ORIGINAL ARTICLE

Probing behavior of aposymbiotic green peach aphid (*Myzus persicae*) on susceptible *Solanum tuberosum* and resistant *Solanum stoloniferum* plants

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Abstract The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the potato important pests; it is the most efficient vector of potato viruses. *Myzus persicae* harbors the endosymbiotic bacteria *Buchnera aphidicola* which supplements their diet. There is increasing evidence that *B. aphidicola* is involved in plant–aphid interactions and we previously demonstrated that *B. aphidicola* disruption (aposymbiosis) affected the probing behavior of *M. persicae* on radish plants, delaying host plant acceptance. In this work, we evaluated the effect of aposymbiosis on the probing behavior of *M. persicae* on 2 *Solanum* species with different compatibility with *M. persicae*, *Solanum tuberosum* (susceptible) and *Solanum stoloniferum* (resistant) with the electrical penetration graph technique (EPG). To disrupt *B. aphidicola*, rifampicin was administered to aphids through artificial diets. Aposymbiotic aphids, on both plant species, showed increased pathway activities, mechanical problems with the stylets, and delayed salivation in the phloem. The extended time in derailed stylet mechanics affected the occurrence of most other probing activities; it delayed the time to the first phloem phase and prevented ingestion from the phloem. The effect of aposymbiosis was more evident in the compatible interaction of *M. persicae–S. tuberosum*, than in the incompatible interaction with *S. stoloniferum*, which generated the *M. persicae–S. tuberosum* interaction to become incompatible. These results confirm that *B. aphidicola* is involved in the plant–aphid interaction in relation to plant acceptance, presumably through a role in stylets penetration in the plant.

Key words aphid; artificial diets; *Buchnera aphidicola*; EPG technique; potato

Introduction

The potato is one of the 4 major food crops in the world together with wheat, rice and maize (FAOSTAT, 2013). This crop comprises mainly *Solanum tuberosum* L. and, to a lesser extent, other species of the genus. The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae),

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is a piercing-sucking insect that feeds exclusively from phloem sap. *Myzus persicae* is a worldwide distributed and highly polyphagous species, which is able to infect over 40 plant families, such as Solanaceae, Asteraceae, Malvaceae, Brassicaceae, Amaranthaceae, Rosaceae, Cucurbitaceae, among others (Blackman & Eastop, 2000). This aphid is one of the most important pests of potato crops, especially for its ability to effectively transmit the main potato viruses (Beemster & De Bokx, 1987).

Plant acceptance by aphids is a complex process that involves a series of behavioral steps that culminate in the sustained ingestion of phloem sap (Fereres & Moreno, 2009). To select a host plant, aphids penetrate the plant tissue by inserting their specialized mouthparts (stylets)

longitudinally through the walls between the cells of the epidermis and the mesophyll (Tjallingii & Hogen Esch, 1993). From the onset of probing, aphids repeatedly excrete gelling and watery saliva (Moreno *et al.*, 2011). On the way to the phloem, the aphids pierce the cells to test their content without killing them; when they reach a sieve element, they repeat these brief punctures several times, and if the plant is accepted, they subsequently ingest phloem sap (Tjallingii, 2006). This process can take a few hours in which the plant and the aphid interact closely (Martin *et al.*, 1997; Cherqui & Tjallingii, 2000; Will *et al.*, 2008, 2009).

Plant responses to aphids are very complex; in *Arabidopsis thaliana* a large number of general metabolism genes change their expression influencing changes in the physiological state of the plants, which is proposed to be a manipulation of the plant physiology for the aphids own benefit (De Vos *et al.*, 2005, 2007; Thompson & Goggin, 2006; Goggin, 2007). Furthermore, the transcriptomes of *S. tuberosum* and *S. stoloniferum* plants infested by *M. persicae* show changes in the expression of a large number of pathogenesis related genes (PR genes) and genes related to signal transduction dependent on salicylic acid (SA) (Alvarez *et al.*, 2013; Alvarez *et al.*, 2014). The manipulation of plant physiology performed by aphids is proposed to be a condition for plant acceptance (Zhu-Salzman *et al.*, 2004; Alvarez *et al.*, 2014).

Myzus persicae, like most aphids, harbors the endosymbiotic bacteria, *Buchnera aphidicola* (Buchner, 1965), which supplements the aphids diet with essential amino acids that they cannot get in sufficient amounts from the phloem sap (Wilkinson & Douglas, 1995; Douglas, 1996, 2008). Although this is the accepted relation of *B. aphidicola* with aphids, evidence has arisen in the last years on they the involvement of *Buchnera* in plant–aphid interactions. Elzinga *et al.* (2014) showed that *Nicotiana tabacum* and *A. thaliana* plants expressing a Buchnera's protein, GroEL, had increased resistance to *M. persicae*. Chaudhary *et al.* (2014) found that the exogenous application of this bacterial protein in *Arabidopsis* plants induced defensive genes, and, the transgenic expression of GroEL in *Arabidopsis* plants, reduced fecundity of *M. persicae*. We have recently demonstrated that in *M. persicae*, the disruption of the symbiosis with *B. aphidicola* negatively affected the feeding behavior on *Raphanus sativus* (which is a very suitable host for this aphid) causing a delay in host plant acceptance of this aphid. The aposymbiotic aphids also showed mechanical problems with stylet penetration into the plant tissues which likely was related to changes in aphid's saliva composition (Machado-Assefh *et al.*, 2015). However, the involvement of *B. aphidicola* in relation to host-plant

acceptance by aphids needs further investigation to better understand the extent of the endosymbiont influence on plant–aphid interaction.

The electrical penetration graph (EPG) technique is a powerful tool to study the plant penetration by the aphid's stylets and, thus, it is possible to study in detail the plant–aphid–endosymbiont inter- and intracellular interaction. The EPG technique was introduced by McLean and Kinsey (1964) and further developed by Tjallingii (Tjallingii, 1978, 1985, 1988). The EPG waveforms have been correlated with aphid activities as well as with tissue locations of the stylet tips (Kimmins & Tjallingii, 1985; Tjallingii, 1985, 1988; Tjallingii & Hogen Esch, 1993).

There are potato crop wild relatives that showed variability in the level of compatibility and responses to *M. persicae* infestation (Alvarez *et al.*, 2006; Askarianzadeh *et al.*, 2012), therefore the system *Solanum* sp.–*M. persicae*–*B. aphidicola* provides an interesting model system to study plant acceptance through the complex interactions between the host plant, the insect and its endosymbiont. We studied the plant acceptance by contrasting the probing behavior of aposymbiotic and control aphids on 2 potato genotypes with different level of compatibility with *M. persicae*, the potato *S. tuberosum* will be compared to the potato crop wild relative *Solanum stoloniferum* Schlechtd which shows resistance to *M. persicae* (Alvarez *et al.*, 2006, 2013).

Materials and methods

Plants

Potato plants of *Solanum tuberosum* and *Solanum stoloniferum* were used. The *S. tuberosum* cultivar PO 97. 11.9 was provided by the INTA Balcarce seed bank (Balcarce, Buenos Aires, Argentina) and the *S. stoloniferum* accession PI 338617 was provided by the Centre for Genetic Resources (Centre for Genetic Resources, Wageningen, The Netherlands); and from this accession we selected a resistant genotype, clone 8. Plants were propagated *in vitro* on 3% Murashige and Skoog medium (with vitamins), pH 5.8. After 2 weeks, the seedlings with developing roots were transplanted to soil in pots of 500 g capacity, and maintained in a growth chamber at $22 \pm 3 \, °C$, 30%–40% relative humidity, and L 16 : D 8 photoperiod.

Aphids

In order to avoid a behavioral bias toward susceptible cultivated potato, we reared the *Myzus persicae* colony on radish (*Raphanus sativus*) so that aphids could
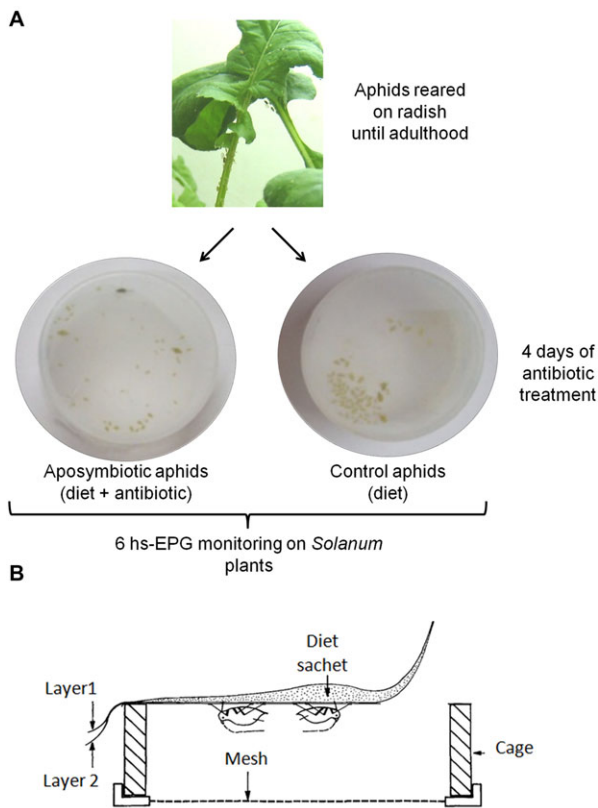
Fig. 1 EPG experiment design. The probing behavior of aposymbiotic and control *Myzus persicae* was monitored on 2 *Solanum* genotypes: *S. stoloniferum* (resistant) and *S. tuberosum* (susceptible). Aphids were reared on radish until they reached adulthood and then they were transferred to artificial diet cages for 4 d. (B) Artificial diet cages: a plastic cylinder of 3 cm height × 2 cm wide, with a mesh on the bottom and the top opened. On this opened side the diet sachet was applied (diet solution between 2 layers of Parafilm) (modified from Cloutier & Mackauer, 1975).

not adapt to any of the *Solanum* genotypes before the evaluation. Aphids used in the experiments came from a colony maintained at the faculty of Natural Sciences (Facultad de Ciencias Naturales, Universidad Nacional de Salta. Salta, Argentina). This colony was initiated from a single virginoparous wingless individual collected in field in 2009. Colonies were maintained in a climate chamber at 22 ± 3 °C, 30%–40% relative humidity, and L 16 : D 8 photoperiod to induce parthenogenesis. A new colony was started every week, and newly molted adult aphids were used for the experiments.

To produce aposymbiotic aphids (aphids whose symbiosis with *B. aphidicola* has been interrupted), recently molted adult wingless aphids were treated with 50 μg/mL of rifampicin for 4 d (Wilkinson & Douglas, 1995;

Douglas, 1996). Rifampicin selectively disrupts *B. aphidicola* (Koga *et al.*, 2007) and it was administrated through artificial diets containing 150 mmol/L amino acids, 500 mmol/L sucrose, vitamins and minerals following the protocol modified by AE Douglas (pers. comm.) after Prosser and Douglas (1992), and used in Machado-Assefh *et al.* (2015). The insects were reared on radish until the first day of adulthood and then they were transferred to artificial diet cages, which consisted on a plastic cylinder of 3 cm height × 2 cm wide, with a mesh on the bottom and the top opened to allow respiration of the insects but preventing them from escaping. On this opened side of the cage, the diet sachet (diet solution between 2 layers of Parafilm) was applied (Fig. 1B, modified from Cloutier & Mackauer, 1975). The control aphids were fed on artificial diets without the antibiotic in the same conditions as aposymbiotic aphids. To evaluate the efficiency of the antibiotic treatment the expression of a *Buchnera*-specific gene, GroEL, was measured by RT-qPCR as in Machado-Assefh *et al.* (2015).

Probing behavior of aposymbiotic and control
M. persicae on S. tuberosum and S. stoloniferum plants

The probing behavior of aposymbiotic and control aphids was evaluated by using an 8-channel DC-EPG device (Wageningen University, the Netherlands). Comparisons were made between the 2 insect treatments, (1) aposymbiotic aphids and (2) control aphids, probing on *S. tuberosum* (susceptible plant, compatible interaction) and *S. stoloniferum* (resistant plant, incompatible interaction) (Fig. 1). Four plants, 2 of each genotype, were placed in a Faraday cage and the probing behavior of 2 wingless aphids on each plant was recorded simultaneously for 6 h. Aphids were placed on the abaxial side of a leaf, which was nearly fully expanded. Before exposure to the plant, the aphid was attached to an electrode while immobilized by a vacuum-suction device. The insect electrode consisted of a 2- to 3-cm-long gold wire (diameter 20 μm), conductively glued (water-based silver glue) to the dorsum. The other end of the gold wire was attached to a 3-cm-long copper wire (diameter 0.2 mm) and connected to the input of the first head stage amplifier with a 1 giga-Ohm input resistance and 50× gain. The plant electrode, a 2-mm-thick, 10-cm-long copper rod, was inserted into the soil of the potted plant and connected to the plant voltage output of the Giga-8 DC-EPG device. The recording was started immediately after wiring the aphids, at 20 ± 4 °C, under constant light in the laboratory, and within an hour after collecting the aphids from the diet cages. Only the aphids that were actually feeding from the diet sachet were collected for the EPG

evaluation. Aphids from each of the 2 treatments were randomly distributed in the rounds of recording.

Data acquisition and waveform analysis was performed with the software Stylet$^+$ v01.23 (EPG Systems, Wageningen, the Netherlands). For each treatment, only the aphids that showed activities in each of 6 h of recording were considered as valid replicates.

EPG waveforms and variables

Among the EPG signals, first a distinction was made between probing (stylet penetration) and nonprobing periods. Then, within probes, 6 waveform (i–vi) were distinguished, considering only uninterrupted periods as an event. Waveforms are generally grouped in 3 behavioral phases related to plant tissue location of the stylets tips; that is, pathway, phloem phase, xylem phase, respectively, each comprising 1 or more waveforms. The waveform events distinguished here were: (i) waveform event C (pathway periods), including the 3 overlapping waveforms A, B, and C, in which waveform A reflects the first electrical stylet contact with the epidermis; B reflects intercellular sheath salivation; C reflects stylet penetration movements. Also, the potential drop (pd) waveform was considered as part of a C event. The pds reflect brief intracellular stylet punctures. Within phloem phase, 2 separate E waveforms occur, (ii) E1, sieve element salivation and (iii) waveform E2, phloem sap ingestion with concurrent salivation. Phloem salivation, E1, always precedes phloem sap ingestion, E2, even if it can occur as a single waveform without a subsequent E2. Also, E1 events may occur intermittently, alternating with E2 events, called E1 fragments. There are 2 waveform events that are considered as belonging to the probing phase as well: (iv) waveform E1e, putative extracellular (watery) salivation, and (v) waveform F, derailed stylet mechanics (stylet penetration difficulties). Finally, (vi) waveform G is the sole waveform in xylem phase that represents active sap ingestion from xylem elements (Tjallingii, 1990). Waveform event variables per treatment (Table 1) were characterized into 6 broad categories following the nomenclature of the list of EPG variables of Tjallingii (www.epgsystems.eu), (i) mean number of times waveform events occurred per insect; (ii) mean of the mean duration of waveform events per insect; (iii) total duration of a waveform event per insect; (iv) mean time to the first occurrence of a waveform event from the start of the probe per insect; (v) the percentage of time in probing spent in a particular activity; and (vi) number or percentage of aphids that show a particular waveform per treatment, with special interest in the percentage of aphids performing sustained phloem ingestion (sE2: uninterrupted period of E2 longer than 10 min).

These variables were calculated for each insect treatment using the Excel® workbook for automatic parameter calculation of EPG data 4.4.3 version by Sarria *et al.* (2009). A total of 63 variables were obtained from EPG analysis and 30 were selected (Table 1). Other 3 variables (16, 21 and 27) were calculated manually. An overview of the representative EPG waveforms of aposymbiotic and control aphids on both plant species is shown in Fig. 2.

Statistical analysis

The EPG variables were analyzed individually for each aphid and then an average was calculated for each insect and for each treatment in order to obtain means and standard errors of the mean (SEM). In the case of multiple events, the mean was calculated from the average of each insect. Individuals that did not show a certain waveform, did not contribute to the calculated variable and thus, n was smaller than the total number of replicates per treatment. The Kruskal–Wallis nonparametric analysis of variance (ANOVA) at one way of classification, was performed to compare the effect of aposymbiosis on both plants species. When a significant effect was detected ($P \leq 0.05$), multiple comparisons between means of treatments were performed with the Conover test (with Bonferroni correction $P \leq 0.008$, Weisstein, 1999). The Fisher's exact test was used to evaluate the significance in the difference in proportions of individuals performing each type of activity. All statistical analyses were performed using InfoStat Professional v2013p (http://www.infostat.com.ar, Di Rienzo *et al.*, 2013).

Results

EPG variables of the probing behavior of aposymbiotic and control *M. persicae* on *S. stoloniferum* and *S. tuberosum* are presented in Table 1, and the percentage of probing spent on each activity is shown in Fig. 3. On both *Solanum* species, aposymbiotic aphids had a different behavior than control aphids in activities related to: probing, pathway and cell puncture (Table 1, variable 5, 9, and 10); derailed stylet mechanics (Table 1, variable 12–16); and phloem salivation and ingestion (Table 1, variables 22, 23, and 26, and Fig. 4).

In *S. tuberosum*, aposymbiotic aphids differed from control in the number of probes, total duration of probing, number of C, number of pd per hour of C, and number of aphids with E1 (Table 1, variables 2, 3, 4, 8, and 27). In *S. stoloniferum*, aposymbiotic aphids differed from control in the mean duration of C, mean duration of

Table 1 Means ± SEM of EPG variables for aposymbiotic (apo) and control (ctrl) aphids on *S. tuberosum* (tbr) and *S. stoloniferum* (sto) plants, during 6 h.

Related to	No.	Variables	Units	Sto-apo aphids, $n = 13$	Sto-ctrl aphids, $n = 14$	Tbr-apo aphids, $n = 14$	Tbr-ctrl aphids, $n = 13$	H	P†
Probing, pathway, and cell puncture	1	Time to 1st probe	min	8.77 ± 4.31	7.53 ± 2.27	3.61 ± 1.28	2.74 ± 0.44	4.74	0.19
	2	Number of probes	#	7.85 ± 1.56^a	13.57 ± 2.72^a	8.71 ± 2.00^a	23.23 ± 2.86^b	16.09	0.001**
	3	Total duration of probing	min	315.74 ± 7.8^b	309.78 ± 9.16^b	330.43 ± 10.8^a	298.07 ± 10.2^a	8.31	0.039*
	4	Number of pathway C	#	10.62 ± 2.19^a	17.43 ± 3.08^a	10.86 ± 2.08^a	28.77 ± 3.02^b	17.53	0.001**
	5	Total duration of C	min	71.57 ± 18.6^a	170.78 ± 22.8^b	76.84 ± 18.6^a	193.52 ± 20.04^b	20.54	<0.001**
	6	Mean duration of C	min	14.85 ± 9.40^b	12.48 ± 2.00^a	7.68 ± 1.56^a	7.33 ± 0.78^a	9.25	0.026*
	7	Mean duration of pd	sec	5.13 ± 0.22^b	4.61 ± 0.2^a	4.75 ± 0.24^a	4.22 ± 0.12^a	10.03	0.018*
	8	Number of pds/h C	#	41.08 ± 9.07^a	36.10 ± 3.41^a	27.9 ± 5.36^a	47.9 ± 2.39^b	9.83	0.020*
	9	Percentage of probing spent in C	%	21.99 ± 5.46^a	55.81 ± 7.19^b	25.63 ± 7.42^a	65.77 ± 6.74^b	19.49	<0.001**
	10	Total duration of nonphloem phase	min	358.53 ± 0.89^b	341.82 ± 5.77^a	357.84 ± 5.77^b	346.24 ± 4.83^a	8.66	0.034*
	11	Time from 1st probe to 1st pd	min	54.56 ± 26.62	6.22 ± 2.57	25.72 ± 21.60	3.71 ± 3.60	1.75	0.63
Derailed stylet mechanics	12	Number of derailed stylets mechanics (F)	#	3.85 ± 0.89^b	1.07 ± 0.56^a	3.91 ± 0.51^b	1.08 ± 0.35^a	16.31	0.001**
	13	Total duration of F	min	256.05 ± 14.46^b	95.03 ± 33.67^a	253.09 ± 31.00^b	20.72 ± 13.71^a	21.08	<0.001**
	14	Mean duration of F	min	126.34 ± 112.78^b	44.87 ± 42.20^ab	111.91 ± 117.42^b	7.34 ± .54^a	16.59	0.001**
	15	Percentage of probing spent in F	%	81.12 ± 57.36^a	16.03 ± 6.7^b	63.99 ± 9.57^a	4.75 ± 3.43^b	30.83	<0.001**
	16	Aphids with F	# (%)	13 (100)^x	7 (50)^w	13 (93)^z	8 (57)^y	–	<0.05‡
Xylem ingestion	17	Number of xylem ingestion (G)	#	0.31 ± 0.14	1.07 ± 0.32	1.00 ± 0.58	1.15 ± 0.25	6.09	0.065
	18	Total duration of G	min	26.30 ± 11.09	141.79 ± 38.09	97.51 ± 30.99	107.00 ± 27.51	6.28	0.099
	19	Mean duration of G	min	26.30 ± 11.09	100.42 ± 34.79	46.47 ± 9.87	83.54 ± 27.34	4.56	0.207
	20	Percentage of probing spent in G	%	2.59 ± 0.00^a	24.9 ± 8.84^a	10.18 ± 4.72^a	26.49 ± 7.19^a	8.60	0.020*
	21	Aphids with G	# (%)	4 (30)^w	8 (57)^w	5 (35)^y	10 (92)^y	–	>0.05‡

(to be continued)

Table 1 Continued.

Related to	No.	Variables	Units	Sto-apo aphids, $n = 13$	Sto-ctrl aphids, $n = 14$	Tbr-apo aphids, $n = 14$	Tbr-ctrl aphids, $n = 13$	H	$P^\dagger$
Phloem salivation and ingestion	22	Time from 1st probe to 1st salivation (E1)	min	329.75 ± 14.38^b	258.22 ± 31.12^a	318.61 ± 25.58^b	150.07 ± 33.47^a	20.09	$<0.001^{**}$
	23	Number of E1	#	0.23 ± 0.12^a	2.36 ± 0.8^b	0.36 ± 0.19^a	4.62 ± 0.2^b	18.45	$<0.001^{**}$
	24	Total duration of E1	min	1.46 ± 0.90^a	14.73 ± 5.35^c	2.16 ± 1.66^b	9.91 ± 3.19^b	8.33	0.040^*
	25	Mean duration of E1	min	1.46 ± 0.89	3.51 ± 0.86	1.13 ± 0.81	3.30 ± 0.89	5.63	0.131
	26	Percentage of probing spent in E1	%	0.11 ± 0.08^a	3.24 ± 0.32^b	0.20 ± 0.18^a	3.48 ± 1.08^b	18.79	$<0.001^{**}$
	27	Aphids with E1	# (%)	$3 (23)^w$	$8 (57)^w$	$3 (21)^y$	$12 (92)^z$	–	$\leq 0.05^\ddagger$
	28	Time from 1st probe to 1st phloem ingestion (E2)	min	–	–	–	58.52 ± 29.47	–	–
	29	Number of E2	#	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.39	–	–
	30	Total duration of E2	min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.56 ± 2.93	–	–
	31	Mean duration of E2	min	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.06 ± 0.76	–	–
	32	Percentage of probing spent in E2	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.63 ± 0.36	–	–
	33	Aphids with E2	# (%)	0 (0)	0 (0)	0 (0)	4 (30)	–	–

†Non-parametric Kruskal-Wallis's ANOVA followed by multiple comparisons with Bonferroni correction ($P \leq 0.008$) for aposymbiotic aphids compared to control aphids on both plant species. For the same row values followed by different letters (a, b, c and d) indicate significant differences. ‡ Fisher's exact test for aposymbiotic and control aphids within plant species, for the same row values followed by different letters (w, x, y and z) indicate significant differences.
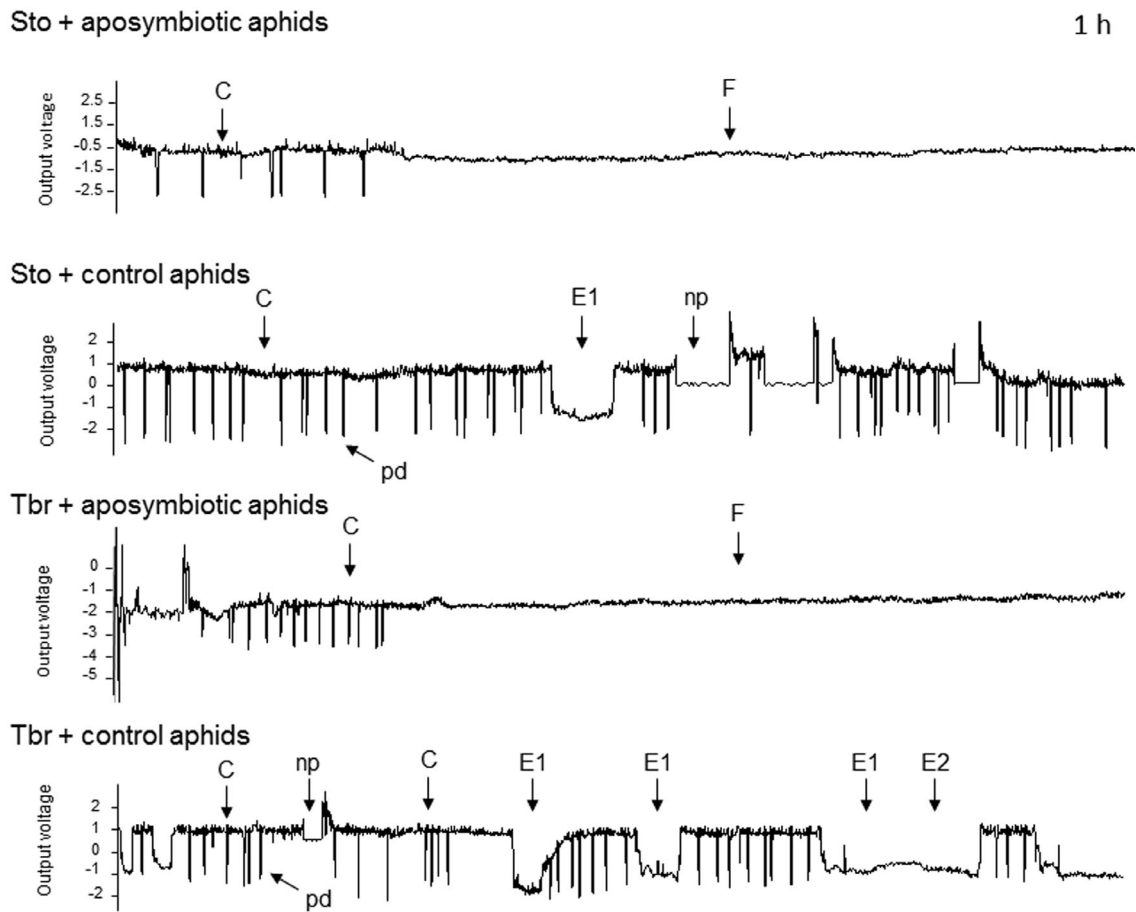$^*P \leq 0.05$ and $^{**}P \leq 0.01$.

Fig. 2 Representative EPG recordings of aposymbiotic and control aphids on *S. stoloniferum* (sto) and *S. tuberosum* (tbr) plants during 1 h; np, nonprobing; pd, potential drop, C, pathway; F, derailed stylet mecanics; E1, phloem salivation; and E2, phloem ingestion. G waveform is not shown.

pd, and the total duration of E1 (Table 1, variables 6, 7, and 24).

In xylem activities, the Kruskal–Wallis's ANOVA test detected significant effect in the percentage of probing spent in G (Table 1, variable 20), however, for the multiple comparisons with Bonferroni correction there were no differences between the means ($P \geq 0.008$).

Discussion

Aposymbiotic aphids showed a different probing behavior than control aphids, on both Solanum species, susceptible and resistant. They showed a strong decrease in pathway (Table 1, variables 5 and 9, and Fig. 3, % in C), and an increase in derailed stylet activities (Table 1, variables 12–16, and Fig. 3, % in F). Waveform F indicates that 1 individual stylet loses the bundle formation by being protruded much further than the other 3 stylets, thus making proper penetration impossible. The intercellular structural composition within plant cell walls seems to be responsible for such derailments, which normally occur at a low frequency (Tjallingii, 1988). Here, the increase in mechanical difficulties in stylet penetration is related to aposymbiosis and therefore the aposymbiotic aphids likely spent considerable more time in trying to restore the stylets bundle order than control aphids. We previously found F waveform in aposymbiotic aphids probing on radish plants; and the mechanical difficulties are presumably due to a deficient saliva composition (Machado-Assefh *et al.*, 2015). The "F" variables of control aphids did not show differences between plant species (Table 1, variables 12–16); the incompatibility of control aphids with *S. stoloniferum* is then likely related to other factors than derailed stylet mechanics.

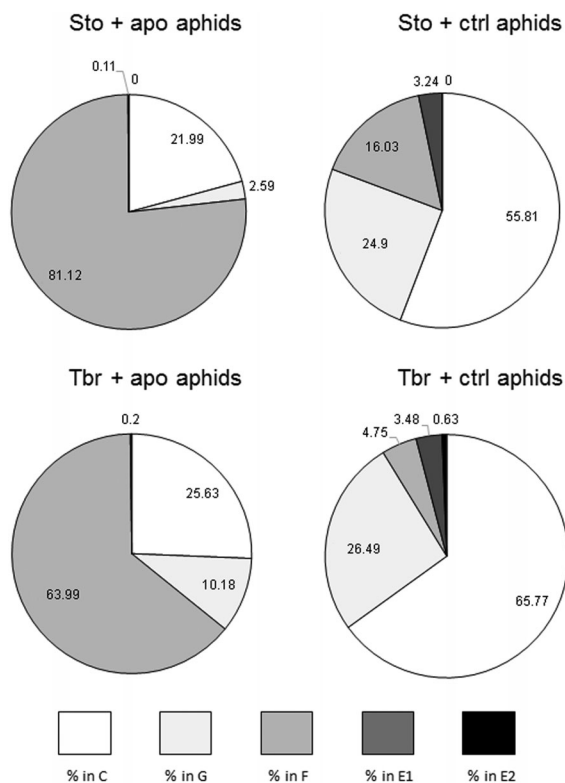Phloem activities started later in aposymbiotic aphids on both Solanum species, susceptible and resistant.

Fig. 3 Mean percentages in probing activities, for aposymbiotic (apo) and control (ctrl) aphids probing on *S. tuberosum* (tbr) and *S. stoloniferum* (sto). C, pathway; G, xylem ingestion; F, derailed stylet mechanics; E1, phloem salivation; and E2, phloem ingestion.

Overall, aposymbiotic aphids required more time than control aphids before first phloem salivation and, in *S. tuberosum* (compatible interaction), the difference was 4 times higher than the difference between aposymbiotic and control aphids in *S. stoloniferum* (incompatible interaction) (Table 1, variable 22 and Fig. 4A). Also, the difference in the percentage of aphids that showed phloem salivation was higher between aposymbiotic and control aphids on *S. tuberosum* than between aposymbiotic and control aphids on *S. stoloniferum* (Table 1, variable 27 and Fig. 4B).

Phloem sap ingestion (E2) only occurred in control aphids on susceptible *S. tuberosum* plants (Table 1, variable 33), and only 30% (4 out of 13) of the evaluated aphids showed this activity in the 6 h recording. This low number of aphids with E2 and its short duration (whereas in the 3 other treatments no E2 ingestion occurred) might be the consequence of the long F periods shown by aposymbiotic aphids that delayed the start of phloem activities. The 6 h of recording may have limited the time needed to reach E1 and E2 and therefore the impact of aposymbiosis on phloem activities as such could not be studied here. Longer EPGs monitoring should be conducted in future experiments, although after 7–8 h wire effects may increase and affect phloem activities as well. Another reason for the decreased E2 phloem ingestion here might be the aphids preceding artificial diet feeding, during which aphids have to actively ingest the diet because it lacks the high phloem sap pressure that enables the passive E2 ingestion. In our previous study of aposymbiotic aphids feeding behavior on radish (Machado-Assefh *et al.*, 2015), the aphids that before the EPG experiment had been feeding on artificial diets spent significantly more time with active ingestion, drinking from xylem as compared to aphids from plants (these treatment was not included here).

The results presented here confirm that *B. aphidicola* is involved in the plant–aphid interaction in relation to host plant acceptance. The interruption of the endosymbiotic relationship with *B. aphidicola* delayed *M. persicae* phloem activities, and in the compatible interaction with *S. tuberosum* the effect of aposymbiosis was more pronounced, which altogether likely generated the *M. persicae–S. tuberosum* interaction to become incompatible. An explanation for this is that *B. aphidicola* plays a role in stylet penetration in the plant tissues, presumably through an influence in salivation or the production of specific proteins, such as GroEL, that act as effectors in the aphid's host plant. It is known that *M. persicae* probing on *S. tuberosum*, *S. stoloniferum*, and *A. thaliana* plants promote the expression of genes related to changes in the physiological state of the plant (Thompson & Goggin, 2006; De Vos *et al.*, 2007; Goggin, 2007; Alvarez *et al.*, 2013, 2014). This changes represent a manipulation of the plant physiology for the aphids own benefit, (Zhu-Salzman *et al.*, 2004; Alvarez *et al.*, 2013) and *B. aphidicola* may be involved in this. To further comprehend the role of *B. aphidicola* in the host plant acceptance process, it would be interesting to study the interaction at a molecular and morphological level.
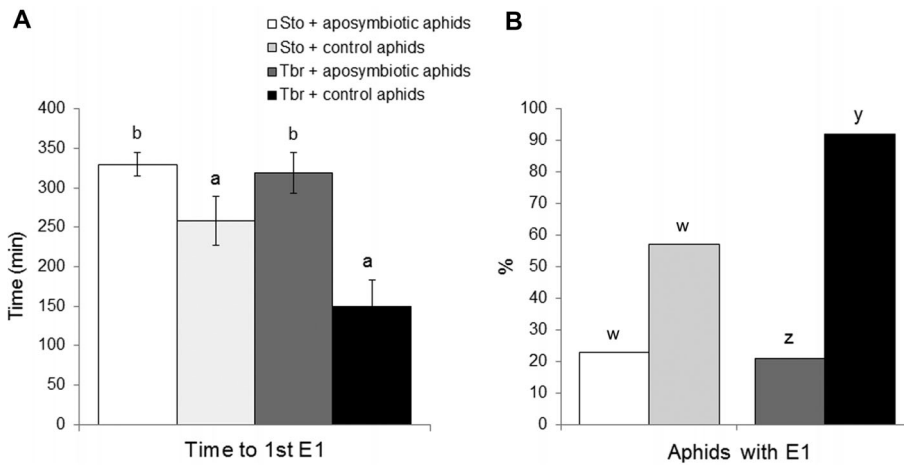
Acknowledgments

Fig. 4 EPG variables differing between aposymbiotic and control aphids on *S. stoloniferum* (sto) and *S. tuberosum* (tbr) plants. (A) Time to first salivation in the phloem (E1); different letters indicate significant differences between treatments (Kruskall–Wallis ANOVA, followed by multiple comparisons with Bonferroni correction, $P \leq 0.008$). (B) Aphids with phloem salivation (E1), different letters indicate significant differences for aposymbiotic and control aphids within a plant species, Fisher's exact test, $P \leq 0.05$.

Disclosure

The authors have declared that no competing interests exist.

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