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Bulletin of Entomological Research

Plant resistance in different cell layers affects aphid probing and feeding behaviour during non-/poor-host interactions

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Abstract:	<p>Aphids are phloem-feeding insects that cause economic losses to crops globally. Whilst aphid interactions with susceptible plants and partially resistant genotypes have been well characterised with regards to aphid probing and feeding behaviour, the interactions with non-natural host species are not well understood. Using aphid choice assays with the broad host range pest <i>Myzus persicae</i> and the cereal pest <i>Rhopalosiphum padi</i> we show that about 10% of aphids settle on non-/poor-host species over a 24h time period. We used the Electrical Penetration Graph technique to assess aphid probing and feeding behaviour during the non-/poor-host interactions. In the <i>Arabidopsis</i> non-host interaction with the cereal pest <i>R. padi</i> aphids were unable to reach and feed from the phloem, with resistance likely residing in the mesophyll cell layer. In the barley poor-host interaction with <i>M. persicae</i>, resistance is likely phloem-based as aphids were able to reach the phloem but ingestion was reduced compared with the host interaction. Overall our data suggests that plant resistance to aphids in non-host and poor-host interactions with these aphid species likely resides in different plant cell layers. Future work will take into account specific cell layers where resistances are based to dissect the underlying mechanisms and gain a better understanding of how we may improve crop resistance to aphids.</p>

1 **Plant resistance in different cell layers affects aphid probing and feeding**
2 **behaviour during non-/poor-host interactions**

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31

32 **Abstract**

33 Aphids are phloem-feeding insects that cause economic losses to crops globally.
34 Whilst aphid interactions with susceptible plants and partially resistant genotypes
35 have been well characterised with regards to aphid probing and feeding behaviour,
36 the interactions with non-natural host species are not well understood. Using aphid
37 choice assays with the broad host range pest *Myzus persicae* and the cereal pest
38 *Rhopalosiphum padi* we show that about 10% of aphids settle on non-/poor-host
39 species over a 24h time period. We used the Electrical Penetration Graph technique
40 to assess aphid probing and feeding behaviour during the non-/poor-host
41 interactions. In the Arabidopsis non-host interaction with the cereal pest *R. padi*
42 aphids were unable to reach and feed from the phloem, with resistance likely residing
43 in the mesophyll cell layer. In the barley poor-host interaction with *M. persicae*,
44 resistance is likely phloem-based as aphids were able to reach the phloem but
45 ingestion was reduced compared with the host interaction. Overall our data suggests
46 that plant resistance to aphids in non-host and poor-host interactions with these
47 aphid species likely resides in different plant cell layers. Future work will take into
48 account specific cell layers where resistances are based to dissect the underlying
49 mechanisms and gain a better understanding of how we may improve crop
50 resistance to aphids.

51

52 **Keywords:** aphid, EPG analyses, nonhost, plant resistance, probing, stylet pathway.

53 Introduction

54 Aphids are important insect pests which cause significant yield losses to crops
55 globally (Blackman R, 2000). There are approximately 5000 aphid species described
56 and around 250 of these are important agricultural and horticultural pests which vary
57 in their host range – the ability to successfully infest different plant species. This host
58 range variation generally applies to secondary hosts during summer months, where
59 aphid populations increase rapidly due to asexual reproduction (Moran, 1992). Whilst
60 the majority of aphid species exhibit a limited host range, dedicated to few closely
61 related plant species, some aphid species, like *Myzus persicae* Sulzer (green peach
62 aphid), have an exceptionally broad host range which includes representatives from
63 more than 40 plant families (Blackman R, 2000, Powell et al., 2006). The
64 evolutionary drivers and molecular determinants of such exceptionally broad host
65 ranges in aphids remain to be elucidated.

66 Host suitability relies on a number of factors, which could be based either at the plant
67 surface or within plant tissues and cells (Powell et al., 2006). Prior to probing the leaf
68 surface aphid behaviour can be influenced by a range of these factors including leaf
69 colour, emitted volatile organic compounds and leaf surface components, such as
70 epicuticular waxes or trichomes (Doring, 2014, Doring & Chittka, 2007, Neal et al.,
71 1990). Regardless of whether the aphid encounters a host or non-host plant species
72 their specialised mouthparts, known as stylets, are utilised to probe into the plant
73 tissue (Escudero-Martinez et al., 2017, Jaouannet et al., 2015, Powell et al., 2006).
74 This probing behaviour is associated with the transmission of important plant viruses
75 during both host and non-host interactions (Debokx & Piron, 1990, Katis & Gibson,
76 1985, Powell et al., 2006, Verbeek et al., 2010) which can substantially reduce crop
77 yields (Perry et al., 2000). During interactions with susceptible plant species the
78 aphid stylets penetrate the plant epidermis and move through the plant tissue
79 towards the vascular bundle. During this process the stylets probe into adjacent plant
80 cells, and saliva is secreted both in the apoplast and into probed cells along the
81 stylet-pathway (Tjallingii, 2006, Tjallingii & Esch, 1993). During compatible plant-
82 aphid interactions the aphid stylets are able to successfully puncture the sieve-tube
83 elements to facilitate ingestion of phloem sap (Tjallingii, 1995, Tjallingii, 2006).

84 The aphid stylet-pathway through the plant tissue has been well-characterised during
85 interactions with susceptible plants using the Electrical Penetration Graph (EPG)
86 technique. This technique uses an electrical circuit to connect the aphid to the plant
87 via a series of electrical probes, allowing distinction between different phases of the
88 stylet pathway from obtained electrical waveforms which correlate with the position of
89 the aphid stylet within plant tissue in real-time (Prado & Tjallingii, 1994, Tjallingii,
90 1985a, Tjallingii, 1985b, Tjallingii & Esch, 1993). Briefly, the aphid is attached to an
91 electrical probe with gold wire, and a copper electrode is placed into the soil to
92 incorporate the plant into the electrical system. Both the plant and the aphid
93 electrodes are attached to a data-logger, which is read by computational software
94 and the whole set-up is contained in a grounded Faraday cage (McClean & Kinsey,
95 1968, Tjallingii, 1978, Tjallingii, 1985a, Tjallingii, 1985b). Once the aphid probes the
96 plant tissue the circuit closes and changes in electrical voltage are displayed as
97 alternating waveforms which can be manually annotated using computational
98 software and translated into time-series data (Tjallingii & Esch, 1993). The biological
99 relevance of the different waveforms that are detected by the EPG technique have
100 been extensively analysed (Prado & Tjallingii, 1994, Tjallingii, 1978, Tjallingii, 1985a,
101 Tjallingii, 1985b). Waveforms associated with aphid probing are: waveform np,
102 representing non-probing behaviour where the stylets are not in contact with the leaf
103 surface; waveform C, which begins upon stylet penetration of leaf tissue and is
104 correlated with the intercellular apoplastic stylet pathway located at the epidermis or
105 the mesophyll cell layers; waveform pd, associated with piercing of a plant cell which
106 leads to a signal potential drop; waveform F, which reflects stylet
107 mechanical/penetration difficulties; and waveform E1e, which represents extracellular
108 saliva secretion into plant tissues other than phloem. Waveforms associated with
109 vascular interactions and which provide intricate information at the aphid feeding site
110 are: waveform G, which represents aphids drinking from the xylem sap; waveform
111 E1, which is linked to aphid salivation into phloem before ingestion; and waveform
112 E2, which corresponds to phloem sap ingestion (Alvarez et al., 2006). A graphical
113 representation of examples of these waveforms, alongside the stylet activity during
114 each, is shown in fig. 1.

115 Although the EPG technique has mainly been used to study aphid interactions with
116 susceptible and (partially-)resistant genotypes of host plant species, it also

117 represents a suitable tool to explore how aphids interact with plants which are not
118 natural hosts, including non-host and poor-host species. Indeed, EPG analyses of
119 *Brevicoryne brassicae* Linnaeus (cabbage aphid) on host Brassicaceae and non-host
120 *Vicia faba* showed that this aphid species was unable to reach the phloem when
121 feeding on the non-host *V. faba*, despite probing the leaf surface (Garbys & Pawluk,
122 1999). Also, epidermis and phloem factors contributed to resistance in different
123 legume species to different pea aphid biotypes (Schwarzkopf et al., 2013). By
124 characterising aphid probing and feeding behaviour across different aphid
125 interactions with non-/poor-host species we aim to generate a better understanding
126 of where associated resistance mechanisms reside. This in turn will facilitate
127 important mechanistic studies to reveal the molecular determinants of plant immunity
128 to aphids.

129 We previously showed that *M. persicae*, which is not a pest of barley, is able to feed
130 and reproduce on this crop under controlled environment conditions, but to a lower
131 extent than on a host species such as oil seed rape or Arabidopsis (Escudero-
132 Martinez et al., 2017). On the contrary, *Rhopalosiphum padi* Linnaeus (bird cherry-
133 oat aphid) is a pest of barley but is unable to feed from, and therefore survive, on
134 Arabidopsis (Jaouannet et al., 2015). However, in both the *M. persicae*-barley poor-
135 host interaction and the *R. padi*-Arabidopsis non-host interaction probing of the leaf
136 surface takes place (Escudero-Martinez et al., 2017, Jaouannet et al., 2015). In line
137 with our previous findings, choice assays showed that both aphid species will settle
138 on and interact with non-/poor-host plant species if given a choice, with 10% of
139 aphids found on non-/poor-hosts after 24h. Using EPG analyses of *M. persicae* and
140 *R. padi* on Arabidopsis and barley we explored differences in aphid probing and
141 feeding behaviour during non-/poor-host versus host interactions. We show that
142 resistance in the non-/poor-host interactions can reside in different plant cell layers,
143 suggesting complex mechanisms may underlie plant immunity to aphids.

144

145 **Materials and Methods**

146 **Aphid rearing**

147 *R. padi* (JHI-JB, genotype G) (Thorpe et al., 2018, Leybourne et al., 2018) was
148 maintained on *Hordeum vulgare* cv Optic and *M. persicae* (JHI_genotype O) was
149 maintained on *Brassica napus* (oilseed rape). All aphid species used in the

150 experiments were maintained in growth chambers under controlled conditions (18°C
151 ± 2°C, 16 h of light).

152 **Plant growth**

153 Barley plants (cv. Golden Promise) were pre-germinated in Petri dishes with wet filter
154 paper for three days in the dark. Then, they were moved to a plant growth cabinet
155 under controlled conditions and grown for 7 days (growth stage 1.10, determined
156 using the staging key (Zadoks et al., 1974)) until the EPG experiments. *Arabidopsis*
157 *thaliana* Col-0 plants were sown directly in soil; the seeds were stratified for 3 days at
158 4°C and placed in the growth cabinet for 4-5 weeks before use in experiments
159 (growth stage 1.10 to 3.90, determined using the Boyes growth key (Boyes et al.,
160 2001)). The cabinet conditions for *Arabidopsis* were 8 hours of light (125 µmol
161 photons/m².s), at 22 °C and 70% humidity. The cabinet conditions for barley were 8
162 hours of light (150 µmol photons/m².s), at 20 °C (+2°C).

163 **Aphid choice experiment**

164 Aphid choice tests were devised to investigate the host plant preference of *R. padi*
165 and *M. persicae*. Three choice test assays were developed: one using 50 *R. padi*
166 aphids, a second using 50 *M. persicae* aphids, and a third using a mixed species
167 population (25 *R. padi*, 25 *M. persicae*). For each assay, fifty aphids (mixed aged: 2nd
168 instar – apterous adult) were placed on a sheet of tissue paper and were placed in
169 the centre of a Perspex cage halfway between two plants (one *Arabidopsis*, one
170 barley). Aphids were 90 mm away from both plants and the two plants were 180 mm
171 apart. Bamboo sticks served as bridges from the cage bottom (where the aphids
172 were placed) to each plant, with additional bamboo sticks acting as bridges between
173 the two plants, similar to the set-up used by Nowak and Komor (Nowak & Komor,
174 2010). Once the aphids were placed between the plants and the ladders were
175 positioned, the cages were closed and the proportion of aphids present on the host,
176 non-/poor-host, or which had not settled were scored three and 24 hours later.
177 Choice assays were carried out in growth chambers under controlled conditions
178 (18°C ± 2°C, 16 h of light).

179

180 Choice tests were carried out simultaneously in separate Perspex cages (440 mm x
181 340 mm x 390 mm). For each replicate the assignment of aphid mixture (*R. padi*, *M.*
182 *persicae*, or mixed) to cage (1, 2, or 3) and the position (1 or 2) of *Arabidopsis* and

183 barley within each cage was randomly assigned. Seven replicates were collected for
184 each aphid mixture. The proportion of aphids detected on each plant were modelled
185 in response to plant type (Host, non-/poor-host, or not settled), aphid mixture (*R.*
186 *padi*, *M. persicae*, mixed species), time-point (three hours and 24 hours) and all
187 interactions using a linear mixed effects model. Cage and block were included as
188 random factors, the model was simplified using manual backward stepwise model
189 selection, and fitted-residual plots were observed at each stage to assess model
190 suitability. Models were analysed using a χ^2 Analysis of Deviance Test. Differences in
191 the Least Squares Mean with Tukey correction for multiple comparison was used as
192 a post-hoc test. Data were analysed in R Studio v. 1.0.143 running R v. 3.4.3 (R Core
193 Team, 2017) with additional packages car v.2.1-4 (Weisberg & Fox, 2011), lme4
194 v.1.1-13, and lsmeans v.2.27-62 (Lenth, 2016).

195

196 **Electrical penetration graph (EPG) analyses**

197 The probing and feeding behaviour of *R. padi* and *M. persicae* on different plant
198 species was assessed using the Electrical Penetration Graph technique (Tjallingii,
199 1995) on a Giga-4 DC-EPG device with 1 Giga Ω resistance (EPG Systems, The
200 Netherlands). We used a randomized block design for all EPG experiments
201 performed here. Aphids were connected to a copper electrode with a golden wire (20
202 μm diameter), attached at the aphid dorsum and connected to the electrode with
203 water-based silver glue. Aphids were lowered onto either an Arabidopsis or barley
204 leaf approximately 1-1.5 hr after being removed from culture, depending on the
205 treatment, and feeding behaviour was recorded over a 6h period. Three recordings
206 were taken simultaneously. Each experiment was initiated between 10-12 am and
207 the experiment was performed over a 6-month period, with 18 host and 17 non-host
208 replicates for *R. padi* and 23 host and 28 poor-host replicates for *M. persicae*. Data
209 were acquired using the Stylet+ D software package version v.01.28 and annotated
210 manually using the Stylet+ A v.01.30 software (EPG-Systems, The Netherlands).
211 Obtained waveforms were annotated with one of the following signals: no penetration
212 (np), stylet penetration into the epidermal and mesophyll tissue (pathway/C phase),
213 cellular punctures during the C phase (pd), watery salivation into sieve elements
214 (E1), ingestion of phloem sap (E2), derailed stylet mechanics/stylet penetration
215 difficulties (waveform F), xylem ingestion (waveform G), or extracellular saliva
216 secretion into mesophyll (E1e) (Alvarez et al., 2006, Tjallingii, 1995). Annotated

217 waveforms were converted into time-series data using the excel macro developed by
218 Dr Schliephake (Julius Kühn-Institut); these converted parameters were used for
219 statistical analysis. Parameters used for comparisons in these experiments are
220 described by Giordanengo et al. (Giordanengo, 2014), and include total time of
221 probing, number of probes, duration of phloem sap ingestion, and duration of xylem
222 sap ingestion, a total of 97 parameters were measured. Statistical analyses were
223 performed in R Studio running R v. 3.2.3. (R Core Team, 2017) using the Wilcoxon
224 rank test, a significance threshold of 0.05 was used.

225

226 **Results**

227

228 **Aphids preferentially settle on their host plant**

229 We used aphid choice assays to examine the host plant preference of
230 *Rhopalosiphum padi* and *Myzus persicae*. We monitored the settling behaviour of *R.*
231 *padi* when provided with a choice between barley (host) and Arabidopsis (non-host),
232 of *M. persicae* when provided with a choice between Arabidopsis (host) and barley
233 (non-host), and of a mixed species population containing *R. padi* and *M. persicae*.

234 The majority of aphids preferentially settled on the host plant, *c.* 50% of aphids
235 settled on the host plant within three hours (Table 1; fig. 2). The number of aphids
236 that settled on the host plant increased to around 80% after 24 hours for all aphid
237 populations assessed ($t = -9.48$; $p = <0.001$) with the number of unsettled aphids
238 decreasing ($t = 8.30$; $p = <0.001$). However, approximately 10% of aphids were found
239 on either the non-host or the poor-host plant at both time-points. No effect of aphid
240 mixture was observed (Table 1), indicating that the presence of additional aphid
241 species did not influence aphid behaviour.

242

243 **The Arabidopsis-*R. padi* non-host interaction is characterised by long no- 244 probing periods and difficulties in locating the vascular tissues**

245

246 We employed the Electrical Penetration Graph (EPG) technique to compare the
247 feeding behaviour of *R. padi* on barley (host) with Arabidopsis (non-host) and of *M.*
248 *persicae* on Arabidopsis (host) with barley (poor-host) over a six hour period in order
249 to identify the tissue layers involved in non-host and poor-host resistance against

250 aphids. We assessed 97 feeding parameters in total, 71 of these were altered during
251 feeding on non/poor-host plants compared with feeding patterns on host plants
252 (Supplementary Table S1) with 26 parameters remaining unaffected (Supplementary
253 Table S2).

254 The majority of feeding parameters that differed between *R. padi* feeding on host
255 compared with non-host plants were related to stylet probing of the plant tissue and
256 interactions with the plant vasculature (fig. 3). In general, probing parameters that
257 differed for *R. padi* when interacting with non-host versus host plants were non-
258 probing periods, number of stylet probes into plant tissue, and time spent in the
259 epidermal/mesophyll cells (C phase) (fig. 3A; Supplementary Table S1).

260 During non-host interactions with Arabidopsis, the total time the aphids were not
261 probing plant tissue during the 6 h recording was 2.5 times greater (4889s) than the
262 host interactions (1767s) (fig. 3A; Supplementary Table S1; $W = 33.00$; $p = <0.001$).
263 However, the overall number of stylet probes into plant tissue was higher on non-host
264 plants (18) than host plants (8) (fig. 3A; Supplementary Table S1; $W = 52.50$, $p =$
265 0.001). Although the total number of C phases (stylet activity at the
266 epidermis/mesophyll, including a return to C phase following stylet interactions in the
267 vasculature) was not significantly different between non-host and host interactions,
268 the overall time spent in the epidermis/mesophyll (C phase) was over two times
269 longer for the non-host (14128s) compared with host interactions (6237s) (fig. 2A;
270 Supplementary Table S1; $W = 37.00$; $p = <0.001$).

271 All the vascular-related parameters (G, E1 salivation and E2 ingestion phases)
272 measured for *R. padi* were significantly reduced during non-host interactions
273 compared with host interactions (fig. 3B; Supplementary Table S1). This included a
274 two-fold reduction in the number of xylem ingestion (G phase) events during the non-
275 host interaction (0.24 times) compared with the host interaction (0.50 times) (fig. 3B;
276 Supplementary Table S1; $W = 2.28.50$; $p = 0.001$) alongside a significant decrease in
277 the total length of xylem ingestion, 1021s for non-host compared with 1483s for host
278 plants (fig. 3B; Supplementary Table S1; $W = 221.50$; $p = 0.003$). We also observed
279 significantly fewer salivation events (E1 phase) during the non-host interaction (0.18
280 events) compared with the host interaction (3.67 events; $W = 282.00$; $p = <0.001$),
281 with salivation events five-fold shorter during the non-host interaction (18s) compared
282 with the host interaction (93s) (fig. 3B; Supplementary Table S1; $W = 278.00$; $p =$

283 <0.001). Ingestion of phloem sap (E2 phase) was rarely observed during the non-
284 host interaction (0.06 times) compared with the host interaction (3 times; $W = 285.00$;
285 $p = <0.001$), and the total duration of this ingestion period was greatly reduced on
286 non-host plants (19s) compared with host plants (10030s, or 2.78 hours) (fig. 3B;
287 Supplementary Table S1; $W = 288.00$; $p = <0.001$).

288

289 **The barley-*M. persicae* poor-host interaction is characterised by a lack of**
290 **sustained phloem ingestion**

291 The majority of feeding parameters that differed between *M. persicae* feeding on host
292 compared with poor-host plants were primarily related to interactions within the plant
293 vasculature, specifically a decrease in interactions with the phloem and an increase
294 in interactions with the xylem (fig. 4; Supplementary Table S1). In general, this
295 involved a decrease in the ability to locate the phloem and initiate ingestion of
296 phloem sap. When feeding on poor-host plants there was a significant increase in the
297 number of probes made into the plant tissue by aphids (19) compared with the
298 number of probes made into host plants (16) (fig. 3A; Supplementary Table S1; $W =$
299 186.00 ; $p = 0.024$). However, the total length of time aphids probed into plant tissue,
300 the number of pathway (C) phase events, and the total time spent within the pathway
301 (C) phase was similar for the host and poor-host interactions (fig. 4A)

302 Aphid stylet activities related to the vascular parameters (G – xylem, E1 – phloem
303 salivation, and E2 – phloem ingestion) were different between host and poor-host
304 interactions (fig. 4B; Supplementary Table S1). The number of times that *M. persicae*
305 reached the xylem (G phase) during the poor-host interaction was higher (1.33 times;
306 $W = 133.50$; $p = <0.001$) and the total time of xylem ingestion was longer (2321s; W
307 $= 142.50$; $p = <0.001$) than during the host interaction, where aphids reached the
308 xylem 0.30 times and spent a total of 691s ingesting xylem sap (fig. 4B;
309 Supplementary Table S1). For the E1 salivation phase the number and duration of
310 events was reduced during the poor-host interaction, 1.73 events ($W = 5.28$; $p =$
311 <0.001) with a total length of time spent salivating into the phloem of 562s ($W =$
312 500.00 ; $p = <0.001$), compared with the host interaction (7 events with a time length
313 of 652s) (fig. 4B; Supplementary Table S1).

314 *M. persicae* showed limited ingestion periods during the poor-host compared with
315 host interactions. The number of E2 phases and their length was greatly reduced on
316 poor-host plants, 0.53 events ($W = 552.50$; $p = <0.001$) with a 40-fold decrease in the
317 total time spent ingesting phloem (126s; $W = 573.50$; $p = <0.001$), compared with
318 host plants (5.7 events with a total length of 5064s) (fig. 4B; Supplementary Table
319 S1). Moreover, on the poor-host sustained phloem ingestion was severely lacking,
320 and aphids spent only 49s in the E2 ingestion phase on poor-host plants ($W =$
321 520.00 ; $p = <0.001$) with events being nearly absent, 0.07 events ($W = 515.00$; $p =$
322 <0.001). In contrast, aphids spent 4322s in the E2 sustained ingestion phase on host
323 plants over 2.1 events during the 6h recording (fig. 4B; Supplementary Table 1).
324 Therefore, the *M. persicae* poor-host interaction features substantially reduced
325 phloem ingestion.

326

327 **Discussion**

328 The overall aim of this study was to gain insight into where resistances against
329 aphids may reside within the plant tissue during host versus non/poor-host
330 interactions by analysing aphid probing and feeding behaviour. We showed that
331 when given a choice aphids do interact with non-/poor-host plants under controlled
332 conditions, and we further explored these interactions using EPG analyses. Common
333 features of the non-host and poor-host interactions were an increased number of
334 probes and longer no-probing periods. Importantly, our data showed differences
335 between *R. padi* and *M. persicae* probing and feeding behaviour on the non-/poor-
336 host plants. During the *R. padi*-*Arabidopsis* (non-host) interaction the aphids only
337 occasionally reached the vascular tissues. On the contrary, during the *M. persicae*-
338 barley interaction (poor-host) aphids successfully reached the vascular tissue and
339 could ingest xylem and phloem, however prolonged periods of phloem ingestion were
340 inhibited. Based on the data generated here for *M. persicae* and *R. padi* we propose
341 a model wherein poor- and non-host plant resistances against these aphid species
342 may reside within the phloem and mesophyll cell layers, respectively (fig. 5).

343 During the *R. padi*-barley interaction (host interaction) the aphids spend less time
344 probing and in the pathway (C) phase and readily reach the phloem where salivation
345 and phloem sap ingestion occurs for several hours (fig. 5A). Occasionally, aphids
346 ingest xylem, which is thought to be important in coping with osmotic effects

347 associated with ingestion of large amounts of phloem sap (Pompon et al., 2010,
348 Spiller et al., 1990). In contrast, during the *R. padi* – Arabidopsis interaction (non-host
349 interaction) aphids exhibit altered probing behaviour, including an increase in the
350 number of plant probes alongside a decrease in the total time probing into plant
351 tissue. Additionally, *R. padi* shows an extended stylet pathway phase, and only rarely
352 does the aphid reach the Arabidopsis phloem or xylem (fig. 5B). On the occasions
353 where the *R. padi* stylets reach the vascular tissue during non-host interactions the
354 ingestion of phloem and xylem sap is ineffective, in line with this aphid being unable
355 to survive on Arabidopsis (Jaouannet et al., 2015).

356 Interestingly, *R. padi* spent less time probing into plant tissue during the non-host
357 interaction. However, during these probes aphids spent an increased time interacting
358 with the mesophyll tissue during the non-host interaction than the host interaction,
359 including an increase in the total time spent in the pathway (C) phase. This indicates
360 that non-host resistance could potentially reside in the mesophyll tissue as the aphids
361 struggled to probe beyond this layer and access to the vascular tissue was limited
362 (fig. 5B), as further indicated by the increased time required for aphids to reach the
363 phloem during non/poor-host interactions compared with the host interactions.
364 Further research will be needed to further understand the mechanisms underlying
365 Arabidopsis non-host resistance to *R. padi*, and to investigate the potential
366 involvement of specific recognition receptors within the mesophyll cell layer.
367 Interestingly, the NADPH oxidase *AtRbohF*, involved in ROS (Reactive Oxygen
368 Species) production, a member of the *LEA* (*Late Embryogenesis Abundant*) family,
369 implicated in abiotic and biotic stress, as well as the *VSP1* (*Vegetative Storage*
370 *Protein 1*), which is activated by jasmonate signalling, contribute to Arabidopsis non-
371 host resistance against *R. padi* (Jaouannet et al., 2015). Whether these genes act
372 within the mesophyll cell layer to activate defences against aphids remains to be
373 determined.

374
375 The *M. persicae*-Arabidopsis (host) interaction, features short probing and pathway
376 times, and prolonged salivation and ingestion once the phloem is reached, as well as
377 occasional xylem drinking (fig. 5C). In contrast, during the *M. persicae*-barley
378 interaction (poor-host interaction) aphids show increased probing but spend a similar
379 time in the stylet pathway phase as aphids on host Arabidopsis plants. The main

380 differences between the Arabidopsis (host) and barley (poor-host) interactions with
381 *M. persicae* are reduced salivation in the phloem and relatively short periods of
382 phloem ingestion (less than 10 minutes) on barley (fig. 5C and 5D). It is likely that
383 this reduced phloem sap ingestion is responsible for the reduced *M. persicae*
384 performance on barley (Escudero-Martinez et al., 2017, Ramirez & Niemeyer, 2000).
385 It is possible that *M. persicae* attempts to compensate for this reduced ingestion of
386 phloem sap with increased xylem drinking, in line with the observation that aphid
387 starvation increases the xylem phase (fig. 5D) (Ramirez & Niemeyer, 2000).

388 Phloem resistance factors are related to the E1 salivation and E2 ingestion
389 parameters, and in particular ingestion phases shorter than 10 minutes (Alvarez et
390 al., 2006, Prado & Tjallingii, 1997). Phloem-mediated defences against aphids
391 include the occlusion of sieve elements, which prevents aphids from ingesting
392 phloem sap (Dreyer & Campbell, 1987, Medina-Ortega & Walker, 2015, Will & van
393 Bel, 2006). This phloem occlusion occurs upon callose deposition and formation of P-
394 protein plugs. The latter is thought to seal off the phloem upon damage and/or to
395 block the aphid food canal (Tjallingii, 2006, Will & van Bel, 2006). Interestingly, PAD4
396 was found to be a component of phloem-based immunity against *M. persicae* in
397 Arabidopsis (Pegadaraju et al., 2007). However, no barley PAD4 (MLOC_1340) or
398 PAD4-related genes were up-regulated during the barley-*M. persicae* interaction
399 (Escudero-Martinez et al., 2017). However, our previous transcriptome analyses
400 showed induction of a barley gene encoding Phloem Protein 2-like (PP2), which is a
401 phloem specific lectin, with the induction being most pronounced during the barley-*M.*
402 *persicae* interaction (Escudero-Martinez et al., 2017). Lectins have carbohydrate-
403 binding properties and function in cell communication, development, and plant
404 defence (Bellande et al., 2017). PP2 is a lectin highly abundant in the phloem and
405 accumulates in damaged phloem sieve pores to form protective plugs (Read &
406 Northcote, 1983). Overexpression of *AtPP2* in Arabidopsis leads to reduced *M.*
407 *persicae* feeding suggesting PP2 may contribute to defences against aphids (Zhang
408 et al., 2011), possibly by interfering with aphid digestion in the midgut (Kehr, 2006).
409 The very infrequent phloem sap ingestion we observed might reflect a rejection of the
410 sieve element, possibly due to the presence of a deterrent factor in the phloem sap
411 (Mayoral et al., 1996). Indeed, lectins, including PP2-like proteins, have been shown
412 to have deterrent activities and insecticidal activities against *M. persicae* (Jaber et al.,

413 2010, Sauvion et al., 1996, Zhang et al., 2011). Whether barley phloem-lectins like
414 PP2 indeed contribute to phloem-based defences of barley against *M. persicae*
415 needs to be further tested.

416 It is important to note that the EPG experimental set-up was of a no-choice nature
417 (i.e. aphids were placed on the plants) and that additional plant resistance
418 components that affect aphid choice may play a role in the interactions studied here
419 (Escudero-Martinez et al., 2017, Powell et al., 2006). For example, we previously
420 showed that the black cherry aphid (*Myzus cerasi* Fabricius), which infests cherry
421 trees as well as several herbaceous plants, displays only limited probing on non-host
422 barley plants, and does not settle on barley leaves (Escudero-Martinez et al., 2017),
423 pointing to a potential role of barley defences that act at the pre-probing level against
424 this aphid species (Nottingham et al., 1991). In addition, some plant induced volatile
425 compounds have been reported to be repellent to aphid pests and attractants of their
426 natural enemies (Dreyer & Jones, 1981, Mallinger et al., 2011, Turlings & Ton, 2006).

427 With limited genetic crop resistance available against aphids, identifying the
428 determinants of non/poor-host resistance is an important area of research that may
429 help the development novel crop protection strategies. Using a detailed assessment
430 of aphid probing and feeding behaviour on different natural host and non-host
431 species we show that resistances may reside in different cell layers depending on the
432 plant species-aphid species interaction.

433

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443

444 **Author contributions**

445 JIBB, CEM and DJL conceived and designed the experiments, CEM and DJL
446 performed the experiments, JIBB, CEM and DJL analysed the data, JIBB and CEM
447 wrote the manuscript with input from DJL. All authors read and approved the final
448 manuscript.

449

450 **Supplementary Material**

451 **Supplementary Table S1.** Results for all obtained Electrical penetration graph
452 (EPG) parameters which were significantly different between host and non/poor-host
453 feeding. Table displays the EPG parameter assessed, a description of the parameter,
454 and the plant tissue layer involved. Results displayed are the mean and standard
455 deviation (SD) for each aphid-plant combination for each parameter alongside the
456 Wilcoxon test statistic (W value) and p value for each pairwise host vs non/poor host
457 comparison. p values in bold represent values significantly different in both host vs
458 non-host and host vs poor-host interactions, italicised p values represent parameters
459 which only differed in one combination. Average and standard deviation of the 97
460 electrical EPG parameters calculated for *R. padi* host (Rp_Hv) and non-host (Rp_At).
461 Average and standard deviation of the 97 electrical EPG parameters calculated for
462 *M. persicae* host (Mp_At) and poor-host (Mp_Hv). Calculations were made with
463 summary statistics in Rstudio. The EPG list of parameters was taken from EPG
464 systems: www.epgsystems.eu/files/List%20EPG%20variables.xls

465 **Supplementary Table S2:** Results for all obtained Electrical penetration graph
466 (EPG) parameters which were not significantly different between host and non/poor-
467 host feeding. Table displays the EPG parameter assessed, a description of the
468 parameter, and the plant tissue layer involved. Results displayed are the mean and
469 standard deviation (SD) for each aphid-plant combination for each parameter
470 alongside the Wilcoxon test statistic (W value) and p value for each pairwise host vs
471 non/poor host comparison. p values in bold represent values significantly different in
472 both host vs non-host and host vs poor-host interactions, italicised p values represent
473 parameters which only differed in one combination. Average and standard deviation
474 of the 26 electrical EPG parameters calculated for *R. padi* host (Rp_Hv) and non-
475 host (Rp_At). Average and standard deviation of the 97 electrical EPG parameters

476 calculated for *M. persicae* host (Mp_At) and poor-host (Mp_Hv). Calculations were
477 made with summary statistics in Rstudio. The EPG list of parameters was taken from
478 EPG systems: www.epgsystems.eu/files/List%20EPG%20variables.xls

479

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634

Tables

Table 1. Statistical results of the choice test assay

Response variable	Test Statistic (degrees of freedom)	p-value
Plant Type	$X^2_{(2)} = 532.65$	P = <0.001
Aphid Mixture	$X^2_{(2)} = 0.01$	P = 0.996
Time-point	$X^2_{(1)} = 0.01$	P = 0.949
Plant Type x Aphid mixture	$X^2_{(4)} = 5.43$	P = 0.245
Plant Type x Time-Point	$X^2_{(2)} = 162.06$	P = <0.001
Aphid Mixture x Time-Point	$X^2_{(2)} = 0.01$	P = 0.996
Plant Type x Aphid Mixture x Time-Point	$X^2_{(4)} = 0.34$	P = 0.986

Figure Legends

Figure 1. Graphical representation of aphid/stylet activities associated with each EPG waveform.

- (a) Example of aphid activity during np (non-probing) period, stylet is not in contact with leaf tissue.
- (b) Initiation of pathway (C) phase - aphid stylet pierces leaf epidermis,
- (c) Potential drop (pd) – aphid stylet penetrates adjacent plant cell
- (d) Stylet penetration difficulties (F phase)
- (e) Extracellular saliva secretion (E1e) phase – salivation into extracellular space.
- (f) Xylem ingestion (G phase) – stylet penetrates vascular xylem cells to initiate xylem drinking.
- (g) Salivation into phloem (E1 phase) – stylet penetrates sieve tube element and aphid initiates salivation into phloem sap.
- (h) Phloem ingestion (E2 phase) – aphid begins passive ingestion of phloem sap. Also includes sustained phloem ingestion (sE2 phase) - a period of phloem sap ingestion lasting > 10 mins.

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Figure 2. Stacked bar charts showing the settling behaviour of aphids in the choice experiment.

- (a) Aphid settling after three hours
- (b) Aphid settling after 24 hours

Graphs show the mean proportion of aphids from the *R. padi* (Rp), *M. persicae* (Mp), and the mixed species population (Mix) which had settled on the host plant (H; green), the non-host plant (NH; red), the poor-host plant (PH; yellow), the non/poor-

host plant (NH.PH; orange) or which has not settled (NS; grey). Letter under each bar indicate differences based on Least Squares Mean post-hoc analysis with Tukey correction.

Figure 3. Box plots showing different EPG parameters associated with *Rhopalosiphum padi*-barley (host) and *Rhopalosiphum padi*-*Arabidopsis* (non-host) interactions.

(a) Probing-related parameters: total number of probing events, total length of no probing time, total number of pathway (C) phase events, total length of pathway (C) phase time.

(b) Vascular-related parameters: number of xylem ingestion (G phase) events, total length of xylem ingestion, number of salivation (E1 phase) events where aphid saliva is secreted into phloem sap, total length of salivation (E1 phase), number of phloem sap ingestion (E2 phase) events and total length of phloem sap ingestion (E2 phase). Green boxes indicate the host (H) interaction and red boxes represent the non-host (NH) interaction. *R. padi* on host plants was replicated 18 times and *R. padi* on non-host plants was replicated 17 times. Significant differences between interactions were assessed by Wilcoxon non-parametric t-test (* = $p \leq 0.05$ and *** = $p \leq 0.01$).

Figure 4. Box plots showing different EPG parameters in *Myzus persicae* interaction with a host (*Arabidopsis*) and a poor-host plant (barley).

(a) Probing-related parameters: total number of probing events, total length of no probing time, total number of pathway (C) phase events, total length of pathway (C) phase time.

(b) Vascular-related parameters: number of xylem ingestion (G phase) events, total length of xylem ingestion, number of salivation (E1 phase) events where aphid saliva is secreted into phloem sap, total length of salivation (E1 phase), total length of phloem sap ingestion (E2 phase) and total length of sustained phloem sap ingestion (sE2 phase).

Green boxes indicate the host (H) interaction and yellow boxes represent the poor-host (PH) interaction. *M. persicae* on host plants was replicated 23 times and *M. persicae* on poor-host plants was replicated 28 times. Significant differences between interactions were assessed statistically by Wilcoxon non-parametric t-test (* = $p \leq 0.05$ and *** = $p \leq 0.01$).

Figure 5. Model showing *R. padi* and *M. persicae* probing and feeding during host, poor-host and non-host plant interactions.

(a) During the host interaction (*R. padi*-barley), the aphids will probe the epidermal and mesophyll cells (pathway C phase), then will drink from the xylem or salivate and feed from the phloem, with feeding lasting for hours.

(b) During the non-host interaction (*R. padi*-*Arabidopsis*), the aphids will spend a long time not probing, and when probing eventually occurs the aphids remain in stylet pathway phase (in epidermis and mesophyll cell layers) most of the time and only occasionally will reach the vascular tissue, either xylem or phloem. No sustained ingestion of phloem sap takes place.

(c) During the host interaction (*M. persicae*-*Arabidopsis*), the aphids will probe the epidermal and mesophyll cells (pathway C phase), then will drink from the xylem or salivate and feed from the phloem, with feeding taking place for hours.

(d) During the poor-host interaction (*M. persicae*-barley), the aphids show increased probing compared to the host interaction, while the stylet pathway phase (in epidermis and mesophyll cell layers) is similar to the interaction with the host plant. At the vascular level, long periods of time will be spent in the xylem, and eventually aphid will reach the phloem, salivate and ingest phloem sap. However, contrary to the host interaction, no sustained (>10 minutes) ingestion of phloem sap takes place.

Figure 1

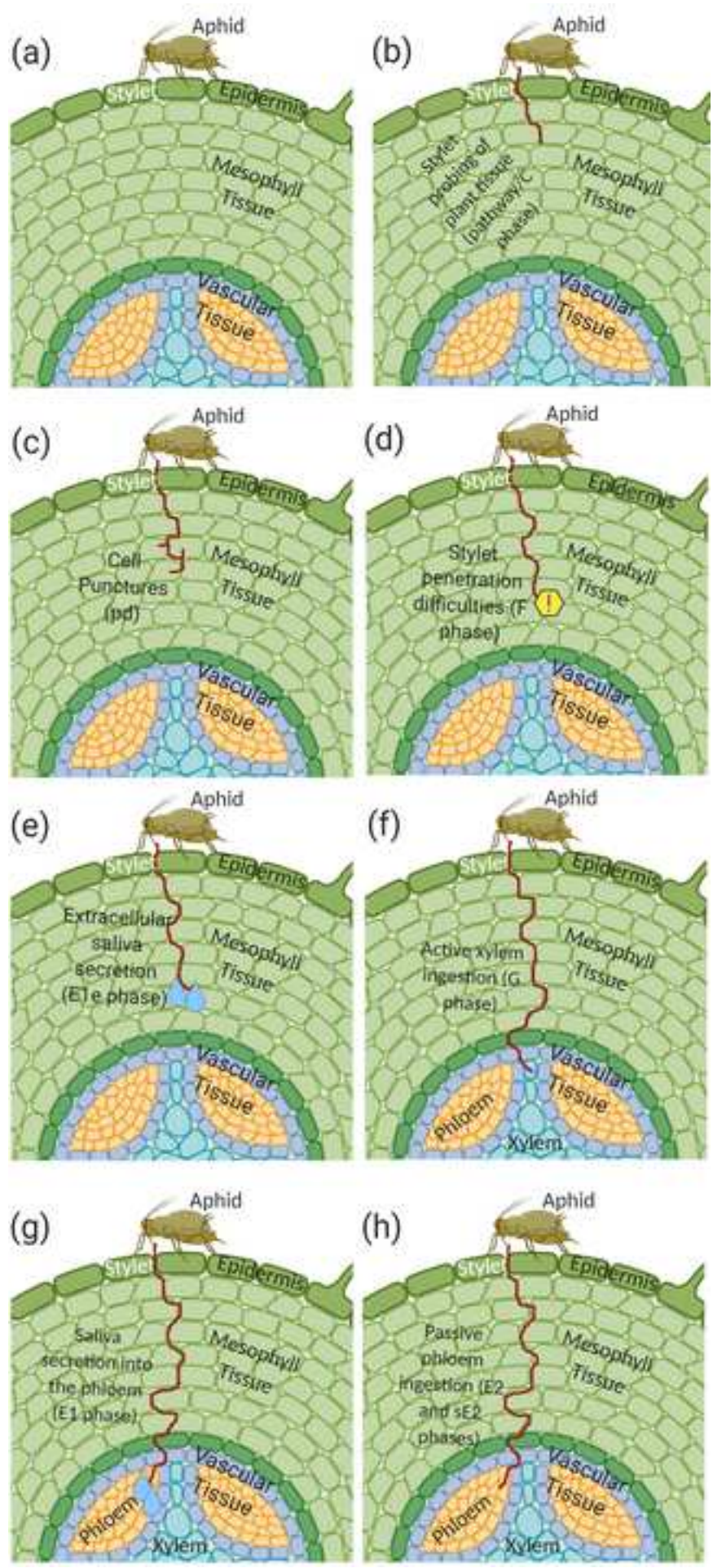


Figure 2

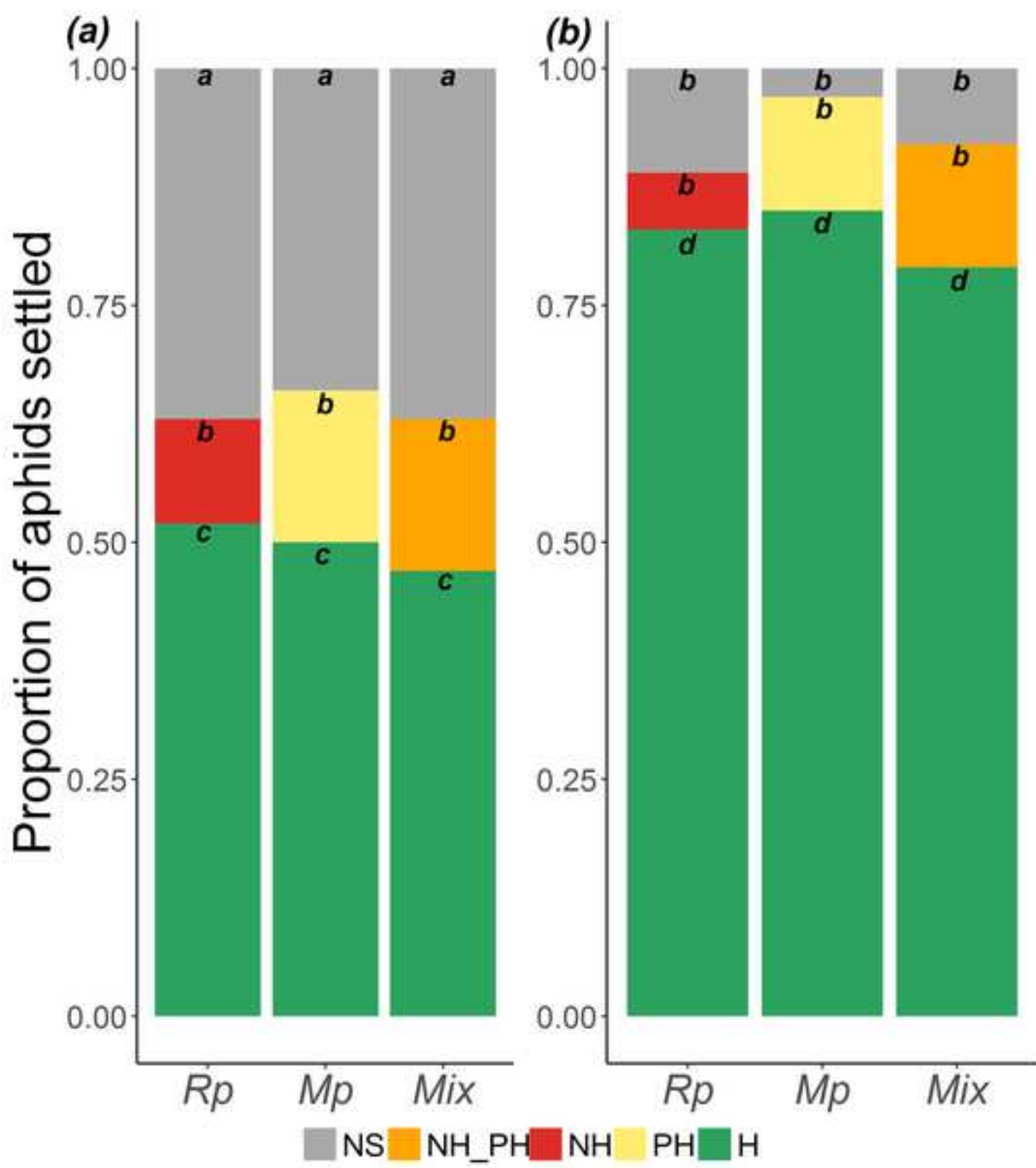


Figure 3

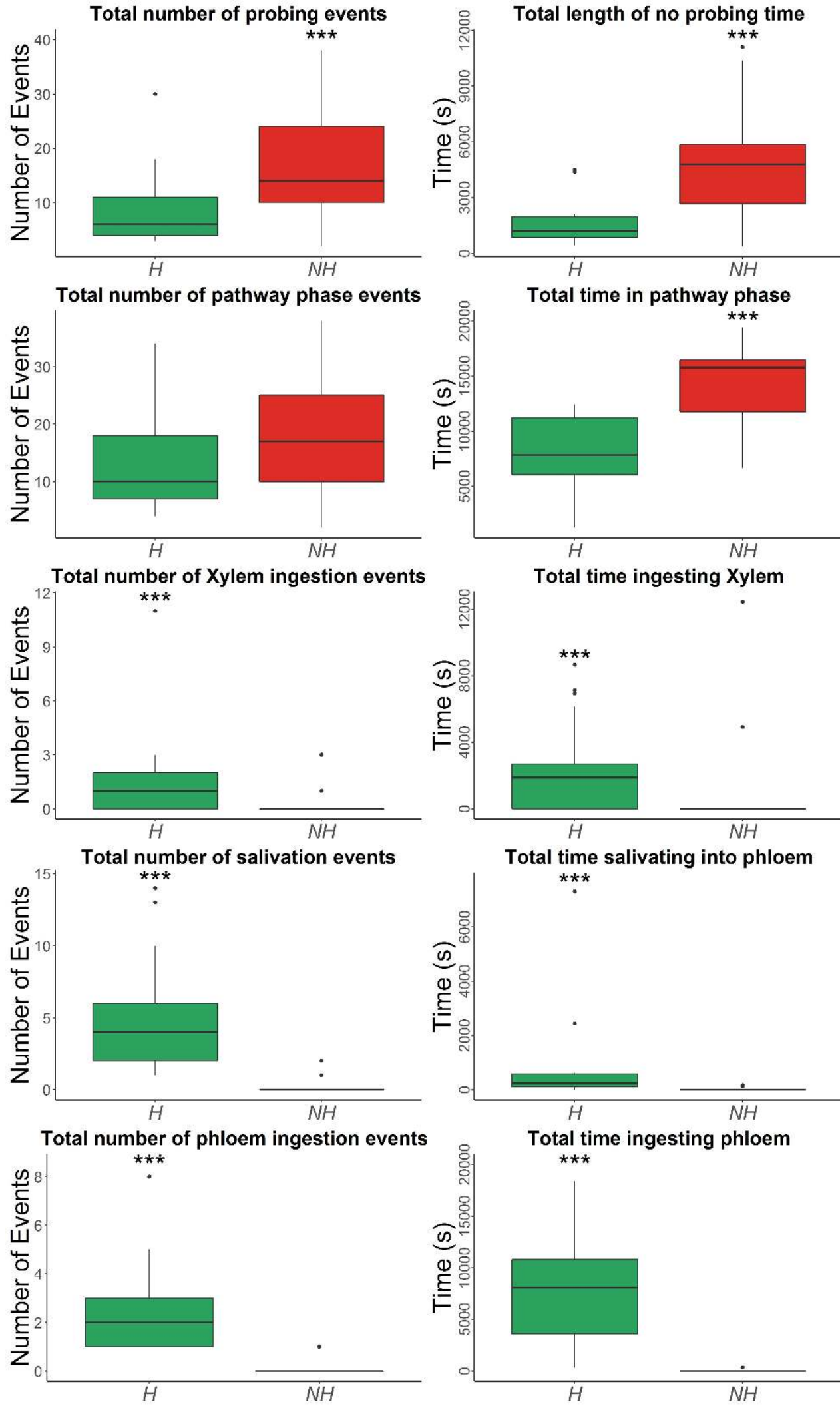


Figure 4

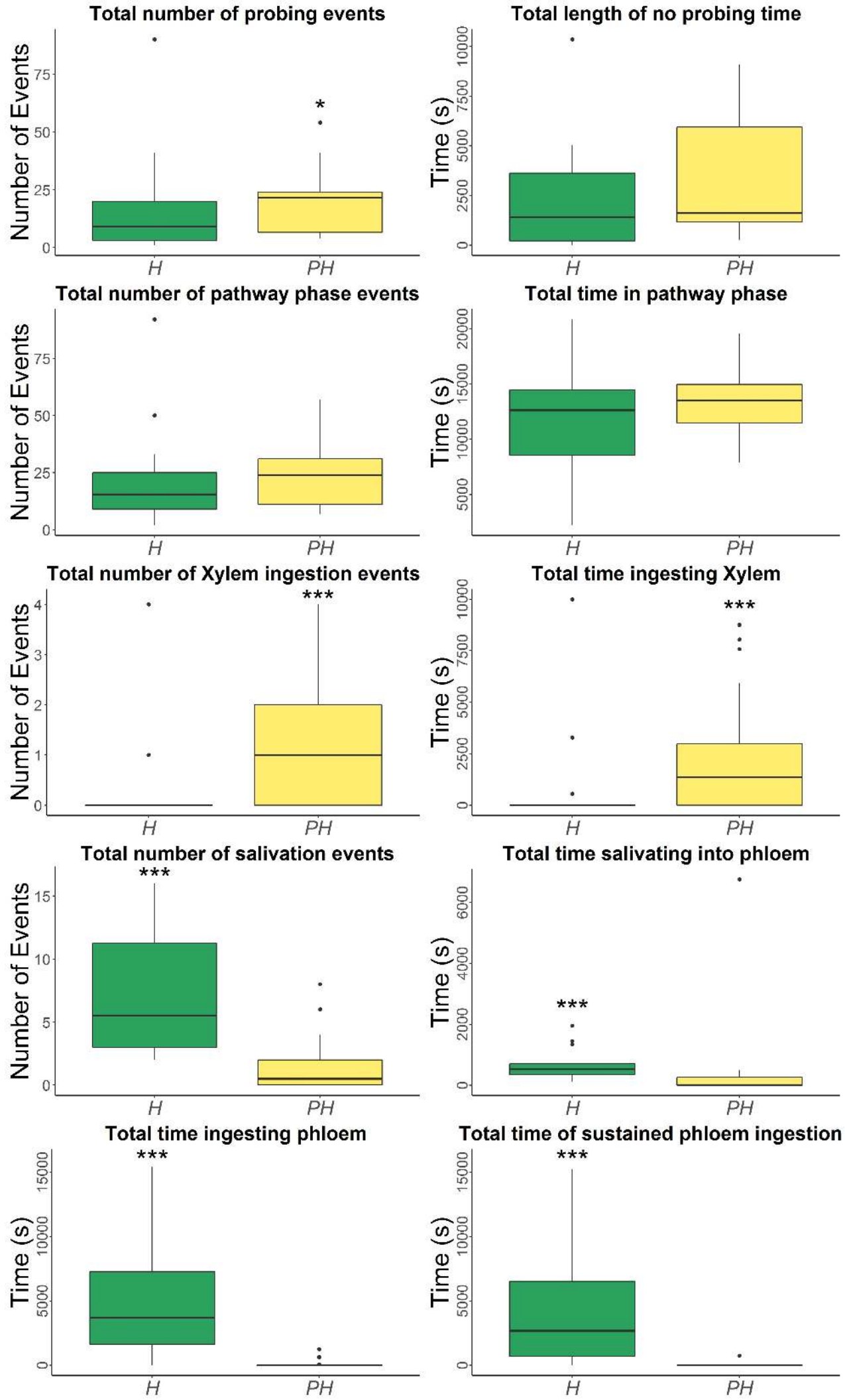


Figure 5

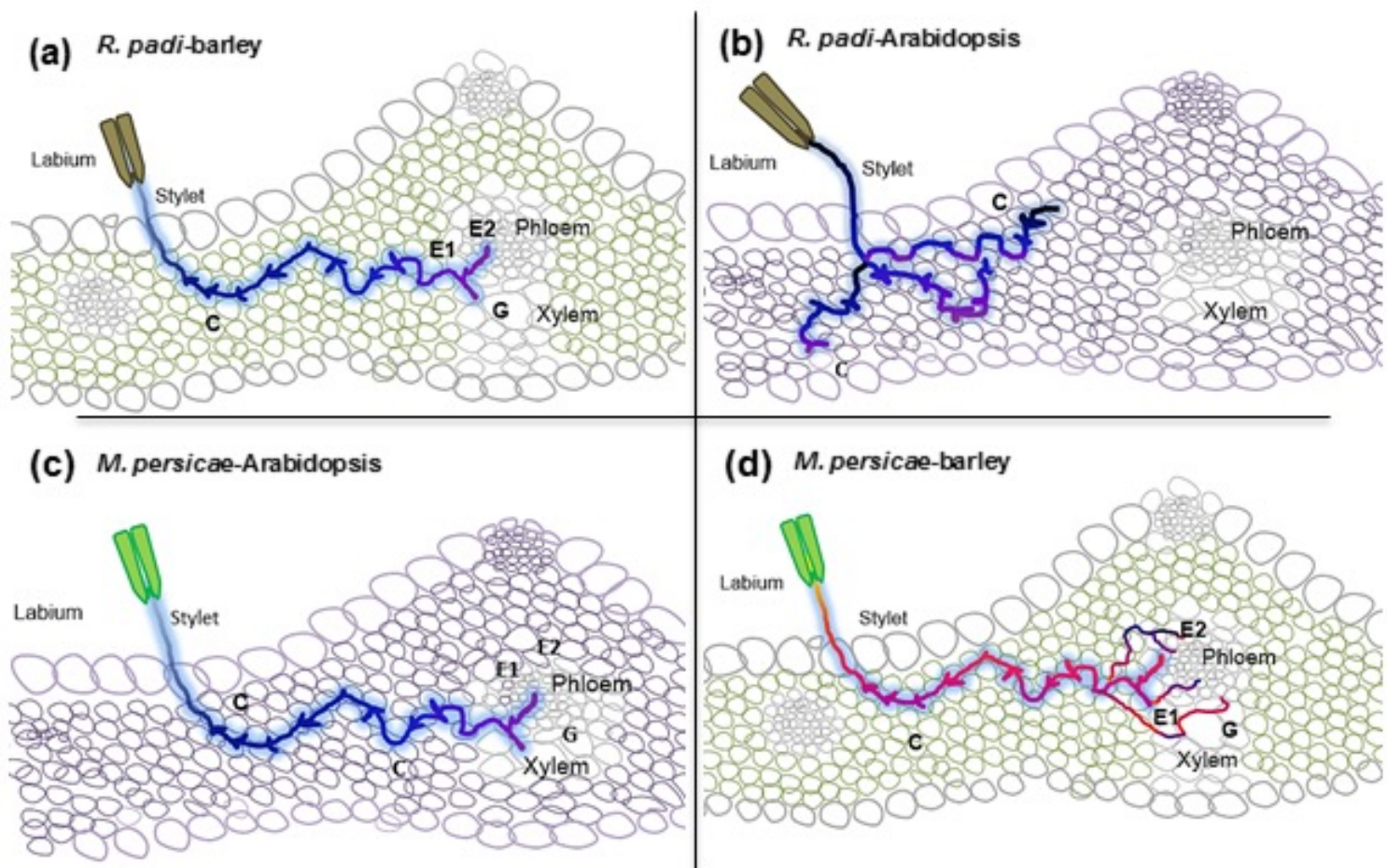


Table S1. Results for an obtained Electrical Penetration Graph (EPG) parameters which were significantly different and the plant tissue layer involved. Results displayed are the mean and standard deviation (SD) for each aphid-plant non/poor host comparison. p values in bold represent values significantly different in both host vs non-host and standard deviation of the 97 electrical EPG parameters calculated for *P. nudi* host (Pn_Hv) and non-host (P

EPG Variables	Description	Plant tissue layer(s) hypothesised to be involved in resistance trait	Average		
			Rp_Hv (Host)	Rp_At (Non host)	Mp_At (Host)
n_Np	number of non probing periods	Epidermis	8.17	17.84	16.15
d_1Pr	duration of 1st probe (s)	Epidermis	4822.06	2907.11	6941.74
s_Np	sum of non probing time (s)	Epidermis	1766.80	4888.50	2275.40
a_Pr	average probe time (s)	Epidermis/Mesophyll	3435.50	1614.10	5961.20
n_Pr	number of probing events	Epidermis/Mesophyll	8.17	17.94	16.05
s_Pr	sum of probing time (s)	Epidermis/Mesophyll	19826.00	16599.00	19322.00
m_Pr	median probe time (s)	Epidermis/Mesophyll	1141.00	981.47	5510.99
n_Pr_1sE2	number of probes before 1st sE2	Epidermis/Mesophyll/Sieve Element/Phloem	5.17	0.00	2.80
n_Pr_1E2	number of probes to the 1st E2	Epidermis/Mesophyll/Sieve Element/Phloem	2.33	1.41	4.50
nPr_1sE2	number of probes after 1st sE2	Epidermis/Mesophyll/Sieve Element/Phloem	2.00	0.00	2.20
n_Pr_1E	number of probes before the 1st E1 phase	Epidermis/Mesophyll/Sieve Element/Phloem	2.33	2.00	3.45
n_bPr_1E	number of brief probes < 3 min before 1st E1 phase (s)	Epidermis/Mesophyll/Sieve Element/Phloem	0.83	0.76	2.10
nPr_1G	number of probes before the first G phase	Epidermis/Mesophyll/Xylem	3.50	0.47	0.75
t_1G	time to the first G phase after first penetration (s)	Epidermis/Mesophyll/Xylem	14208.00	19191.00	19916.00
n_E1e	number of E1 extracellular salivation (E1e) events	Mesophyll	0.17	0.12	0.00
at_1pd_Pr	average time to 1st pd in all probes with a pd (s)	Mesophyll	97.04	193.83	1382.10
mt_1pd_Pr	median time to 1st pd in all probes with a pd (s)	Mesophyll	59.58	99.32	1361.34
a_E1e	average length of E1e event (s)	Mesophyll	98.06	24.05	0.00
m_E1e	median length of E1e event (s)	Mesophyll	98.06	24.05	0.00
n_pd_minC	no. pd per min of C, C phases with pd	Mesophyll	0.68	0.34	0.73
s_C	sum time in C (s)	Mesophyll	6237.00	14128.00	11879.00
n_pd_minC_pd	no. pd per min of C	Mesophyll	0.73	0.42	0.81
rel_E2_1E2	E2 index: % (all time of E2 / Time to the start of 1st E2 from first penetration)	Mesophyll/Sieve Element/Phloem	0.62	0.04	0.49

rel_E2_C	SE ingestion/pathway ratio as %	Mesophyll/Sieve Element/Phloem	3.42	0.00	0.81
n_frE1_n_E12	phloem phase fractioning	Mesophyll/Sieve Element/Phloem	1.00	0.12	1.01
rel_E1_allE	E1 index:duration E1/ allE as %	Mesophyll/Sieve Element/Phloem	0.01	0.02	0.17
t_1E	time to 1st E1 phase from the 1st probe (s)	Epidermis/Mesophyll/Sieve Element/Phloem	4969.60	20794.00	6393.00
n_G	number of G phase events	Mesophyll/Xylem	0.50	0.24	0.30
a_G	average time in G (s)	Mesophyll/Xylem	2966.01	828.10	316.70
m_G	median time in G (s)	Mesophyll/Xylem	2966.01	829.50	314.10
s_G	sum of time spent in G (s)	Mesophyll/Xylem	1483.00	1021.00	691.30
t_C_1E_1Pr	time to 1st E1 within the 1st probe with an E phase (s)	Mesophyll/Sieve Element/Phloem	887.40	478.60	2212.00
at_C_1E_Pr	average time to 1st E1 phase within probes (s)	Mesophyll/Sieve Element/Phloem	1172.20	478.60	2074.00
mn_C_1E_Pr	minimum time to 1st E1 phase within probes (s)	Mesophyll/Sieve Element/Phloem	967.00	478.60	1791.30
s_E2	sum of E2 (s)	Sieve Element/Phloem	10030.00	19.36	5064.00
mx_E2	maximum E2 phase (s)	Sieve Element/Phloem	9256.00	19.36	2250.40
m_E2	median length of E2 phase (s)	Sieve Element/Phloem	5149.57	19.36	924.60
a_E2	average length of E2 phase (s)	Sieve Element/Phloem	6379.00	19.36	1108.30
n_E12	E12: number of phloem periods with both E1 and E2	Sieve Element/Phloem	3.00	0.06	5.60
n_E2	number of E2 phases	Sieve Element/Phloem	3.00	0.06	5.70
s_E12	sum of E12 (s)	Sieve Element/Phloem	10107.00	26.42	5512.00
n_frE1	number of fractions of E1; E1 followed/preceded by E2	Sieve Element/Phloem	3.00	0.12	5.50
s_sE2	sum of duration of sE2 (s)	Sieve Element/Phloem	9760.00	0.00	4321.60
a_sE2	mean duration of sE2 phase (s)	Sieve Element/Phloem	9175.00	0.00	1699.50
m_sgE2	median duration of sE2 phase (s)	Sieve Element/Phloem	9175.00	0.00	1589.30
n_sE2	number of sustained E2 phases, sE2 - longer than 10 min	Sieve Element/Phloem	1.17	0.00	2.10
a_E12	average length of E12 (s)	Sieve Element/Phloem	6404.00	26.42	1295.90
mx_E12	maximum E12 period (s)	Sieve Element/Phloem	9280.00	26.42	2425.00
m_E12	median length of E12 (s)	Sieve Element/Phloem	5178.62	26.42	1116.40
a_frE1	average fraction of E1 (s)	Sieve Element/Phloem	25.38	3.53	80.75
m_frE1	median fraction of E1 (s)	Sieve Element/Phloem	23.88	3.53	66.27
s_E1_1sE2	sum of E1 before 1st sE2 (s)	Sieve Element/Phloem	57.48	0.00	239.37

mx_frE1	maximum duration of a fraction of E1 (s)	Sieve Element/Phloem	31.82	5.67	186.60
s_frE1	sum of fractions of E1 (s)	Sieve Element/Phloem	77.19	7.06	447.20
n_E1	number of all E1 periods (sgE1 + frE1)	Sieve Element/Phloem	3.67	0.18	7.00
t_1E12	time to 1st E phase with E1 and E2 (s)	Sieve Element/Phloem	4969.60	21389.00	10116.00
t_1E2	time to 1st E2 phase (s)	Sieve Element/Phloem	4994.60	21394.00	10226.00
t_1E1_1E2	time from the 1st E1 to 1st E2 (s)	Sieve Element/Phloem	25.05	19504.42	3832.95
s_E1	sum of E1 (sgE1 and E1) (s)	Sieve Element/Phloem	93.08	16.79	651.80
t_1sE2	time to 1st sE2 phase (E2 > 10 min) (s)	Sieve Element/Phloem	8953.90	21423.00	13792.00
t_1E1_1sE2	time from the 1st E1 to 1st sE2 (s)	Sieve Element/Phloem	8953.90	21423.00	13792.00
mx_E1	maximum E1 phase (either sgE1 or frE1) (s)	Sieve Element/Phloem	31.82	15.39	287.57
a_E1	average E1 length (s)	Sieve Element/Phloem	24.65	13.26	112.30
m_E1	median E1 (sgE1 and E1fr) length (s)	Sieve Element/Phloem	23.78	13.26	70.37
a_E2_1sE2	mean duration of E2 periods before the 1st sE2 (s)	Sieve Element/Phloem	101.51	0.00	78.22
m_sgE1	median length of single E1 phase (s)	Sieve Element/Phloem	24.20	9.73	65.21
n_sgE1	number of single E1 phases (without E2)	Sieve Element/Phloem	0.67	0.06	1.50
n_E2_1sE2	number of E2 before the 1st sE2	Sieve Element/Phloem	0.83	0.00	1.10
s_sgE1	sum of single E1 phase (s)	Sieve Element/Phloem	15.88	9.73	204.58
a_sgE1	average length of single E1 phase (s)	Sieve Element/Phloem	24.20	9.73	90.61
mx_sgE1	maximum duration of a single E1 phase (s)	Sieve Element/Phloem	25.78	9.73	157.13

in between host and non/poor-host feeding. Table displays the EPG parameter assessed, a distinct plant combination for each parameter alongside the Wilcoxon test statistic (W value) and p value for host vs poor-host interactions, italicised p values represent parameters which only differed in (Poor-At). Average and standard deviation of the 97 electrical EPG parameters calculated for M. persicae on 10 plant combinations.

Aphid-Plant combination					W value
	SD				
Mp_Hv (Poor-host)	Rp_Hv (Host)	Rp_At (Non-host)	Mp_At (Host)	Mp_Hv (Poor-host)	Rp Host vs Non-host
19.37	6.85	10.29	21.40	12.73	52.50
2706.55	5857.48	5349.83	7703.36	3811.21	146.00
3129.90	1194.35	2856.64	2613.81	2674.35	33.00
1781.90	1887.40	2095.19	7332.87	1590.60	241.00
19.30	6.85	10.29	21.28	12.70	52.50
18468.00	1198.61	2809.03	2613.68	2673.50	256.00
798.70	1596.02	2195.37	7682.26	955.55	224.00
0.20	7.43	0.00	4.72	0.76	255.00
0.43	6.75	5.82	7.24	1.04	249.00
1.33	3.12	0.00	4.48	5.07	255.00
2.10	2.80	6.16	7.16	4.16	233.50
0.73	1.63	2.22	6.28	1.72	196.00
5.43	3.79	1.70	2.90	5.91	231.00
12294.00	8540.93	6274.34	4790.88	7598.14	62.00
0.40	0.39	0.49	0.00	1.10	160.00
194.73	529.91	169.20	4028.53	126.87	104.00
121.75	69.74	96.34	4035.52	88.73	141.00
80.62	142.06	99.15	0.00	211.95	161.00
68.81	142.06	99.15	0.00	178.45	161.00
0.55	0.20	0.22	0.28	0.26	235.00
13328.00	3253.10	3847.96	4553.67	3007.80	37.00
0.63	0.22	0.21	0.35	0.29	226.00
0.01	0.33	0.16	0.34	0.02	282.00

0.01	3.30	0.01	1.54	0.03	288.00
0.37	0.16	0.49	0.36	0.89	272.00
0.11	0.20	0.06	0.18	0.28	144.50
13851.00	4972.13	2422.84	5262.30	8546.79	2
1.33	2.59	0.75	0.92	1.32	228.50
1394.30	2095.70	3018.18	897.82	1885.06	224.50
1333.00	2095.42	3018.58	891.22	1901.30	223.00
2321.00	2907.64	3173.97	2309.20	2825.99	221.50
583.44	704.96	1621.98	3422.58	737.43	257.00
528.70	1078.38	1621.98	3399.93	768.22	229.00
462.10	817.26	1621.98	3461.42	712.01	228.00
126.00	4554.83	79.81	4515.20	339.29	288.00
79.52	4959.91	79.81	2049.53	209.71	288.00
31.78	5519.01	79.81	1105.35	85.51	285.00
38.45	5210.59	79.81	1139.63	97.60	288.00
0.33	2.24	0.24	4.10	0.76	285.50
0.53	2.31	0.24	4.16	1.36	285.50
584.00	4657.25	108.92	4789.43	1791.34	289.00
0.73	2.31	0.49	3.79	1.78	280.00
49.11	4666.90	0.00	4669.20	186.91	280.50
50.81	5133.28	0.00	1755.88	189.98	280.50
50.81	5192.50	0.00	1745.54	189.98	280.50
0.07	0.94	0.00	2.13	0.25	280.50
292.00	5554.52	108.92	1329.75	895.67	288.00
529.30	5116.08	108.92	2099.45	1722.44	288.00
292.00	5902.09	108.92	1321.60	895.67	284.00
112.40	1752.36	14.56	47.26	401.35	278.00
16.34	1754.06	14.56	42.54	40.97	278.00
26.88	1765.18	0.00	213.80	102.29	280.50

425.90	1768.30	23.37	190.29	1548.02	277.00
458.00	1752.36	29.11	399.26	1605.58	280.00
1.73	4.13	0.53	4.27	2.55	282.00
18509.00	5685.82	326.91	7042.07	6799.75	0.00
18532.00	5746.30	313.90	7028.75	6746.74	0.00
15452.05	4242.11	5707.93	5656.93	8278.80	10.00
561.93	1758.33	48.06	508.89	1690.16	278.00
20407.00	6344.60	270.10	7385.88	4159.06	5.00
20407.00	6344.60	270.10	7385.88	4159.06	5.00
471.21	1758.45	45.13	389.14	1510.05	270.00
111.86	1750.70	41.80	132.40	220.20	266.00
60.24	1755.11	41.80	39.60	94.92	264.00
8.18	155.53	0.00	181.71	31.14	170.00
54.66	62.08	40.10	46.68	95.86	226.00
1.00	3.17	0.24	1.15	1.44	232.50
0.27	0.71	0.00	2.95	1.01	187.00
103.90	181.44	40.10	428.62	145.42	229.00
57.38	61.97	40.10	138.98	95.99	226.00
71.00	99.70	40.10	380.98	105.74	226.00

description of the parameter,
 a for each pairwise host vs
 one combination. Average
 vs poor host (Mp, At) and

value	p value	
	Mp Host vs Poor-host	Rp Host vs Non-host
187.00	0.0016	0.02564
412.00	0.9729	0.0272
222.00	4.20E-05	0.1248
422.00	0.0005557	0.0161
186.00	0.0016	0.02432
378.00	4.20E-05	0.1248
371.00	0.005394	0.1626
520.00	1.44E-05	2.61E-07
473.50	7.61E-05	8.53E-05
434.00	1.44E-05	0.000414
340.50	0.0009351	0.3873
308.00	0.03419	0.8597
122.50	0.0008919	0.0001523
483.00	0.004674	0.0003003
528.00	0.3555	4.67E-06
133.00	0.1705	0.0009735
149.00	0.9188	0.002872
410.00	0.3248	0.02791
408.00	0.3248	0.03089
409.00	0.001326	0.03162
242.00	8.94E-05	0.2547
395.00	0.00425	0.06122
578.50	5.94E-07	2.87E-09

570.50	1.96E-07	8.02E-09
499.00	8.90E-07	1.54E-05
491.50	NA	4.50E-05
148	9.844E-07	0.002691
133.50	0.001187	0.0003352
137.50	0.002158	0.0005387
139.50	0.002453	0.0006307
142.50	0.003159	0.0007967
486.00	5.48E-05	0.0001956
474.00	0.001454	0.0004085
455.00	0.001656	0.001647
573.50	1.96E-07	5.47E-09
570.50	1.96E-07	8.02E-09
566.50	3.51E-07	1.33E-08
562.50	1.96E-07	2.18E-08
560.00	2.53E-07	2.83E-08
552.50	2.54E-07	7.06E-08
551.50	1.61E-07	8.22E-08
544.50	7.61E-07	1.81E-07
520.00	4.27E-07	2.61E-07
502.50	4.27E-07	3.83E-07
502.50	4.27E-07	3.83E-07
515.00	2.46E-07	4.58E-07
534.50	1.96E-07	5.76E-07
515.00	1.96E-07	9.37E-07
526.50	4.25E-07	1.38E-06
525.50	1.30E-06	1.53E-06
520.50	1.30E-06	2.60E-06
500.00	4.27E-07	2.86E-06

504.00	1.56E-06	3.10E-06
517.50	7.48E-07	3.56E-06
528.00	7.46E-07	4.67E-06
80.00	6.92E-07	1.37E-05
80.00	6.92E-07	1.37E-05
84.00	3.86E-06	1.97E-05
500.00	1.66E-06	6.18E-05
120.50	1.66E-06	0.0003904
120.50	1.66E-06	0.0003904
449.00	6.72E-06	0.002867
410.00	1.31E-05	0.02791
408.00	1.82E-05	0.03089
370.00	0.0121	0.03103
405.50	0.001088	0.0316
400.00	0.0003946	0.03871
366.00	0.01877	0.04206
382.50	<i>0.0007049</i>	0.09323
390.50	<i>0.001088</i>	0.06542
376.50	<i>0.001088</i>	0.1198

Table S2. Results for an obtained electrical penetration graph (EPG) parameters which were not significantly different between host and non/pd description of the parameter, and the plant tissue layer involved. Results displayed are the mean and standard deviation (SD) for each aphid-p test statistic (W value) and p value for each pairwise host vs non/poor host comparison. p values in bold represent values significantly different. Italicised p values represent parameters which only differed in one combination. Average and standard deviation of the 26 electrical EPG parameters calculated for *M. persicae* host (*Mp_At*) and poor host (*Mp_Hv*) (*Rp_At*). Average and standard deviation of the 07 electrical EPG parameters calculated for *M. persicae* host (*Mp_At*) and poor host (*Mp_Hv*)

EPG Variable	Description	Plant tissue layer(s) hypothesised to be involved	Aphid-Plant combination					
			Average				SI	
			<i>Rp_Hv</i> (Host)	<i>Rp_At</i> (Non-host)	<i>Mp_At</i> (Host)	<i>Mp_Hv</i> (Poor-host)	<i>Rp_Hv</i> (Host)	<i>Rp_At</i> (Non-host)
t_1Pr	time to 1st probe (s)	Epidermis	106.28	176.79	67.52	95.30	137.95	270.10
a_Np	average non probing time (s)	Epidermis	210.91	388.73	203.69	156.43	106.58	388.88
m_Np	median non probing time (s)	Epidermis	204.61	226.01	79.21	86.06	113.44	312.37
n_bPr	number of brief probes (probes < 180 s)	Epidermis/Mesophyll	2.67	5.82	8.85	8.33	4.51	

d_2pd	duration of the second pd (s)	Mesophyll	6.09	4.93	5.37	4.99	2.01	3.90
n_C	number of C phase events	Mesophyll	12.33	18.59	22.90	23.03	8.33	10.09
m_pd	median duration of pd (s)	Mesophyll	4.81	4.39	4.77	4.95	0.42	1.76
n_pd_1Pr	no. pd in 1st probe	Mesophyll	10.83	17.18	23.45	11.60	8.65	37.78
n_Pr_1pd	number of probes before 1st pd	Mesophyll	0.88	0.65	0.60	0.47	0.33	0.49
d_1pd	duration of the first pd (s)	Mesophyll	4.17	11.40	5.55	5.30	1.75	24.15
s_E1e	sum of E1e (s)	Mesophyll	16.34	48.09	0.00	254.40	142.06	198.29
t_1pd	time to 1st pd (from start of 1st probe) (s)	Mesophyll	423.13	652.07	1617.90	325.52	414.62	1125.28
s_pd	sum of pd (s)	Mesophyll	346.73	403.60	678.00	593.02	175.87	456.21
a_C	average C phase length (s); with pd without E1e, F and G	Mesophyll	505.00	1361.80	1108.10	754.60	383.54	2132.67
a_pd	average duration of pd (s)	Mesophyll	5.10	5.30	5.01	5.18	0.55	2.92
n_pd	number of pd	Mesophyll	68.00	78.12	136.80	117.50	37.41	54.21

a_F	average length of F (s)	Mesophyll	1963.90	717.10	1017.00	680.30	1453.37	1506.79
m_F	median length of F (s)	Mesophyll	1963.90	695.00	1017.00	636.20	1453.37	1500.47
n_F	number of F phase events	Mesophyll	1.00	0.59	0.20	0.93	0.80	1.33
t_1pd_1pr	time to 1st pd in 1st probe with a pd (s)	Mesophyll	284.76	223.57	1399.98	215.15	196.54	209.65
mnt_1pd_1Pr	min. time to 1st pd in 1st probe (s)	Mesophyll	7.78	29.23	1337.09	46.45	55.27	49.32
s_F	sum of time in F (s)	Mesophyll	1963.90	1366.00	1017.00	1870.00	2065.16	2586.60
m_C	median C time (s)	Mesophyll	318.35	982.60	688.67	426.58	370.70	2193.29
d_pd5	mean duration of the first 5 pd (s)	Mesophyll	5.03	6.32	5.23	5.07	1.18	5.46
rel_Prob_pd	relation of probes with pd	Mesophyll	1.00	1.00	1.00	1.00	0.00	0.00

poor-host feeding. Table displays the ERG parameter assessed, a plant combination for each parameter alongside the Wilcoxon test in both host vs non-host and host vs poor-host interactions, parameters calculated for R. padi host (Rp_Hv) and non-host (Mp_Hv). Calculations were made with summary statistics in Rstudio. The

D		W value		p-value	
Mp_At (Host)	Mp_Hv (Poor-host)	Rp Host vs Non-host	Mp Host vs Poor-host	Rp Host vs Non-host	Mp Host vs Poor-host
97.78	115.15	189	238.5	0.128	0.2265
335.34	91.76	111.00	223.00	0.2594	0.1297
55.72	39.66	157.00	254.00	0.6832	0.3674
17.39	7.92	120.50	204.50	0.4112	0.05712

1.06	1.80	201.5	351	0.05163	0.3172
21.03	364, 12.44	90.50	247.00	0.06471	0.2978
0.53	0.72	198	257	0.06749	0.3997
20.38	11.00	193	391	0.09542	0.07223
0.50	0.51	178.5	340	0.1165	0.3661
1.69	1.72	105	331.5	0.1816	0.5392
0.00	737.29	160	260	0.3559	0.09633
4041.70	333.96	171	254	0.3753	0.3674
356.37	231.82	171	320	0.3753	0.6993
1841.61	394.12	120.00	293.00	0.4134	0.8976
0.60	0.83	161	250	0.5861	0.3268
68.15	44.40	153.5	335	0.7696	0.4942

2852.89	1247.40	151.00	256.00	0.7901	0.2878
2852.89	1193.74	151.00	258.00	0.7901	0.3106
0.41	1.60	150.00	234.00	0.8239	0.1087
4023.20	201.69	149	240	0.8919	0.2386
4043.69	68.18	148	221.5	0.9177	0.1223
2852.89	3984.15	147.00	246.00	0.9293	0.1911
1895.77	351.70	142.00	239.00	0.9458	0.2308
0.74	1.00	142	329	0.9458	0.5724
0.00	0.00	144.5	300	NA	NA

```
wilcox.test(Mp_host$t_1Pd, Mp_poorhost$t_1Pd, conf.int = FALSE, conf.level = 0.95)
```