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

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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

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

Results

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

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

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

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

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

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

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

For the E1 salivation phase the number and duration of events was reduced during the poor-host interaction, with 1.73 events (W = 5.28; p = <0.001), with 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.

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

It is important to note that the EPG experimental set-up was of a no-choice nature (i.e. aphids were placed on the plants) and that additional plant resistance components that affect aphid choice may play a role in the interactions studied here [3,7]. For example, we previously showed that the 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

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

Electrical penetration graph (EPG) analyses

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

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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.

Competing interests

The author(s) declare no competing interests.

Figure Legends

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

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

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

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

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

e) Stylet penetration difficulties (F phase) – waveform characterised by rapid oscillations in positive voltage between +1 and +5 volts, waveforms generally appear smooth.

f) Extracellular saliva secretion (E1e) phase – salivation into extracellular space, waveform is characterised by short fin-shapes waves in a positive voltage, around +2 to +4 volts.

g) Xylem ingestion (G phase) – stylet penetrates vascular xylem cells to initiate xylem drinking. Waveform is characterised by oscillating positive waveforms between +2 and +4 volts.

h) Salivation into phloem (E1 phase) – stylet penetrates sieve tube element and aphid initiates salivation into phloem sap. Waveform is preceded by a series of rapid potential drops (cellular probes) with a final probe into a sieve element, salivation waveform characterised by fin-shaped waveforms in the negative voltage.

i) Phloem ingestion (E2 phase) – aphid begins passive ingestion of phloem sap. Waveform always follows on from E1 waveform and is characterised by brief and sharp negative peaks.

Figure 2. Box plots showing different EPG parameters associated with *Rhopalosiphum padi*-barley (host) and *Rhopalosiphum padi*-Arabidopsis (non-host) interactions.

a) Probing-related parameters: non-probing period length, number of probing events, number of pathway (C phase) events and pathway phase length.

b) Vascular-related parameters: number of xylem (G phase) events, xylem 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.**

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.

Supplementary Data

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

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

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

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

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Figure 1

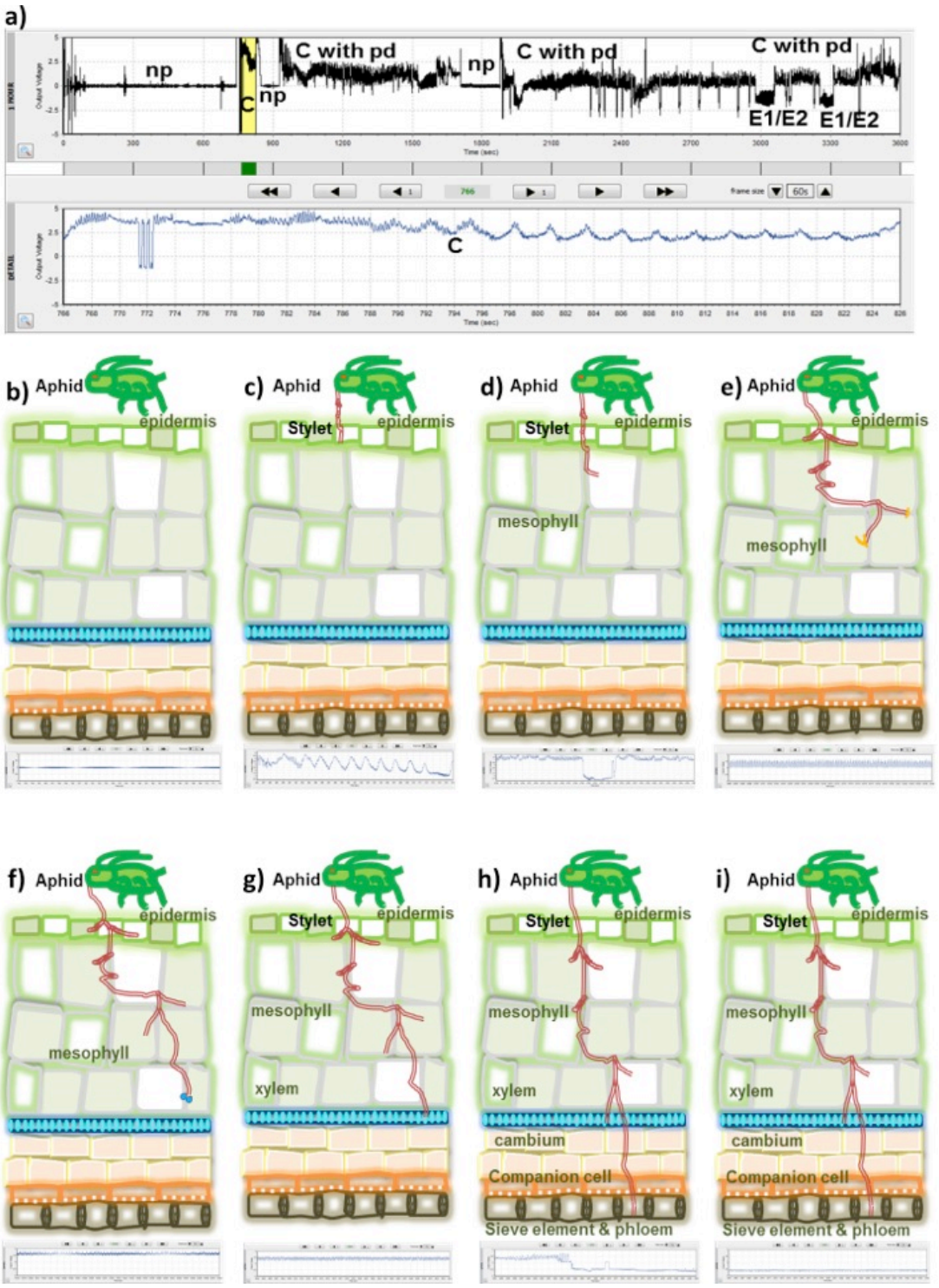

Figure 2

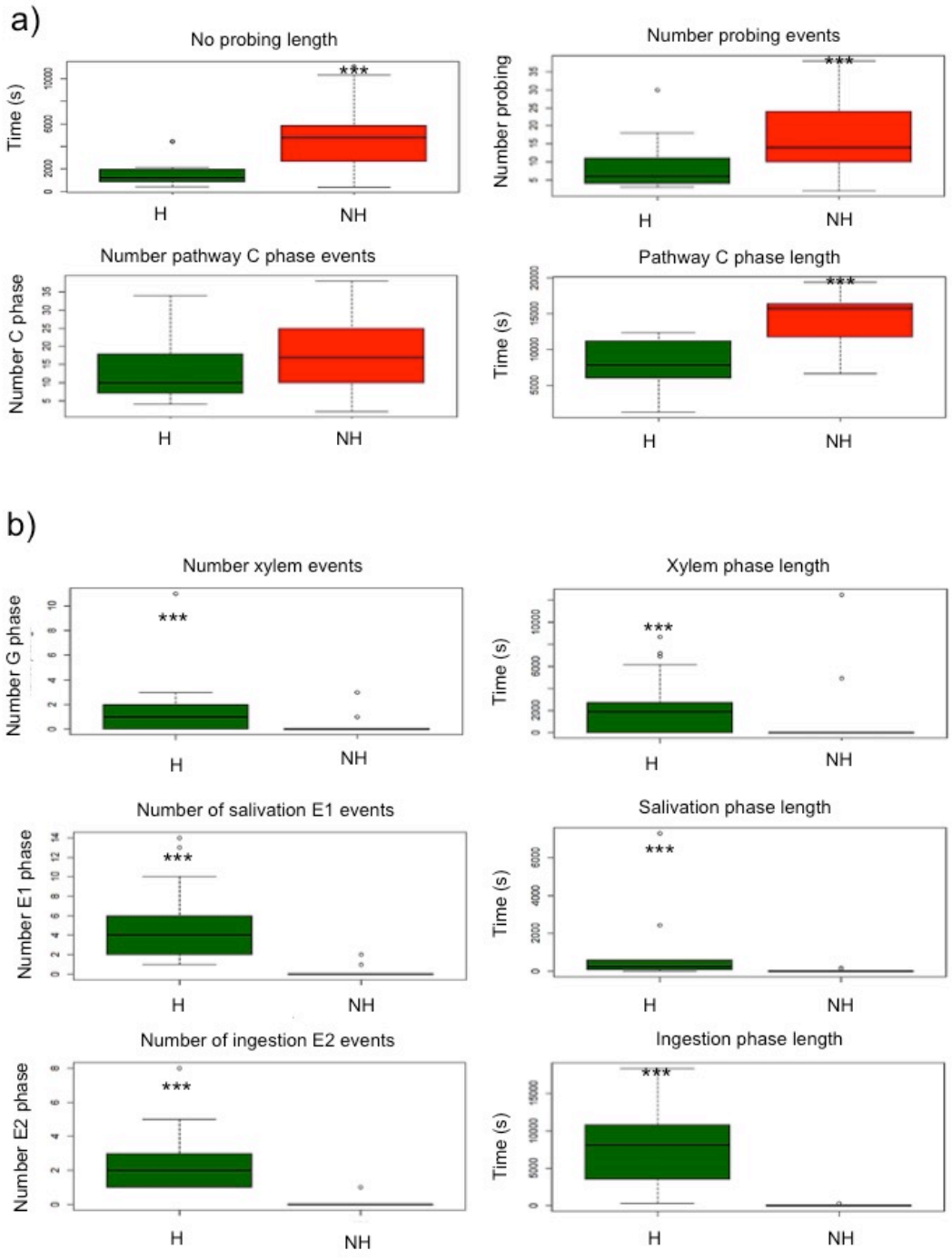

Figure 3

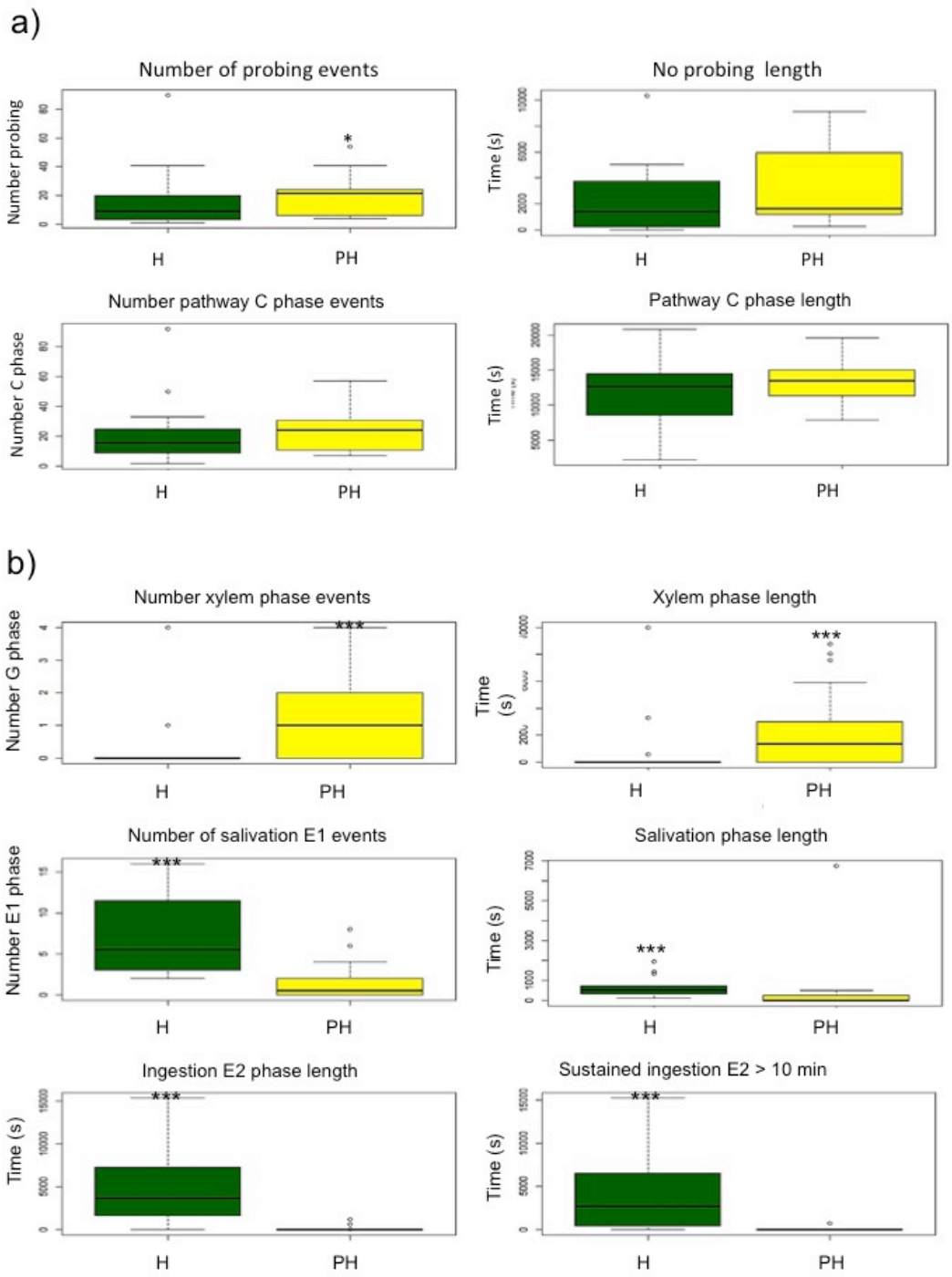

Figure 4