Non-host and poor-host resistance against aphids may reside in different plant cell layers depending on the plant species-aphid species interaction

Carmen Escudero-Martinez[1,2§], Daniel J. Leybourne[1,2§], Jorunn I.B. Bos[1,2*]

[1]Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

[2]Division of Plant Sciences, School of Life Sciences, University of Dundee, Dundee

§these authors contributed equally

*Corresponding Author:

j.bos@dundee.ac.uk

Abstract

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. Here we use the Electrical Penetration Graph technique to study aphid probing and feeding behaviour on poor- and non-host plants for the broad host range pest *Myzus persicae* and the cereal pest *Rhopalosiphum padi*. In the Arabidopsis non-host interaction with the cereal pest *R. padi* 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 *M. persicae*, resistance is likely to be 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.

Introduction

Aphids are important insect pests which cause significant yield losses to crops globally [1]. There are approximately 5000 aphid species described and around 250 of these are important agricultural and horticultural pests which vary in their host range – the ability to successfully infest different plant species. This host range variation generally applies to secondary hosts during summer

41 months, where aphid populations increase rapidly due to asexual reproduction
42 [2]. Whilst the majority of aphid species exhibit a limited host range, dedicated
43 to few closely related plant species, some aphid species, like *Myzus persicae*
44 (green peach aphid), have an exceptionally broad host range which includes
45 representatives from more than 40 plant families [1,3]. The evolutionary drivers
46 and molecular determinants of such exceptionally broad host ranges in aphids
47 remain to be elucidated.

48 Host suitability relies on a number of factors, which could be based either at
49 the plant surface or within plant tissues and cells [3]. Prior to probing the leaf
50 surface aphid behaviour can be influenced by a range of these factors
51 including leaf colour, emitted volatile organic compounds and leaf surface
52 components, such as epicuticular waxes or trichomes [4-6]. Regardless of
53 whether the aphid encounters a host or non-host plant species their
54 specialised mouthparts, known as stylets, are utilised to probe into the plant
55 tissue [3,7,8]. This probing behaviour is associated with the transmission of
56 important plant viruses during both host and non-host interactions [3,9-11] which
57 can substantially reduce crop yields [12]. During interactions with susceptible
58 plant species the aphid stylets penetrate the plant epidermis and move
59 through the plant tissue towards the vascular bundle. During this process the
60 stylets probe into adjacent plant cells, and saliva is secreted both in the
61 apoplast into probed cells along the stylet-pathway [13,14]. During compatible
62 plant-aphid interactions the aphid stylets are able to successfully puncture the
63 sieve-tube elements to facilitate ingestion of phloem sap [13,15].

The aphid stylet-pathway through the plant tissue has been well-characterised during interactions with susceptible plants using the Electrical Penetration Graph (EPG) technique. This technique uses an electrical circuit to connect the aphid to the plant via a series of electrical probes, allowing distinction between different phases of the stylet pathway from obtained electrical waveforms which correlate with the position of the aphid stylet within plant tissue in real-time [14,16-18]. Briefly, the aphid is attached to an electrical probe with gold wire, and a copper electrode is placed into the soil to incorporate the plant into the electrical system. Both the plant and the aphid electrodes are attached to a data-logger which is read by computational software and the whole set-up is contained in a grounded Faraday cage [17-20]. Once the aphid probes the plant tissue the circuit closes and changes in electrical voltage are displayed as alternating waveforms which can be manually annotated using computational software and translated into time-series data [14]. The biological relevance of the different waveforms that are detected by the EPG technique have been extensively analysed [16-18,20]. Waveforms associated with aphid probing are: waveform np, representing non-probing behaviour where the stylets are not in contact with the leaf surface; waveform C, which begins upon stylet penetration of leaf tissue and is correlated with the intercellular apoplastic stylet pathway located at the epidermis or the mesophyll cell layers; waveform pd, associated with piercing of a plant cell which leads to a signal potential drop; waveform F, which reflects stylet mechanical/penetration difficulties; and waveform E1e, which represents extracellular saliva secretion into plant tissues other than phloem. Waveforms associated with vascular interactions and which provide intricate information at the aphid feeding site

are: waveform G, which represents aphids drinking from the xylem sap; waveform E1, which is linked to aphid salivation into phloem before ingestion; and waveform E2, which corresponds to phloem sap ingestion [21]. A graphical representation of examples of these waveforms, alongside the stylet activity during each, is shown in Fig. 1.

Although the EPG technique has mainly been used to study aphid interactions with susceptible and (partially-)resistant genotypes of host plant species, it also represents a suitable tool to explore how aphids interact with plants which are not natural hosts, including non-host and poor-host species. By characterising aphid probing and feeding behaviour on non-/poor-host species we will generate a better understanding of where associated resistance mechanisms reside. This in turn will facilitate important mechanistic studies to reveal the molecular determinants of plant immunity to aphids.

We previously showed that *M. persicae*, which is not a pest of barley, is able to feed and reproduce on this crop under controlled environment conditions, but to a lower extent than on a host species such as oil seed rape or Arabidopsis [7]. On the contrary, *Rhopalosiphum padi* (bird cherry-oat aphid) is a pest of barley but is unable to feed from, and therefore survive, on Arabidopsis [8]. However, in both the *M. persicae*-barley poor-host interaction and the *R. padi*-Arabidopsis non-host interaction probing of the leaf surface takes place [7,8]. Here, we made use of the different host and non-/poor-host combinations of *M. persicae* and *R. padi* with Arabidopsis and barley to explore aphid probing and feeding behaviour during these different interactions. We show that resistance in the non-/poor-host interactions can

113 reside in different plant cell layers, suggesting complex mechanisms may
114 underlie plant immunity to aphids.

115 **Results**

116 **The Arabidopsis-*R. padi* non-host interaction is characterised by long**
117 **no-probing periods and difficulties to locate the vascular tissues**

118 We used the EPG technique to monitor *R. padi* probing and feeding behaviour
119 for the first 6 hours on either Arabidopsis (non-host) or barley cv. Golden
120 Promise (host) and found significant differences with regards to parameters
121 relating to probing and interactions with the plant vasculature (Fig. 2). The
122 statistical results for all EPG parameters, which were significantly different in
123 host vs non/poor-host interactions (71/97 tested parameters) are displayed in
124 Table S1, with the statistical results for all non-significant parameters (26/97
125 tested) shown in Table S2.

126 In general, probing parameters which differed for *R. padi* when interacting
127 with non-host versus host plants were non-probing periods, number of probing
128 events, and time spent in the epidermal/mesophyll cells (C phase) (Fig. 2a;
129 Table S1). In the non-host interaction, the total time the aphids were not
130 probing during the 6 h recording was over 2.5 times greater (4889s) than the
131 host interaction (1767s) (Fig. 2a; Table S1; $W = 33.00$; $p = <0.001$). Also,
132 aphids probed non-host plants more frequently (18 attempts) than host plants
133 (8 attempts) (Fig. 2a; Table S1; $W = 52.50$, $p = 0.001$). Although the total
134 number of C phases (stylet activity at the epidermis/mesophyll) was not
135 different between non-host and host interactions, the overall time spent in the
136 epidermis/mesophyll (C phase) was over two times longer in the non-host

137 (14128s) compared with the host interaction (6237s) (Fig. 2a; Table S1; W =
138 37.00; p = <0.001). All the vascular-related parameters (G, E1 salivation and
139 E2 ingestion phases) measured for *R. padi* were significantly reduced during
140 the non-host compared with the host interaction (Fig. 2b; Table S1).
141 Additionally, the number of xylem events was halved during the non-host
142 interaction (0.24 times) compared with the host interaction (0.50 times) (Fig.
143 2b; Table S1; W = 2.28.50; p = 0.001). The total length of xylem ingestion (G
144 phase) was significantly shorter on the non-host (1021s) compared with the
145 host plants (1483s) (Fig. 2b; Table S1; W = 221.50; p = 0.003). We observed
146 significantly fewer salivation events (E1 phase) during the non-host interaction
147 (0.18 events) compared with the host interaction (3.67 events; W = 282.00; p
148 = <0.001), and salivation events were five-fold shorter during the non-host
149 (18s) versus host (93s) interactions (Fig. 2b; Table S1; W = 278.00; p =
150 <0.001). Ingestion of phloem sap (E2 phase) was rarely observed during the
151 non-host interaction (0.06 times) compared with the host interaction (3 times;
152 W = 285.00; p = <0.001), and the total duration of this ingestion period was
153 greatly reduced on non-host (19s) versus host plants (10030s, or 2.78 hours)
154 (Fig. 2b; Table S1; W = 288.00; p = <0.001).

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

157 Similar to the EPG assays performed with *R. padi*, we also assessed probing
158 and feeding of *M. persicae* on host plants (Arabidopsis) and poor-host plants
159 (barley cv. Golden Promise) over a 6-hour period (Fig. 3). Within the probing
160 parameters, we observed significant differences in the number of probing
161 attempts on poor-host compared with host plants, while the non-probing time,

162 and number and time of events associated with stylet activity in the epidermal
163 and mesophyll tissue (C phase) were similar (Table S2; Fig. 3b). In contrast,
164 the differences between *M. persicae* interactions with poor-host versus host
165 plants were primarily at the vascular level with aphids spending extensive
166 periods of time in the xylem and showing reduced phloem ingestion on poor-
167 host plants (Table S1; Fig. 3b). During the poor-host interaction, there was a
168 significant increase in the number of aphid probing attempts (19 attempts)
169 compared with the host interaction (16 attempts) (Fig. 3a; Table S1; W =
170 186.00; p = 0.024). The time spent in the non-probing phase was longer in the
171 poor-host interaction (3130s) than the host interaction (2275s), but this
172 difference was not statistically significant (Fig. 3a; Table S2). The aphids
173 spent a similar amount of time in the C phase, which lasted 13328s during the
174 poor-host and 11879s during the host interaction. Aphid stylet activities
175 related to the vascular parameters (G – xylem, E1 – phloem salivation, and
176 E2 – phloem ingestion) were different between host and poor-host
177 interactions (Fig. 3b; Table S1). The number of times that *M. persicae*
178 reached the xylem (G phase) during the poor-host interaction was higher
179 (1.33 times; W = 133.50; p = <0.001) and time of interaction was longer
180 (2321s; W = 142.50; p = <0.001) than during the host interaction, where
181 aphids reached the xylem 0.30 times and spent a total of 691s in the xylem
182 (Fig. 3b; Table S1).

183 For the E1 salivation phase the number and duration of events was reduced
184 during the poor-host interaction, with 1.73 events (W = 5.28; p = <0.001), with
185 a total length of time spent salivating into the phloem of 562s (W = 500.00; p =

186 <0.001), compared with the host interaction (7 events with a time length of
187 652s) (Fig. 3b; Table S1).

188 *M. persicae* showed limited ingestion periods during the poor-host compared
189 with host interactions. The number of E2 phases and their length was greatly
190 reduced on poor-host plants, with 0.53 events (W = 552.50; p = <0.001) with a
191 40-fold decrease in the total time spent ingesting phloem (126s; W = 573.50;
192 p = <0.001), compared with host plants (5.7 events with a total length of
193 5064s) (Fig. 3b; Table S1). Moreover, on the poor-host sustained phloem
194 ingestion was severely lacking, and aphids spent only 49s in the E2 ingestion
195 phase on poor-host plants (W = 520.00; p= <0.001) with events being nearly
196 absent, 0.07 events (W = 515.00; p = <0.001). In contrast, aphids spent 4322s
197 in the E2 sustained ingestion phase on host plants over 2.1 events during the
198 6h recording (Fig. 3b; Table 1). Therefore, the *M. persicae* poor-host
199 interaction features substantially reduced phloem ingestion.

200 **Discussion**

201 The overall aim of this study was to characterise aphid probing and feeding
202 behaviour during host versus non/poor-host interactions in order to gain
203 insight into where resistances against aphids may reside within the plant
204 tissue. Our EPG analyses revealed that common features of the non-host and
205 poor-host interactions were an increased number of probing events and
206 longer no-probing periods. Importantly our data showed differences between
207 *R. padi* and *M. persicae* probing and feeding behaviour on the non/poor-host
208 plants. During the *R. padi*-Arabidopsis (non-host) interaction the aphids only
209 occasionally reached the vascular tissues. On the contrary, during the *M.*

210 *persicae*-barley interaction (poor-host) aphids reached the phloem but were
211 unable to ingest sap for prolonged periods of time. Based on the data
212 generated here for *M. persicae* and *R. padi* we propose a model wherein
213 poor- and non-host plant resistances against these aphid species may reside
214 within the phloem and mesophyll cell layers, respectively (Fig. 4).

215 During the *R. padi*-barley interaction (host interaction) the aphids spend less
216 time probing and in the pathway phase compared to the non-host interaction
217 with Arabidopsis, and readily reach the phloem where salivation and phloem
218 sap ingestion takes place for several hours (Fig. 4a). Occasionally, aphids will
219 drink from the xylem, which is thought to be important in coping with osmotic
220 effects associated with ingestion of large amounts of phloem sap [22,23]. In
221 contrast, *R. padi* shows increased probing behaviour on the non-host plant
222 Arabidopsis, as well as an extended stylet pathway phase, and only rarely
223 does the aphid reach the Arabidopsis phloem or xylem (Fig. 4b). On the
224 occasions where the *R. padi* stylets reach the vascular tissue during non-host
225 interactions the ingestion of sap on these occasions is not effective, in line
226 with this aphid being unable to survive on Arabidopsis [8]. Interestingly, *R. padi*
227 encountered more frequent stylet penetration difficulties when interacting with
228 Arabidopsis, as reflected by the F phase. This F phase is known to occur
229 exclusively at the mesophyll cell layers [20], suggesting that the non-host
230 resistance could reside there (Fig. 4b). Further research will be needed to
231 further understand the mechanisms underlying Arabidopsis non-host
232 resistance to *R. padi*, and to investigate the potential involvement of specific
233 recognition receptors within the mesophyll cell layer. Interestingly, the
234 NADPH oxidase *AtRbohF*, involved in ROS (Reactive Oxygen Species)

235 production, a member of the *LEA* (*Late Embryogenesis Abundant*) family,
236 implicated in abiotic and biotic stress, as well as the *VSP1* (*Vegetative*
237 *Storage Protein 1*), which is activated by jasmonate signalling, contribute to
238 Arabidopsis non-host resistance against *R. padi* [8]. Whether these genes act
239 within the mesophyll cell layer to activate defences against aphids remains to
240 be determined.

241 The *M. persicae*-Arabidopsis (host) interaction, features short probing and
242 pathway times, and prolonged salivation and ingestion once the phloem is
243 reached, as well as occasional xylem drinking (Fig. 4c). In contrast, during the
244 *M. persicae*-barley interaction (poor-host interaction) aphids show increased
245 probing but spend a similar time in the stylet pathway phase as aphids on
246 host Arabidopsis plants. The main differences between the Arabidopsis (host)
247 and barley (poor-host) interactions with *M. persicae* are reduced salivation in
248 the phloem and relatively short periods of phloem ingestion (less than 10
249 minutes) on barley (Fig. 4c and d). It is likely that this reduced phloem sap
250 ingestion is responsible for the reduced *M. persicae* performance on barley
251 [7,24]. It is possible that *M. persicae* attempts to compensate for this reduced
252 ingestion of phloem sap with increased xylem drinking, in line with the
253 observation that aphid starvation increases the xylem phase (Fig. 4d) [24].

254 Phloem resistance factors are related to the E1 salivation and E2 ingestion
255 parameters, and in particular ingestion phases shorter than 10 minutes [21,25].
256 Phloem-mediated defences against aphids include the occlusion of sieve
257 elements, which prevents aphids from ingesting phloem sap [26-28]. This phloem
258 occlusion occurs upon callose deposition and formation of P-protein plugs.

259 The latter is thought to seal off the phloem upon damage and/or to block the
260 aphid food canal [13,28]. Interestingly, PAD4 was found to be a component of
261 phloem-based immunity against *M. persicae* in Arabidopsis [29]. However, no
262 barely PAD4 (MLOC_1340) or PAD4-related genes were up-regulated during
263 the barley-*M. persicae* interaction [7]. However, our previous transcriptome
264 analyses showed induction of a barley gene encoding Phloem Protein 2-like
265 (PP2), which is a phloem specific lectin, with the induction being most
266 pronounced during the barley-*M. persicae* interaction [7]. Lectins have
267 carbohydrate-binding properties and function in cell communication,
268 development, and plant defence [30]. PP2 is a lectin highly abundant in the
269 phloem and accumulates in damaged phloem sieve pores to form protective
270 plugs [31]. Overexpression of *AtPP2* in Arabidopsis leads to reduced *M.*
271 *persicae* feeding suggesting PP2 may contribute to defences against aphids
272 [32], possibly by interfering with aphid digestion in the midgut [33]. The very
273 infrequent phloem sap ingestion we observed might reflect a rejection of the
274 sieve element, possibly due to the presence of a deterrent factor in the
275 phloem sap [34]. Indeed, lectins, including PP2-like proteins, have been shown
276 to have deterrent activities and insecticidal activities against *M. persicae*
277 [32,35,36]. Whether barley phloem-lectins like PP2 indeed contribute to phloem-
278 based defences of barley against *M. persicae* needs to be further tested.

279 It is important to note that the EPG experimental set-up was of a no-choice
280 nature (i.e. aphids were placed on the plants) and that additional plant
281 resistance components that affect aphid choice may play a role in the
282 interactions studied here [3,7]. For example, we previously showed that the
283 black cherry aphid (*Myzus cerasi*), which infests cherry trees as well as

284 several herbaceous plants, displays only limited probing on non-host barley
285 plants, and does not settle on barley leaves [7], pointing to a potential role of
286 barley defences that act at the pre-probing level against this aphid species [37].
287 In addition, some plant induced volatile compounds have been reported to be
288 repellent to aphid pests and attractants of their natural enemies [38-40].

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

295 **Methods**

296 **Aphid rearing**

297 *R. padi* (JHI-JB, genotype G) [41,42] was maintained on *Hordeum vulgare* L. cv
298 Optic and *M. persicae* (JHI_genotype O) was maintained on *Brassica napus*
299 (oilseed rape). All aphid species used in the experiments were maintained in
300 growth chambers under controlled conditions (18°C, 16 h of light).

301 **Plant growth**

302 Barley plants (cv. OpticGolden Promise) were pre-germinated in Petri dishes
303 with wet filter paper for three days in the dark. Then, they were moved to a
304 cabinet under controlled conditions and grown for 7 days (growth stage 1.10,
305 determined using the staging key [43]) until the EPG experiments. Arabidopsis
306 plants were sown directly in soil; the seeds were stratified for 3 days at 4ºC
307 and placed in the growth cabinet for 4-5 weeks before use in experiments

308 (growth stage 1.10 to 3.90, determined using the Boyes growth key [44]). The
309 cabinet conditions for Arabidopsis were 8 hours of light (125 µmol
310 photons/m^2.s), at 22 °C and 70% humidity. The cabinet conditions for barley
311 were 8 hours of light (150 µmol photons/m2.s), at 20 °C (+-2°C).

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

313 The probing and feeding behaviour of *R. padi* and *M. persicae* on different
314 plant species was assessed using the Electrical Penetration Graph technique
315 [15] on a Giga-4 DC-EPG device with 1 Giga Ω resistance (EPG Systems, The
316 Nethelands). We used a randomized block design for all EPG experiments
317 performed here. Aphids were connected to a copper electrode with a golden
318 wire (20 µm diameter), attached at the aphid dorsum and connected to the
319 electrode with water-based silver glue. Aphids were lowered onto either an
320 Arabidopsis or barley leaf approximately 1-1.5 hr after being removed from
321 culture, depending on the treatment, and feeding behaviour was recorded
322 over a 6h period. Three recordings were taken simultaneously. Each
323 experiment was initiated between 10-12 am and the experiment was
324 performed over a 6-month period, with 18 host and 17 non-host replicates for
325 *R. padi* and 23 host and 28 poor-host replicates for *M. persicae*. Data were
326 acquired using the Stylet+ D software package version v.01.28 and annotated
327 manually using the Stylet+ A v.01.30 software (EPG-Systems, The
328 Netherlands). Obtained waveforms were annotated with one of the following
329 signals: no penetration (np), stylet penetration into the epidermal and
330 mesophyll tissue (pathway/C phase), cellular punctures during the C phase
331 (pd), watery salivation into sieve elements (E1), ingestion of phloem sap (E2),
332 derailed stylet mechanics/stylet penetration difficulties (waveform F), xylem

ingestion (waveform G), or extracellular saliva secretion into mesophyll (E1e) [15,21]. Annotated waveforms were converted into time-series data using the excel macro developed by Dr Schliephake (Julius Kühn-Institut); these converted parameters were used for statistical analysis. Parameters used for comparisons in these experiments are described by Giordanengo et al. (2014) [45], and include total time of probing, number of probes, duration of phloem sap ingestion, and duration of xylem sap ingestion, a total of 97 parameters were measured. Statistical analyses were performed in R Studio running R v. 3.2.3. (R Core Team, 2017) [46] using the Wilcoxon rank test.

Acknowledgements

Dr. Freddy Tjallingii (EPG Systems, The Netherlands), Professor Alberto Fereres (CSIC, Spain) and Professor Gregory Walker (University of California, Riverside, USA) for providing EPG training, and additional thanks to Dr Tjallingii for helpful comments on non-host EPG waveforms. We also thank Dr. Nick Birch for allowing us to use the EPG equipment. This work was support by the European Research Council (310190-APHIDHOST to JIBB), and the James Hutton Institute and Universities of Aberdeen and Dundee through a Scottish Food Security Alliance (Crops) PhD studentship to DJL.

Author contributions

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

359

360 **Competing interests**

361 The author(s) declare no competing interests.

362

363

364 **Figure Legends**

365

366 **Figure 1. Graphical representation of aphid/stylet activities during each**
367 **EPG waveform.**

368 a) Example of obtained EPG waveform, top panel shows waveform over a
369 1 hour period and lower panel is the magnified waveform for the highlighted
370 section over a 60s period. np = non-probing, C = C phase (stylet activity in
371 epidermis/mesophyll), pd = potential drop (cellular puncture) E1/E2 indicates
372 phloem phases, saliva secretion (E1) and phloem ingestion (E2).

373 b) Example of aphid activity during np waveform, stylet is not in contact
374 with leaf tissue therefore voltage is approximately 0. The magnified panels
375 cover only a 30s period.

376 c) Initiation of pathway (C) phase - aphid stylet pierces leaf epidermis,
377 voltage becomes positive as aphid stylet moves through epidermis. Waveform
378 characterised by large initial waves, voltage is generally between 0 and +4
379 volts.

380 d) Potential drop (pd) – aphid stylet penetrates adjacent plant cell leading
381 to rapid decrease in voltage. Waveform characterised by a reduction in
382 voltage to around – 3 volts for 2 – 3s before stylet retraction from pierced cell
383 and return to C phase.

384 e) Stylet penetration difficulties (F phase) – waveform characterised by
385 rapid oscillations in positive voltage between +1 and +5 volts, waveforms
386 generally appear smooth.
387 f) Extracellular saliva secretion (E1e) phase – salivation into extracellular
388 space, waveform is characterised by short fin-shapes waves in a positive
389 voltage, around +2 to +4 volts.
390 g) Xylem ingestion (G phase) – stylet penetrates vascular xylem cells to
391 initiate xylem drinking. Waveform is characterised by oscillating positive
392 waveforms between +2 and +4 volts.
393 h) Salivation into phloem (E1 phase) – stylet penetrates sieve tube
394 element and aphid initiates salivation into phloem sap. Waveform is preceded
395 by a series of rapid potential drops (cellular probes) with a final probe into a
396 sieve element, salivation waveform characterised by fin-shaped waveforms in
397 the negative voltage.
398 i) Phloem ingestion (E2 phase) – aphid begins passive ingestion of
399 phloem sap. Waveform always follows on from E1 waveform and is
400 characterised by brief and sharp negative peaks.
401
402 **Figure 2. Box plots showing different EPG parameters associated with**
403 ***Rhopalosiphum padi*-barley (host) and *Rhopalosiphum padi*-Arabidopsis**
404 **(non-host) interactions.**
405 **a**) Probing-related parameters: non-probing period length, number of probing
406 events, number of pathway (C phase) events and pathway phase length.
407 **b**) Vascular-related parameters: number of xylem (G phase) events, xylem
408 phase length, number salivation (E1 phase) events, salivation phase length,

409 number of ingestion (E2 phase) events and ingestion phase length. Green
410 boxes indicate the host interaction and red boxes represent the non-host
411 interaction. *R. padi* on host plants was replicated 18 times and *R. padi* on
412 non-host plants was replicated 17 times. Significant differences between
413 interactions were assessed by Wilcoxon non-parametric t-test (*= p ≤0.05 and
414 *** = p ≤0.01).
415
416 **Figure 3: Box plots showing different EPG parameters in *Myzus persicae***
417 **interaction with a host (Arabidopsis) and a poor-host plant (barley).**
418 **a**) *M. persicae* probing-related parameters: number of probing events, no--
419 probing period length, number of pathway (C phase) events and pathway
420 phase length.
421 **b**) *M. persicae* vascular-related parameters: number of xylem (G phase)
422 events, xylem phase length, number of salivation (E1 phase) events,
423 salivation phase length, number of ingestion (E2 phase) events and ingestion
424 phase length. Green boxes indicate the host interaction and red boxes
425 represent the poor-host interaction. *M. persicae* on host plants was replicated
426 23 times and *M. persicae* on poor-host plants was replicated 28 times.
427 Significant differences between interactions were assessed statistically by
428 Wilcoxon non-parametric t-test (*= p ≤0.05 and *** = p ≤0.01).
429
430 **Figure 4. Model showing *R. padi* and *M. persicae* probing and feeding**
431 **during host, poor-host and non-host plant interactions.**

432 **a**) During the host interaction (*R. padi*-barley), the aphids will probe the
433 epidermal and mesophyll cells (pathway C phase), then will drink from the
434 xylem or salivate and feed from the phloem, with feeding lasting for hours.
435 **b**) During the non-host interaction (*R. padi*-Arabidopsis), the aphids will spend
436 a long time not probing, and when probing eventually occurs the aphids
437 remain in stylet pathway phase (in epidermis and mesophyll cell layers) most
438 of the time and only occasionally will reach the vascular tissue, either xylem or
439 phloem. No sustained ingestion of phloem sap takes place.
440 **c**) During the host interaction (*M. persicae*-Arabidopsis), the aphids will probe
441 the epidermal and mesophyll cells (pathway C phase), then will drink from the
442 xylem or salivate and feed from the phloem, with feeding taking place for
443 hours.
444 **d**) During the poor-host interaction (*M. persicae*-barley), the aphids show
445 increased probing compared to the host interaction, while the stylet pathway
446 phase (in epidermis and mesophyll cell layers) is similar to the interaction with
447 the host plant. At the vascular level, long periods of time will be spent in the
448 xylem, and eventually aphid will reach the phloem, salivate and ingest phloem
449 sap. However, contrary to the host interaction, no sustained (>10 minutes)
450 ingestion of phloem sap takes place.

451 **Supplementary Data**

452 **Table S1**. Results for all obtained Electrical penetration graph (EPG)
453 parameters which were significantly different between host and non/poor-host
454 feeding. Table displays the EPG parameter assessed, a description of the
455 parameter, and the plant tissue layer involved. Results displayed are the

456 mean and standard deviation (SD) for each aphid-plant combination for each
457 parameter alongside the Wilcoxon test statistic (W value) and p value for each
458 pairwise host vs non/poor host comparison. p values in bold represent values
459 significantly different in both host vs non-host and host vs poor-host
460 interactions, italicised p values represent parameters which only differed in
461 one combination. Average and standard deviation of the 97 electrical EPG
462 parameters calculated for *R. padi* host (Rp_Hv) and non-host (Rp_At).
463 Average and standard deviation of the 97 electrical EPG parameters
464 calculated for *M. persicae* host (Mp_At) and poor-host (Mp_Hv). Calculations
465 were made with summary statistics in Rstudio. The EPG list of variables was
466 taken from EPG systems:
467 www.epgsystems.eu/files/List%20EPG%20variables.xls

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

481 were made with summary statistics in Rstudio. The EPG list of variables was
482 taken from EPG systems:
483 www.epgsystems.eu/files/List%20EPG%20variables.xls

References:

1 Blackman R, E. V. 466 (Wiley & sons, Chichester, 2000).

2 Moran, N. A. The evolution of aphid life-cycles. *Annual Review of Entomology* **37**, 321-348 (1992).

3 Powell, G., Tosh, C. R. & Hardie, J. Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annu Rev Entomol* **51**, 309-330, doi:10.1146/annurev.ento.51.110104.151107 (2006).

4 Doring, T. F. How aphids find their host plants, and how they don't. *Annals of Applied Biology* **165**, 3-26, doi:10.1111/aab.12142 (2014).

5 Doring, T. F. & Chittka, L. Visual ecology of aphids: a critical review on the role of colours in host finding. *Arthropod-Plant Interactions* **1**, 3-16, doi:10.1007/s11829-006-9000-1 (2007).

6 Neal, J. J., Tingey, W. M. & Steffens, J. C. Sucrose esters of carboxylic-acids in glandular trichomes of *Solanum-berthaultii* deter settling and probing by green peach aphid. *Journal of Chemical Ecology* **16**, 487-497, doi:10.1007/bf01021780 (1990).

7 Escudero-Martinez, C. M., Morris, J. A., Hedley, P. E. & Bos, J. I. B. Barley transcriptome analyses upon interaction with different aphid species identify thionins contributing to resistance. *Plant Cell and Environment* **40**, 2628-2643, doi:10.1111/pce.12979 (2017).

8 Jaouannet, M., Morris, J. A., Hedley, P. E. & Bos, J. I. Characterization of arabidopsis transcriptional responses to different aphid species reveals genes that contribute to host susceptibility and non-host resistance. *PLoS Pathog* **11**, e1004918, doi:10.1371/journal.ppat.1004918 (2015).

9 Debokx, J. A. & Piron, P. G. M. Relative efficiency of a number of aphid species in the transmission of potato virus-YN in the Netherlands. *Netherlands Journal of Plant Pathology* **96**, 237-246 (1990).

10 Katis, N. & Gibson, R. W. Transmission of potato virus-Y by cereal aphids. *Potato Research* **28**, 65-70, doi:10.1007/bf02357571 (1985).

11 Verbeek, M., Piron, P. G. M., Dullemans, A. M., Cuperus, C. & van der Vlugt, R. A. A. Determination of aphid transmission efficiencies for N, NTN and Wilga strains of Potato virus Y. *Annals of Applied Biology* **156**, 39-49, doi:10.1111/j.1744-7348.2009.00359.x (2010).

12 Perry, K. L. *et al.* Yield effects of Barley yellow dwarf virus in soft red winter wheat. *Phytopathology* **90**, 1043-1048, doi:10.1094/phyto.2000.90.9.1043 (2000).

13 Tjallingii, W. F. Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany* **57**, 739-745, doi:10.1093/jxb/erj088 (2006).

14 Tjallingii, W. F. & Esch, T. H. Fine-structure of aphid stylet routes in plant-tissues in correlation with epg signals. *Physiological Entomology* **18**, 317-328, doi:10.1111/j.1365-3032.1993.tb00604.x (1993).

15 Tjallingii, W. F. 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 (N.E.V.), Vol 6, 1995*, 163-169 (1995).

16 Prado, E. & Tjallingii, W. F. Aphid activities during sieve element punctures. *Entomologia Experimentalis Et Applicata* **72**, 157-165, doi:10.1111/j.1570-7458.1994.tb01813.x (1994).

17 Tjallingii, W. F. Electrical nature of recorded signals during stylet penetration by aphids. *Entomologia Experimentalis Et Applicata* **38**, 177-186, doi:10.1111/j.1570-7458.1985.tb03516.x (1985).

18 Tjallingii, W. F. Membrane-potentials as an indication for plant-cell penetration by aphid stylets. *Entomologia Experimentalis Et Applicata* **38**, 187-193, doi:10.1111/j.1570-7458.1985.tb03517.x (1985).

19 Mclean, D. L. & Kinsey, M. G. Probing Behavior of Pea Aphid *Acyrthosiphon pisum* .2. Comparisons of Salivation and Ingestion in Host and Non-Host Plant Leaves. *Annals of the Entomological Society of America* **61**, 730-& (1968).

20 Tjallingii, W. F. Electronic recording of penetration behavior by aphids. *Entomologia Experimentalis Et Applicata* **24**, 721-730, doi:10.1111/j.1570-7458.1978.tb02836.x (1978).

21 Alvarez, A. E. *et al.* 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, doi:10.1111/j.1570-8703.2006.00464.x (2006).

22 Pompon, J., Quiring, D., Giordanengo, P. & Pelletier, Y. Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). *Journal of Insect Physiology* **56**, 610-615, doi:10.1016/j.jinsphys.2009.12.009 (2010).

23 Spiller, N. J., Koenders, L. & Tjallingii, W. F. Xylem ingestion by aphids: a strategy for maintaining water-balance. *Entomologia Experimentalis Et Applicata* **55**, 101-104, doi:10.1111/j.1570-7458.1990.tb01352.x (1990).

24 Ramirez, C. C. & Niemeyer, H. M. The influence of previous experience and starvation on aphid feeding behavior. *Journal of Insect Behavior* **13**, 699-709, doi:10.1023/a:1007844027368 (2000).

25 Prado, E. & Tjallingii, W. F. Effects of previous plant infestation on sieve element acceptance by two aphids. *Entomologia Experimentalis Et Applicata* **82**, 189-200, doi:10.1046/j.1570-7458.1997.00130.x (1997).

26 Dreyer, D. L. & Campbell, B. C. Chemical basis of host-plant resistance to aphids. *Plant Cell and Environment* **10**, 353-361 (1987).

27 Medina-Ortega, K. J. & Walker, G. P. Faba bean forisomes can function in defence against generalist aphids. *Plant Cell and Environment* **38**, 1167-1177, doi:10.1111/pce.12470 (2015).

28 Will, T. & van Bel, A. J. E. Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany* **57**, 729-737, doi:10.1093/jxb/erj089 (2006).

29 Pegadaraju, V. *et al.* Phloem-based resistance to green peach aphid is controlled by Arabidopsis PHYTOALEXIN DEFICIENT4 without its signaling partner ENHANCED DISEASE SUSCEPTIBILITY1. *Plant Journal* **52**, 332-341, doi:10.1111/j.1365-313X.2007.03241.x (2007).

30 Bellande, K., Bono, J. J., Savelli, B., Jamet, E. & Canut, H. Plant lectins and lectin receptor-like kinases: How do they sense the outside? *International Journal of Molecular Sciences* **18**, doi:10.3390/ijms18061164 (2017).

31 Read, S. M. & Northcote, D. H. Subunit structure and interactions of the phloem proteins of *Cucurbita-maxima* (pumpkin). *European Journal of Biochemistry* **134**, 561-569, doi:10.1111/j.1432-1033.1983.tb07603.x (1983).

32 Zhang, C. L. *et al.* Harpin-induced expression and transgenic overexpression of the phloem protein gene AtPP2-A1 in Arabidopsis repress phloem feeding of the green peach aphid *Myzus persicae*. *Bmc Plant Biology* **11**, 19, doi:10.1186/1471-2229-11-11 (2011).

33 Kehr, J. Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. *Journal of Experimental Botany* **57**, 767-774, doi:10.1093/jxb/erj087 (2006).

34 Mayoral, A. M., Tjallingii, W. F. & Castanera, P. Probing behaviour of *Diuraphis noxia* on five cereal species with different hydroxamic acid levels. *Entomologia Experimentalis Et Applicata* **78**, 341-348, doi:10.1111/j.1570-7458.1996.tb00799.x (1996).

35 Jaber, K., Haubruge, E. & Francis, F. Development of entomotoxic molecules as control agents: illustration of some protein potential uses and limits of lectins. *Biotechnologie Agronomie Societe Et Environnement* **14**, 225-241 (2010).

36 Sauvion, N. *et al.* Effects of GNA and other mannose binding lectins on development and fecundity of the peach-potato aphid *Myzus persicae*. *Entomologia Experimentalis Et Applicata* **79**, 285-293, doi:10.1111/j.1570-7458.1996.tb00836.x (1996).

37 Nottingham, S. F. *et al.* Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology* **17**, 1231-1242, doi:10.1007/bf01402946 (1991).

38 Dreyer, D. L. & Jones, K. C. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: aphid feeding deterrents in wheat. *Phytochemistry* **20**, 2489-2493, doi:10.1016/0031-9422(81)83078-6 (1981).

39 Mallinger, R. E., Hogg, D. B. & Gratton, C. Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: *Aphididae*) in soybean agroecosystems. *Journal of Economic Entomology* **104**, 115-124, doi:10.1603/ec10253 (2011).

40 Turlings, T. C. J. & Ton, J. Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. *Current Opinion in Plant Biology* **9**, 421-427, doi:10.1016/j.pbi.2006.05.010 (2006).

41 J., L. D., B., B. J. I., A., V. T. & J., K. A. The price of protection: a defensive endosymbiont impairs nymph growth in the bird cherry-oat aphid, *Rhopalosiphum padi*. *Insect Science* **0**, doi:doi:10.1111/1744-7917.12606.

42 Thorpe, P. *et al.* Shared transcriptional control and disparate gain and loss of aphid parasitism genes and loci acquired via horizontal gene transfer. *bioRxiv*, doi:10.1101/246801 (2018).

43 Zadoks, J. C., Chang, T. T. & Konzak, C. F. Decimal code for growth stages of cereals. *Weed Research* **14**, 415-421, doi:10.1111/j.1365-3180.1974.tb01084.x (1974).

44 Boyes, D. C. *et al.* Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. *Plant Cell* **13**, 1499-1510, doi:10.1105/tpc.13.7.1499 (2001).

45 Giordanengo, P. EPG-Calc: a PHP-based script to calculate electrical penetration graph (EPG) parameters. *Arthropod-Plant Interactions* **8**, 163-169, doi:10.1007/s11829-014-9298-z (2014).

46 R Core Team (2017) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. *URL http://www.R-project.org/*. (2017).

Figure 1

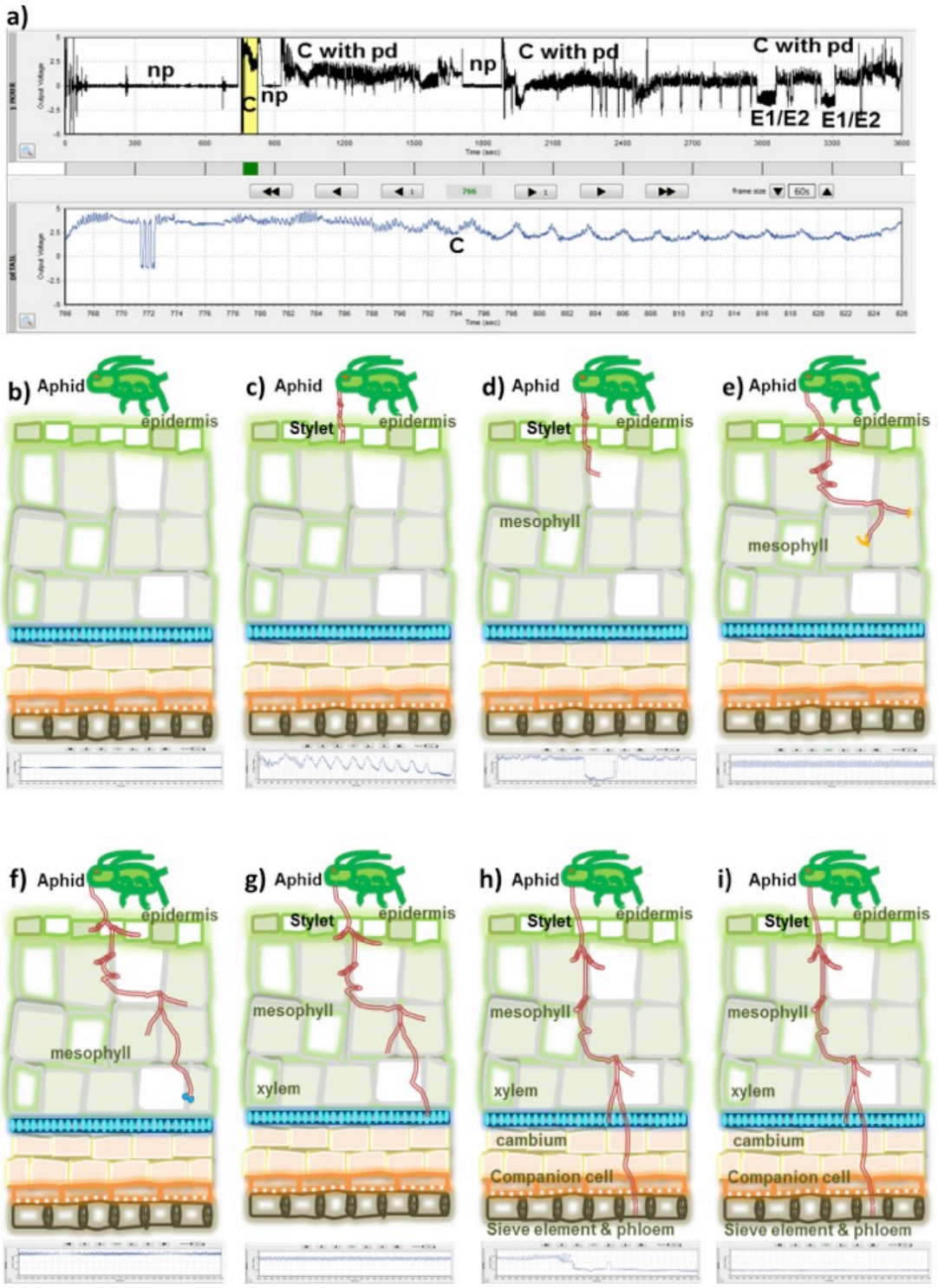

a)
np
C
np
C with pd
np
C with pd
C with pd
E1/E2
E1/E2
C
b) Aphid
epidermis
c) Aphid
Stylet
epidermis
d) Aphid
Stylet
epidermis
mesophyll
e) Aphid
epidermis
mesophyll
f) Aphid
epidermis
mesophyll
g) Aphid
Stylet
epidermis
mesophyll
xylem
h) Aphid
Stylet
epidermis
mesophyll
xylem
cambium
Companion cell
Sieve element & phloem
i) Aphid
Stylet
epidermis
mesophyll
xylem
cambium
Companion cell
Sieve element & phloem

Figure 2

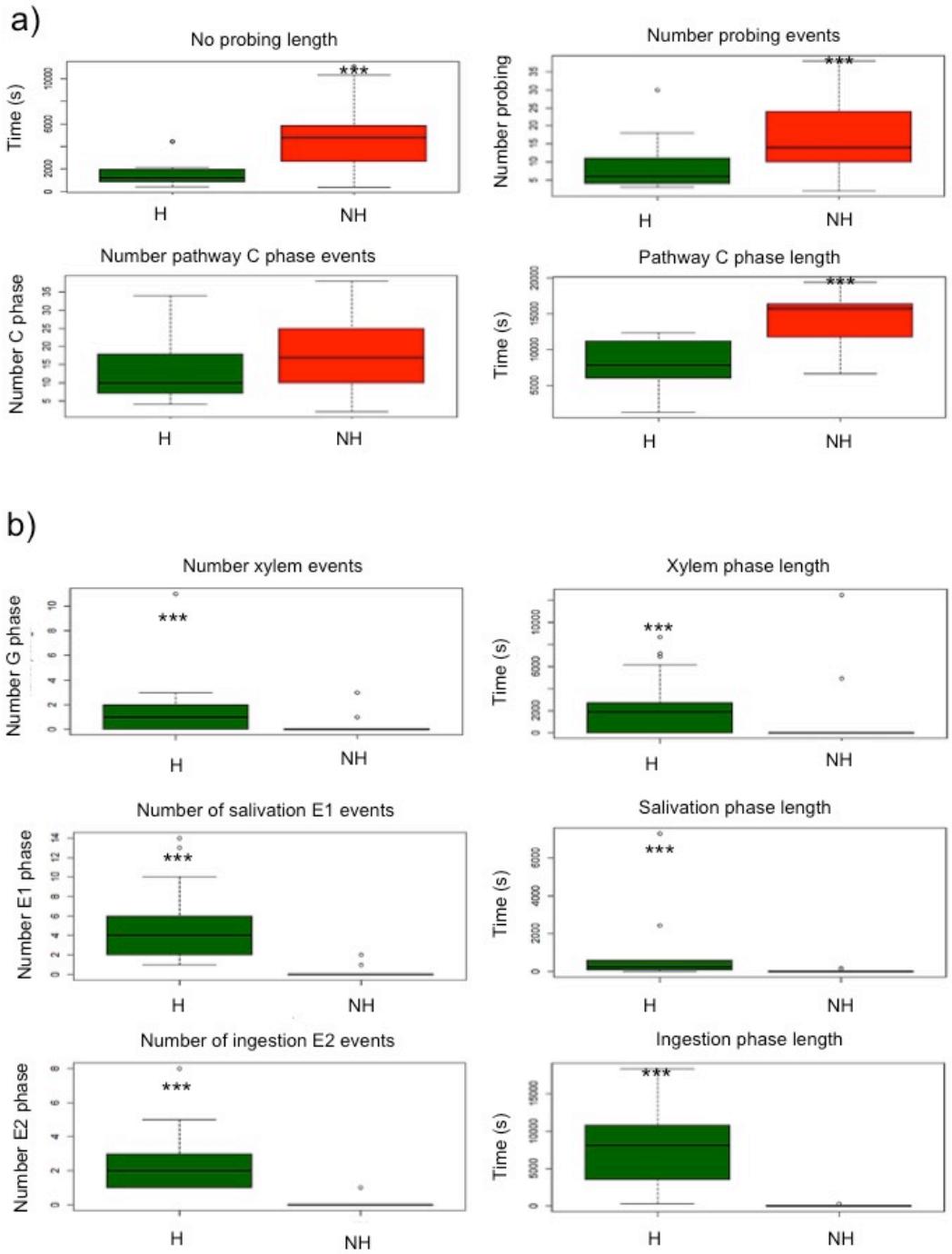

a)
No probing length
Time (s)
***
H
NH
Number probing events
Number probing
***
H
NH
Number pathway C phase events
Number C phase
H
NH
Pathway C phase length
Time (s)
***
H
NH
b)
Number xylem events
Number G phase
***
H
NH
Xylem phase length
Time (s)
***
H
NH
Number of salivation E1 events
Number E1 phase
***
H
NH
Salivation phase length
Time (s)
***
H
NH
Number of ingestion E2 events
Number E2 phase
***
H
NH
Ingestion phase length
Time (s)
***
H
NH

Figure 3

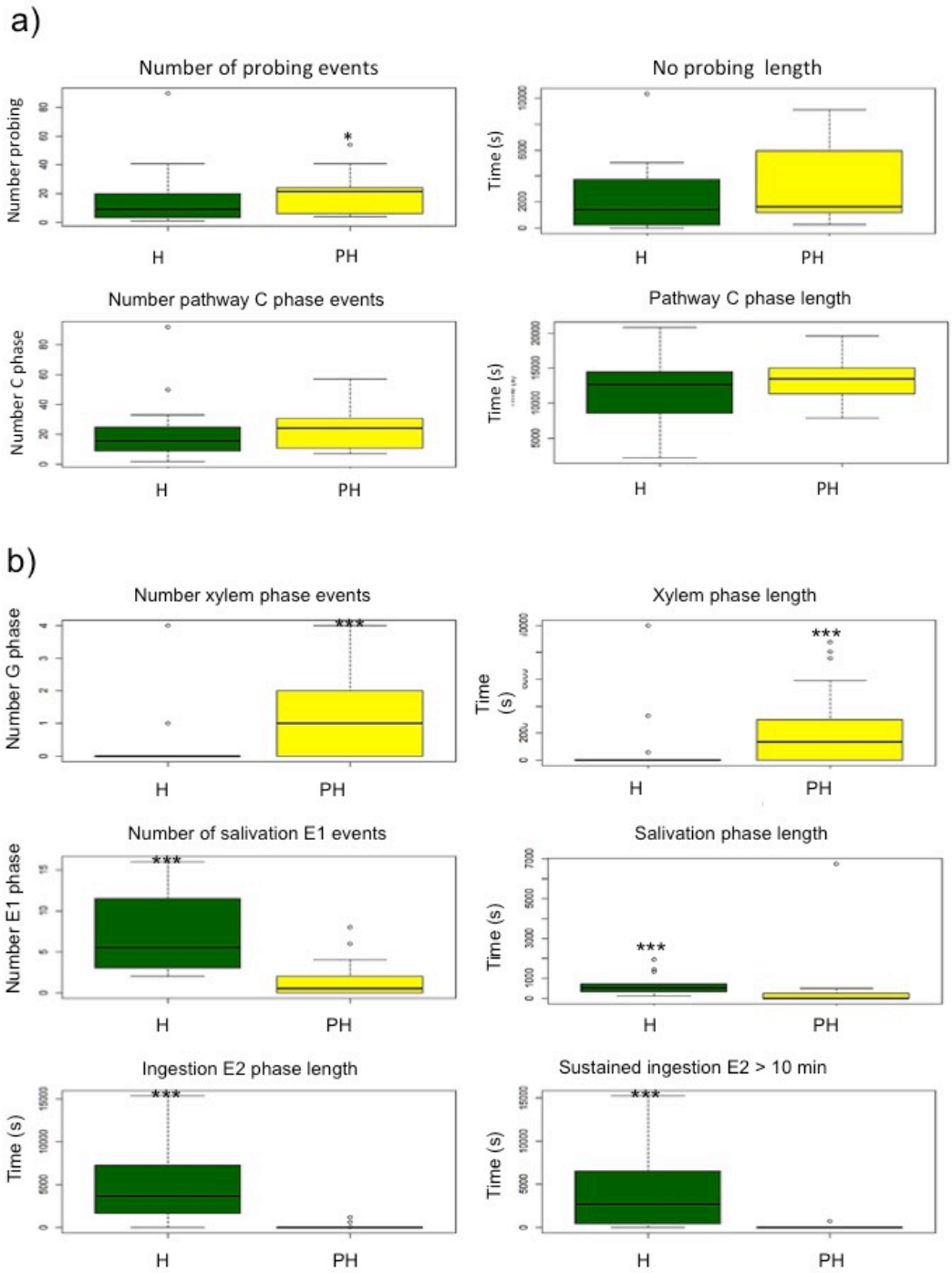
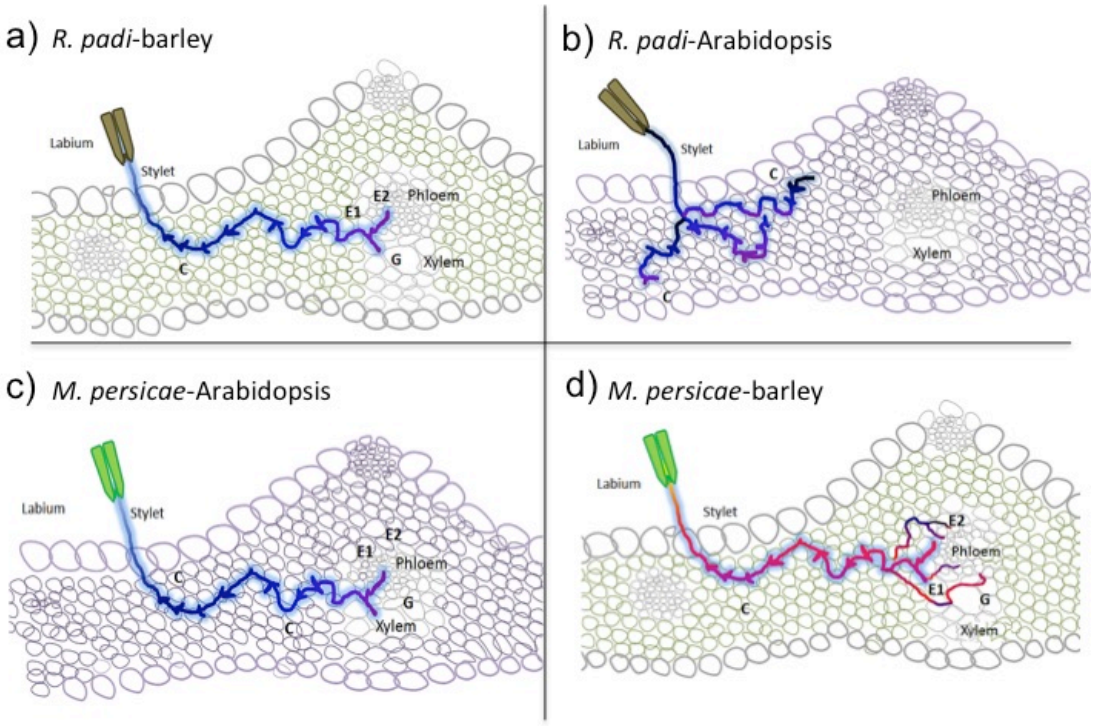

a)
Number of probing events
Number probing
*
H
PH
No probing length
Time (s)
H
PH
Number pathway C phase events
Number C phase
H
PH
Pathway C phase length
Time (s)
H
PH
b)
Number xylem phase events
Number G phase
***
H
PH
Xylem phase length
Time (s)
***
H
PH
Number of salivation E1 events
Number E1 phase
***
H
PH
Salivation phase length
Time (s)
***
H
PH
Ingestion E2 phase length
Time (s)
***
H
PH
Sustained ingestion E2 > 10 min
Time (s)
***
H
PH

Figure 4


a) R. padi-barley
Labium
Stylet
E2
Phloem
E1
C
G
Xylem
b) R. padi-Arabidopsis
Labium
Stylet
C
Phloem
Xylem
C
c) M. persicae-Arabidopsis
Labium
Stylet
E2
E1
Phloem
C
G
C
Xylem
d) M. persicae-barley
Labium
Stylet
E2
Phloem
E1
G
C
Xylem