Press.

Radcliffe, E.B. (1982) Insect pests of potato. *Annual Review of Entomology* **27**, 173–204.

Ramírez, C.C. & Niemeyer, H.M. (1999) Salivation into sieve elements in relation to plant chemistry: The case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*. *Entomologia Experimentalis et Applicata* **91**, 111–114.

Ramírez, C.C. & Niemeyer, H.M. (2000) The influence of previous experience and starvation on aphid feeding behavior. *Journal of Insect Behavior* **13**, 699–709.

Ramírez, C.C., Caballero, P.P. & Niemeyer, H.M. (1999) Effect of previous exposure to hydroxamic acids in probing behavior of aphid *Sitobion fragariae* on wheat seedlings. *Journal of Chemical Ecology* **25**, 771–779.

Robert, Y. (1988) Dispersion and migration. pp. 299–313 *in* Minks, A.K. & Harrewijn, P. (*Eds*) *Aphids: Their Biology, Natural Enemies and Control*. Amsterdam, The Netherlands, Elsevier Science.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. 3rd edn. New York, USA, Freeman.

Spiller, N.J., Koenders, L. & Tjallingii, W.F. (1990) Xylem ingestion by aphids: A strategy for maintaining water balance. *Entomologia Experimentalis et Applicata* **55**, 101–104.

Tjallingii, W.F. (1986) Wire effects on aphids during electrical recording of the stylet penetration. *Entomologia Experimentalis et Applicata* **40**, 89–98.

Tjallingii, W.F. (1987) Stylet penetration activities by aphids: new correlations with electrical penetration graphs. pp. 301–306 *in* Labeyrie, V., Fabres, G. & Lachaise, D. (*Eds*) *Proceedings of the 6th international Symposium on Insect-Plant Relationship*. Pau, France, Dr W. Junk.

Tjallingii, W.F. (1988) Electrical recording of stylet penetration activities. pp. 95–108 *in* Minks, A.K. & Harrewijn, P. (*Eds*) *Aphids: Their Biology, Natural Enemies and Control*. Amsterdam, The Netherlands, Elsevier Science.

Tjallingii, W.F. (1995) Aphid-plant interactions: what goes on in the depth of the tissues? *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society (NEV)* **6**, 189–200.

Tjallingii, W.F. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany* **57**, 739–745.

Tosh, C.R., Powell, G., Holmes, N.D. & Hardie, J. (2003) Reproductive response of generalist and specialist aphid morphs with the same genotype to plant secondary compounds and amino acids. *Journal of Insect Physiology* **49**, 1173–1182.

Will, T., Tjallingii, W.F., Thonnessen, A. & van-Bel, A.-J.E. (2007) Molecular sabotage of plant defence by aphid saliva. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 10536–10541.